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**ANNEXES TO THE REPORT ON THE VALIDATION OF THE UPDATED TEST GUIDELINE 407:
REPEAT DOSE 28-DAY ORAL TOXICITY STUDY IN LABORATORY RATS**

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No. 59**

**REPORT OF THE VALIDATION OF
THE UPDATED TEST GUIDELINE 407
REPEAT DOSE 28-DAY ORAL TOXICITY STUDY
IN LABORATORY RATS**

- ANNEXES -

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

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**OECD Environment Directorate,
Environment, Health and Safety Division
2 rue André-Pascal
75775 Paris Cedex 16
France**

**Fax: (33-1) 44 30 61 80
E-mail: ehscont@oecd.org**

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ANNEX 1

Model Protocol for Phase-2 Updated TG 407 Studies

INTRODUCTION

1. This protocol is based on the current OECD 407 protocol (1) and is intended to extend the scope of the current OECD 407 Test Guideline to examine i) the suitability of sub-acute tests in rodents as screening methods for detection of endocrine-mediated effects, and ii) the sensitivity of several new, endocrine-related, biochemical and morphological parameters when measured as part of a 28-day repeated dose toxicity study.
2. With some limitations, the current OECD 407 protocol also may serve as a basis for detection of adverse effects on the endocrine system (or mediated by the endocrine system). In order to identify these additional endocrine effects the guideline would need enhancement by the addition of additional protocols and measurements which have to be validated for their feasibility and reproducibility. However, where ever possible, the inclusion of these protocol enhancements should not interfere with the measurements specified in the original protocol.
3. This prevalidation exercise is designed primarily to evaluate the feasibility of the updated protocol and determine which of the additional endpoints are useful for the detection of adverse effects on (or mediated by) the endocrine system, and could be measured without interfering with, or detracting from, the basic OECD 407 Test Guideline. As a secondary goal, this exercise will provide preliminary information on interlaboratory reproducibility and on the response of the updated protocol to a variety of different endocrine-affecting agents.
4. An updated TG407 protocol developed in July 1999, in collaboration with the participating laboratories, was employed by the lead laboratory, to test Flutamide. Other laboratories used various aspects of the protocol to test flutamide and other substances. The results of the testing were summarised and discussed at a meeting of the participating laboratories in Wuppertal, Germany, in December 1999. The results, and the recommendations of these participants, were presented to the OECD Validation Management Group on 20 - 21 January 2000 for approval and recommendations for future validation work with this assay.
5. Based on the results of the initial phase of the validation study, and the recommendations of the VMG, the next phase (Phase-2) of the validation study is designed to develop data on a series of strongly acting substances and to assess the sensitivity of the updated 407 procedure against a number of weakly acting hormonal substances. In addition, this phase will provide preliminary information on the reproducibility of the assay with strong and weakly acting substances. Laboratories agreeing to test more than one chemical will be asked to test one strong and one weak, or inactive, chemical.
6. The lead laboratory will be the Institute of Toxicology, Bayer AG, Wuppertal, Germany (Dr. A. Freyberger). This laboratory will have the responsibility to assign chemicals to test laboratories, and approve the test doses and vehicle to be used.
7. The protocol described here is designed solely for the purposes described above. Because the current OECD 407 test guideline is widely used, it is not necessary for the evaluation of the updated protocol that some of the non-endocrine-related tissues be examined histopathologically.

8. The purpose of this phase of the validation study is the assessment of the effectiveness and sensitivity of the updated 407 protocol to identify endocrine-acting substances and their putative target tissues. Another purpose is to provide preliminary information on the intra- and inter-laboratory reproducibility of the endpoints for both strong and weakly active substances. Therefore, it is mandatory that all participating laboratories follow the same test protocol, including dose levels and routes of administration, and endpoints evaluated.

PRINCIPLE OF THE TEST

9. In addition to the effects addressed by the current OECD 407 guideline, this protocol includes several enhancements to investigate, on a qualitative and quantitative basis, effects on the endocrine system.

10. The Updated OECD 407 protocol, as described here, adds emphasis on hormonal effects. The need for careful observations of the animals, so as to obtain as much information as possible, is stressed. The updated protocol should identify chemicals that affect the estrogen, androgen, or thyroid systems, and which may give an indication of reproductive organ toxicity.

11. The test substance is administered daily in graduated doses to several groups of experimental animals, one dose level per group, for a period of 28 days. During the period of administration the animals are observed closely each day for signs of toxicity. Animals that die or are killed during the test are necropsied, and surviving animals are killed and necropsied at the conclusion of the test.

DESCRIPTION OF THE TEST

Test substances

12. The test substance will be administered by the most appropriate route. In most cases this will be determined by the available toxicity literature on which the dose selection is based.

13. The test substance will be suspended or dissolved in a suitable carrier (preferably water or corn oil). The correct concentration, stability, and homogeneity of the test substance in the carrier should be determined.

Test animals

14. Young, male and female rats will be used. The females shall be nulliparous and non-pregnant. Dosing should begin when the animals are 7 weeks old.

15. Healthy, young adult, male and female rats are randomly assigned to the control and treatment groups. Cages shall be arranged in such a way that possible effects due to cage placement are minimised. The animals are identified uniquely and kept in their cages for at least five days prior to the start of the study to allow for acclimatisation to the laboratory conditions.

16. At the commencement of the study the weight variation of the individual animals used should not exceed $\pm 20\%$ of the mean weight of each sex.

17. Ten animals (five female and five male) will be used at each dose level. During the treatment period, animals will be housed one per cage. At the option of the testing laboratory, two groups of 5 male and 5 female animals (twenty animals at each dose level) will be used. This will allow an evaluation of the

sensitivity of the test for all endpoints, and a determination of whether 5 animals are sufficient for a valid test. It will also provide data on intra-laboratory reproducibility.

Dose levels

18. At least three test groups and a vehicle or untreated control group shall be used. Animals in the control group shall be handled in an identical manner to the test group subjects. If a vehicle is used in administering the test substance, the control group shall receive the vehicle in the highest volume used. The test chemicals, and the doses selected for each, are described in Table 1, below.

19. Dose levels should be selected taking into account existing toxicity and (toxico-) kinetic data for the test substance or related materials. The highest dose level shall be chosen with the aim of inducing minimal toxic effects, but not death or severe suffering. Thereafter, a descending sequence of dose levels should be selected with a view to demonstrating a dosage related response and no-observed-adverse effects at the lowest dose level (NOAEL). Two to four-fold intervals are frequently optimal for setting the descending dose levels, and the addition of a fourth test group may be preferable to using very large intervals (e.g., more than a factor of 10) between dosages. In order to avoid the measurement of endocrine-mediated effects that may be a secondary consequence of toxicity, there should be at least 2 doses below the overtly toxic dose.

20. Test substances will be administered by the most appropriate routes of exposure. Chemicals administered by gavage will be given in a single, daily dose to the animals at similar times each day, and adjusted as necessary to maintain a constant dose level in terms of animal body weight. For gavage studies, the maximum volume of liquid administered at one time should not exceed 1 ml/100 g body weight, except in the case of aqueous solutions where 2 ml/100 g body weight may be used. Except for irritating or corrosive substances which will normally produce exacerbated effects with higher concentrations, variability in test volume should be minimised by adjusting the concentration to ensure a constant volume at all dose levels. Efforts should be taken during dosing to minimize animal handling stress, which may affect hormonal parameters.

Dosing procedure

21. The animals are dosed with the test substance daily, seven days each week, for a period of 28 days, and the animals are observed daily. Animals in a satellite group scheduled for follow-up observations should be kept for at least a further 14 days without treatment to detect delayed occurrence, persistence of, or recovery from, toxic effects.

General observations

22. General clinical observations shall be made at least once a day, preferably at the same time(s) each day and considering the peak period of anticipated effects after dosing. The health condition of the animals shall be recorded. At least twice daily, all animals are observed for morbidity and mortality. Moribund animals and animals in severe distress or pain shall be removed when noticed, humanely killed and necropsied.

23. Once before the first exposure (to allow for within-subject comparisons), and at least once a week thereafter, detailed clinical observations shall be made in all animals. These observations should be made outside the home cage in a standard arena and preferably at the same time, each time. They should be carefully recorded, preferably using scoring systems explicitly defined by the testing laboratory. Efforts should be made to ensure that variations in the test conditions are minimal and that observations are preferably conducted by observers unaware of the treatment. Signs noted should include but not be limited

to, changes in skin, fur, eyes, or mucous membranes, occurrence of secretions and excretions, and automatic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture, response to handling, the presence of clonic or tonic movements, stereotypes (e.g., excessive grooming, circling), or bizarre behaviour (e.g., self-mutilation, walking backwards), should also be recorded.

24. Early in the fourth exposure week, sensory reactivity to stimuli of different types (e.g. auditory, visual, and proprioceptive), assessment of grip strength and motor activity assessment should be conducted. Further details of the procedures that could be followed are given in the literature (2)(3)(4)(5)(6)(7)(8).

25. Functional observations may be omitted for groups that reveal signs of toxicity to an extent that would significantly interfere with the functional test performance.

26. At the end of the fourth week the oestrous cycle of all females shall be determined daily by vaginal smears for at least 5 consecutive days in order to determine when they are in dioestrus. All females will be sacrificed during dioestrus following 28 - 32 days chemical administration.

Body weight and food/water consumption

27. All animals should be weighed at least once a week. Food or water consumption should also be measured at least weekly if the test substance is administered via the feed or drinking water.

Haematology

28. Blood samples should be taken from a named site just prior to or as part of the procedure for killing the animals, and stored under appropriate conditions. Animals will not be fasted prior to killing.

29. The following haematological examinations should be made at the end of the test period: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leucocyte count, platelet count, and a measure of blood clotting time/potential.

Clinical biochemistry

30. Animals of all groups should be semi-randomly allocated to a scheduled sacrifice to minimise potentially misleading results caused by diurnal rhythms.

31. Clinical biochemistry determinations shall be performed to the current OECD 407 test guideline, using blood samples obtained from all animals as part of the procedure for killing the animals (apart from those found moribund and/or intercurrently killed). Investigations of plasma or serum shall include sodium, potassium, glucose, total cholesterol, urea, creatinine, total protein and albumin, at least two enzymes indicative of hepatocellular effects (such as, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, and sorbitol dehydrogenase). Measurements of additional enzymes (of hepatic or other origin) and bile acids may provide useful information under certain circumstances. In addition, studies to investigate serum markers of general tissue damage should be considered. Other determinations that should be carried out if the known properties of the test substance may, or are suspected to, affect related metabolic profiles include calcium, phosphate, triglycerides, methaemoglobin, and cholinesterase.

32. For the determination of test chemical effects on endocrine-mediated functions, serum levels of the thyroid hormones T3, T4, and TSH shall be measured. Blood shall be collected from females at the same stage of the oestrous cycle.

33. The following urine analysis determinations shall be performed during the last week of the study using timed urine volume collection; appearance, volume, osmolality or specific gravity, pH, protein, glucose and blood/blood cells.

Gross necropsy

34. All animals in the study shall be subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. The liver, kidneys, adrenals, testes, epididymides, thymus, spleen, brain and heart, ovaries, uterus, thyroid (after fixation to avoid tissue damage while trimming), as well as pituitary, seminal vesicles (including coagulating glands), prostate (dorso-lateral and ventral part combined), and mammary glands (males and females) of all animals should be trimmed of any adherent tissue, as appropriate, and their wet weights taken as soon as possible after dissection to avoid drying.

35. After being blotted and weighed, the prostate shall be fixed, and the dorso-lateral and ventral parts will be surgically separated. The two resulting sections will be weighed.

36. The following tissues shall be preserved in the most appropriate fixation medium for both the type of tissue and the intended subsequent histopathological examination: all gross lesions, brain (representative regions including cerebrum, cerebellum and pons), spinal cord, stomach, small and large intestines (including Peyer's patches), liver, kidneys, adrenals, spleen, heart, thymus, thyroid, trachea and lungs (preserved by inflation with fixative and then immersion), pituitary, gonads and accessory sex organs (ovaries, uterus, vagina, one testis, one epididymidis, seminal vesicles including coagulation glands, ventral prostate, mammary gland), urinary bladder, lymph nodes (preferably one lymph node covering the route of administration and another one distant from the route of administration to cover systemic effects), peripheral nerve (sciatic or tibial) preferably in close proximity to the muscle, and a section of bone marrow (or, alternatively, a fresh-mounted bone marrow aspirate). The clinical and other findings may suggest the need to examine additional tissues. Also all organs considered likely to be target organs based on the known properties of the test substance shall be preserved.

Sperm analysis

37. For males at termination, sperm from one epididymis will be collected for enumeration of cauda epididymal sperm reserves, respectively. In addition, sperm from the cauda epididymis (or vas deferens) will be collected and evaluated for sperm morphology (9)(10)(11)(12)(13).

38. The total number of cauda epididymal sperm will be enumerated. Cauda sperm reserves can be derived from the concentration and volume of sperm in the suspension used to complete the qualitative evaluations, and the number of sperm recovered by subsequent mincing and/or homogenizing the remaining cauda tissue. Enumeration may be performed only in control and high-dose males unless treatment-related effects are observed; in that case, the lower dose groups should also be evaluated.

39. A morphological evaluation of epididymal (or vas deferens) sperm shall be performed. Sperm (at least 200 per sample) shall be examined as fixed, wet preparations and classified as either normal (both head and midpiece/tail appear normal) or abnormal. Examples of morphologic sperm abnormalities would include fusion, isolated heads, and misshapen heads and/or tails. Evaluation of only control and high-dose males may be performed unless treatment-related effects are observed; in that case, the lower dose groups should also be evaluated.

Histopathological examination

40. Full histopathology of endocrine-relevant tissues and known or suspect target tissues shall be carried out on the preserved organs and tissues of all animals in the control and high dose groups. These examinations should be extended to animals of all other dosage groups, if treatment-related changes are observed in the high dose group. The recommended tissues for histopathology include adrenals, brain, epididymis, kidneys, liver, mammary gland (females only), ovaries (including oviduct), pancreas, pituitary, prostate (dorsolateral and ventral), seminal vesicles including coagulation gland, testes, thymus, thyroid and parathyroid, mammary gland, uterus, and vagina.

41. All gross lesions shall be examined.

42. When a satellite group is used, histopathology should be performed on tissues and organs identified as showing effects in the treated groups.

DATA

43. Individual animal data shall be provided. Additionally, all data shall be summarised in tabular form showing for each test group the number of animals at the start of the test, the number of animals found dead during the test or killed for humane reasons and the time of any death or humane kill, the number showing signs of toxicity, a description of the signs of toxicity observed, including time onset, duration, and severity of any toxic effects, the number of animals showing lesions, the types of lesions and the percentage of animals displaying each type. The clinical signs of animals selected for humane killing shall be recorded.

44. Numerical results should be evaluated by an appropriate and generally acceptable statistical method. The statistical methods should be selected during the design of the study.

REPORTING

45. The test report shall include the following information:

Test animals:

- species/strain used;
- number, age, and sex of animals;
- source, housing conditions, diet, etc.;
- individual weights of animals at the start of the test, at weekly intervals thereafter, and at the end of the test

Test conditions:

- justification for choice of vehicle, if other than water;
- doses administered and rationale for dose selection;
- details of test substance source, purity, achieved concentration, stability and homogeneity of the preparation;
- details of the administration of the test substance, including volumes, timing, etc.;
- details of food and water sources and quality;
- information on any difficulties encountered in preparing the test substance or administering it to the animals

Results:

- body weight/body weight changes;
- food consumption, and water consumption, if applicable;
- clinical response data by sex and dose level, including signs of toxicity;
- nature, severity, and duration of clinical signs (whether reversible or not);
- sensory activity, grip strength and motor activity assessments;
- haematological tests with relevant base-line values;
- clinical biochemistry tests with relevant base-line values;
- hormonal analyses with relevant base-line values;
- body weight at killing and organ weight data;
- necropsy findings;
- sperm analysis in males
- female oestrus cyclicity data
- a detailed description of all histopathological findings
- absorption data if available;
- statistical treatment of results, where appropriate, and description of statistical methods.

Discussion of results.

Conclusions.

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APPENDIX

SUMMARY TEST PROTOCOL (PHASE 2)

All testing shall be conducted as described in this protocol. The following Table is provided for clarification, and should aid in achieving conformity among the participating laboratories. All other investigations shall be performed according to the updated OECD 407 protocol described above.

Test Substances:	<u>Test Chemical</u> Flutamide Ethinyl estradiol Tamoxifen Methyl testosterone Thyroxin 6- <i>n</i> -Propyl-2-thiouracil Genistein p,p'-DDE Nonylphenol Fenarimol or Fadrazole
Animal Species:	Rat (strain as preferred by the conducting laboratory)
Number of Animals:	5/sex/dose: animals are randomly assigned to the groups (including controls). At the option of the laboratory, 10 (2 x 5)/sex/dose can be used to evaluate the sensitivity of the endpoint and to provide data to determine intra-laboratory reproducibility.
Age:	7 weeks at the start of treatment
Route and frequency of treatment:	Orally via gavage, or other appropriate route; daily, on 28 consecutive days
Terminal sacrifice:	Males and females are necropsied after having been treated at least 28 days, with sacrifice on days 28-32 to allow for estrus cycling in females. All females shall be necropsied during the dioestrus stage of the oestrous cycle; if necessary the treatment period for females should be extended to 32 days (females showing no cyclicity should be terminated on day 28).
Dose levels and numbers of groups:	The highest dose and descending dose levels shall be selected as described in OECD 407 (i.e., on the basis of existing knowledge and a pilot study, if necessary). The interval factor should not exceed 4 fold. One control and 3 treatment groups are sufficient, however a higher number of treatment groups may be employed. In compliance with the current OECD 407 the lowest dose level should demonstrate a NOAEL.
Female cycle:	Investigate during the fourth week of the study including the day of terminal sacrifice.
Hormone assays:	T ₃ , T ₄ , and TSH. Blood shall be collected at the time of terminal sacrifice.
Sperm analysis:	At time of sacrifice, the total number of cauda epididymal sperm shall be enumerated. Sperm from the cauda epididymis (or vas deferens) shall be collected and evaluated for sperm morphology
Observations:	Histopathology should be restricted to target organs, endocrine sensitive organs and tissues, as well as pituitary, liver, kidneys, and adrenals.

ANNEX 2

Participating Laboratories in Phase-2 Updated TG 407 Validation Studies

This information is available to Government representatives of OECD member countries only.

ANNEX 3

Mortality and Body Weight Data Generated for the Updated TG 407 Validation Studies

1. The purposes of this Annex are to record for the mortalities and body weight observations in the updated TG 407 validation studies:

- whether the mortalities were judged to be treatment related, and whether any action, particularly the alteration of the administered doses, was taken as a result;
- the basic statistical findings for each sex from each Subgroups and the combined Subgroups, when these data were available from the final reports; and
- the means and standard deviations (SDs) for each sex from each Subgroup and the combined Subgroups, when these data were available from the final reports.

2. Additional purposes of this Annex are to compare the reproducibility and reliability of the experimental findings for body weights:

- in regards to a dose response or possible random findings;
- between Subgroups in the same lab;
- the added value and power of combined the Subgroups (i.e., a group size of five versus ten animals per sex); and
- between the two laboratories studying each chemical.

3. This Annex is organized in the following manner:

- each of the ten chemicals tested in the updated TG 407 validation program has its own individual section. The chemicals are roughly grouped as:
 - Estrogens: ethinyl oestradiol, genistein, and nonylphenol.
 - Antiestrogens: tamoxifen.
 - Aromatase inhibitors: CGS 18320B.
 - Androgens: methyl testosterone.
 - Antiandrogens: flutamide and *p,p'*-DDE.
 - Thyroid toxicants and hormones: propylthiouracil and l-thyroxine.
- in each section, the mortalities and body weight data of both laboratories testing the chemical are presented with the laboratories in alphabetical order;
- a summary table is presented with the body weight data for the combined Subgroups of each sex first, then for the two individual Subgroups, when these data are available;
- the dose groups are arranged from left to right in the following order: the vehicle control, the low dose, the mid-dose, and the high dose of each given test substance; and
- the achievement of statistical significance, either an increase or a decrease in the values, is denoted with an asterisk, usually one asterisk for $p < 0.05$ and two asterisks for $p < 0.01$, as most laboratories reported the statistics in this manner.

4. Overall integration and interpretation of the body weight data with other findings are noted in the body of this report, not this or other Annexes. Likewise, discussion, recommendations, and conclusions are in the body of this report, not this or other Annexes.

Ethinyl Estradiol

Laboratory 2 – Ethinyl oestradiol

5. No mortalities or clinical abnormal signs were observed during the study period. Body weights were significantly decreased in the males at the high dose. The body weight decrease in the male high dose group was 12.5% versus the control group. Absolute food consumption was decreased in the males at the high dose, but did not achieve statistical significance. Body weight means and standard deviations (SDs) are in Table 1.

Table 1. Body Weight Means and SD's of Combined and Individual Subgroups for EE in Laboratory 2 Study

Group		Control	Low	Middle	High
Ethinyl Oestradiol Dose ($\mu\text{g}/\text{kg}/\text{day}$)		0	10	50	200
Body weight	Males - combined	311 \pm 24.9	320 \pm 29.1	295 \pm 29.5	**272 \pm 24.7
Body weight	Females - combined	198 \pm 13.8	205 \pm 14.8	205 \pm 13.8	200 \pm 19.3
Body weight	Males – Subgroup A	321 \pm 25.2	311 \pm 20.7	293 \pm 10.6	**277 \pm 22.1
Body weight	Males – Subgroup B	302 \pm 23.0	330 \pm 35.3	297 \pm 42.8	266 \pm 28.3
Body weight	Females – Subgroup A	198 \pm 9.6	203 \pm 21.0	207 \pm 16.1	204 \pm 27.4
Body weight	Females – Subgroup B	198 \pm 18.3	208 \pm 5.6	204 \pm 12.8	196 \pm 7.0

Values expressed as mean only, the Standard Deviations were not provided in the report. *, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively). $n = 10$ Combined Subgroups; $n = 5$ individual Subgroups.

6. Food consumption in the A and B Subgroups was decreased during certain observational periods, but there was no overall significant decrease in either Subgroup. Although body weights were significantly decreased in males at the high dose in Subgroup A, the decrease in high dose male body weights in Subgroup B did not achieve overall statistical significance even though the percentage decrease in the means in Subgroup B versus the controls was 11.9%.

Laboratory 5 – Ethinyl oestradiol

7. No mortalities or clinical abnormal signs were observed during the study period. Body weights were significantly decreased in males at the high dose. The decrease in the male high dose group was 14% versus the control group. Absolute food consumption was decreased and was significant at certain times in both sexes, but there was no overall significant decrease. Body weight means and SDs are in Table 2.

Table 2. Body Weight Means and SD's of Combined and Individual Subgroups for EE in Laboratory 5 Study

Group		Control	Low	Middle	High
Ethinyl Oestradiol Dose ($\mu\text{g}/\text{kg}/\text{day}$)		0	10	50	200
Body weight	Males - combined	371.0 \pm 24.9	367.2 \pm 36.4	360.0 \pm 22.0	**319.2 \pm 30.9
Body weight	Females - combined	250.3 \pm 14.7	248.9 \pm 21.0	248.4 \pm 20.5	233.1 \pm 22.5
Body weight	Males – Subgroup A	378.4 \pm 21.7	372.0 \pm 40.4	368.6 \pm 24.5	331.4 \pm 39.4
Body weight	Males – Subgroup B	363.7 \pm 28.0	362.4 \pm 36.1	351.5 \pm 17.6	**306.9 \pm 14.7
Body weight	Females – Subgroup A	256.2 \pm 16.9	253.1 \pm 26.3	245.4 \pm 25.4	231.3 \pm 17.5
Body weight	Females – Subgroup B	244.4 \pm 10.8	244.6 \pm 16.0	251.4 \pm 16.6	235.0 \pm 28.8

Values were expressed as mean \pm SD. *, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively). $n = 10$ Combined Subgroups; $n = 5$ individual Subgroups.

8. Food consumption in the A and B Subgroups was decreased during certain observational periods, but there was no overall significant decrease in either Subgroup. Although body weights were significantly decreased in males at the high dose in Subgroup B, the decrease in high dose male body

weights in Subgroup A did not achieve overall statistical significance even though the percentage decrease in the means in Subgroup A versus the controls was 12.4%.

Summary – Ethinyl Oestradiol

9. Body weight and food consumption observations were consistent for both sexes in the combined Subgroups of the two laboratories administering ethinyl estradiol.

Genistein

Laboratory 4 – Genistein

10. No mortalities were observed during the study period. No clinical signs related to treatment were observed during the study period. There were no significant differences in body weights or food consumption for either sex. Body weight means and SDs are in Table 3.

Table 3. Body Weight Means and SD's of Combined and Individual Subgroups for Genistein in the Laboratory 4 Study

Group		Control	Low	Middle	High
Genistein (mg/kg/day)		0	120	400	1000
Body weight	Males - combined	409 ± 34	408 ± 24	415 ± 28	410 ± 28
Body weight	Females - combined	249 ± 17	243 ± 18	242 ± 15	249 ± 16
Body weight	Males – Subgroup A	414 ± 28	407 ± 16	419 ± 24	402 ± 30
Body weight	Males – Subgroup B	404 ± 42	409 ± 32	411 ± 34	419 ± 26
Body weight	Females – Subgroup A	243 ± 5	249 ± 20	239 ± 15	240 ± 13
Body weight	Females – Subgroup B	255 ± 23	236 ± 16	246 ± 17	258 ± 13

Values were expressed as mean ± SD. n = 10 Combined Subgroups; n = 5 individual Subgroups.

11. Overall food consumption for the study was not significantly changed in either Subgroup A or B. There were no significant body weight changes at termination in either Subgroup A or B.

Laboratory 12 – Genistein

12. No mortalities were observed during the study period, and no clinical signs related to treatment were observed during the study period. There were no significant differences in body weights or food consumption for either sex. Body weight means and SDs are in Table 4.

Table 4. Body Weight Means and SD's of Combined and Individual Subgroups for Genistein in the Laboratory 12 Study

Group		Control	Low	Middle	High
Genistein (mg/kg/day)		0	120	400	1000
Body weight	Males - combined	363.6 ± 33.0	372.7 ± 18.5	351.8 ± 25.7	354.8 ± 16.7
Body weight	Females - combined	222.9 ± 15.5	224.2 ± 10.1	218.2 ± 10.9	219.1 ± 16.5
Body weight	Males – Subgroup A	370.8 ± 22.0	371.0 ± 25.0	358.2 ± 24.5	350.2 ± 8.2
Body weight	Males – Subgroup B	356.4 ± 42.9	374.4 ± 11.9	345.4 ± 27.9	359.4 ± 22.5
Body weight	Females – Subgroup A	222.4 ± 18.1	223.0 ± 14.1	216.2 ± 12.0	218.0 ± 17.2
Body weight	Females – Subgroup B	223.4 ± 14.6	225.4 ± 5.0	220.2 ± 10.7	220.2 ± 17.7

Values were expressed as mean ± SD. n = 10 Combined Subgroups; n = 5 individual Subgroups.

13. Overall food consumption for the study was not significantly changed in either Subgroup A or B. There were no significant body weight changes at termination in either Subgroup A or B.

Summary – Genistein

14. Body weight and food consumption observations were consistent for their lack of effects with both sexes in the combined Subgroups of the two laboratories with genistein.

Nonylphenol

15. The high dose in the laboratory 1 study began at 200 and decreased to 150 mg/kg/d while the high dose in the laboratory 6 study began at 300 and decreased to 250 mg/kg/d. Therefore, these studies are not fully comparable at the high dose, and more severe effects may be plausibly expected to be observed in the laboratory 6 study.

Laboratory 1 – Nonylphenol

16. There was one female mortality at the high dose on day 14, and this was assessed as treatment related. In accordance with observations of a poorer general condition of the animals, the dosage in the high group was then reduced to 150 mg NP/kg bw/d for the remainder of the study. Clinical signs included, at the high dose, a reduced general condition in 1/10 male and 3/9 females, salivation in 10/10 males and 10/10 females (before mortality), urine staining in the abdominal and anogenital regions in 4 females, and nasal crust formation in one male and one female and, at the mid-dose, salivation in 9/10 males and 7/10 females. There was no significant change in the body weights or food consumption for either sex. Body weight means and SDs are in Table 5.

Table 5. Body Weight Means and SD's of Combined and Individual Subgroups for Nonylphenol in the Laboratory 1 Study

Group		Control	Low	Middle	High
Nonylphenol Dose (mg/kg/day)		0	20	80	200/150
Body weight	Males - combined	306.6 ± 12.3	302.3 ± 19.9	298.9 ± 13.4	300.7 ± 25.6
Body weight	Females - combined	193.9 ± 9.9	204.0 ± 12.2	185.9 ± 13.0	190.9 ± 13.8
Body weight	Males – Subgroup A	310.8 ± 13.9	296.5 ± 17.1	297.8 ± 14.9	312.1 ± 26.6
Body weight	Males – Subgroup B	302.3 ± 10.3	308.2 ± 22.7	299.9 ± 13.4	289.4 ± 21.0
Body weight	Females – Subgroup A	193.8 ± 14.3	197.5 ± 5.1	184.0 ± 15.7	184.6 ± 11.5
Body weight	Females – Subgroup B	194.1 ± 4.3	210.5 ± 14.3	187.7 ± 11.4	195.9 ± 14.5

Values expressed as mean ± SD. *, Significantly different from control (p<0.05). n = 10 Combined Subgroups; n = 5 individual Subgroups.

17. Overall food consumption for the study was not significantly changed in either Subgroup A or B. There were no significant body weight changes at termination in either Subgroup A or B.

Laboratory 6 – Nonylphenol

18. There was one female mortality at the high dose on day 5, and this was assessed as treatment related. The dosage in the high group was then reduced to 250 mg NP/kg bw/d for the remainder of the study. Body weights were significantly decreased in males at the high dose. The decrease in the male high dose group was 17.9% versus the control group. Clinical signs included, at the high dose, emaciation, hypoactivity, anogenital staining, little sign of stool, and nasal crust formation in individuals in both sexes. Food consumption was not significantly decreased. Body weight means and SDs are in Table 6.

Table 6. Body Weight Means and SD's of Combined and Individual Subgroups for Nonylphenol in the Laboratory 6 Study

Group		Control	Low	Middle	High
Nonylphenol Dose (mg/kg/day)		0	20	80	300/250
Body weight	Males - combined	401.0 ± 26.5	383.6 ± 25.3	378.8 ± 25.2	**336.5 ± 41.0
Body weight	Females - combined	233.6 ± 14.5	240.4 ± 9.4	232.4 ± 14.8	236.6 ± 23.7
Body weight	Males – Subgroup A	410.8 ± 25.5	*371.8 ± 16.6	392.4 ± 21.1	**337.2 ± 25.4
Body weight	Males – Subgroup B	391.2 ± 26.2	395.4 ± 28.6	365.2 ± 22.8	335.8 ± 56.0
Body weight	Females – Subgroup A	230.8 ± 11.5	240.4 ± 8.9	238.0 ± 12.3	233.8 ± 18.2
Body weight	Females – Subgroup B	236.4 ± 18.0	240.4 ± 10.9	226.8 ± 16.3	238.8 ± 29.3

Values expressed as mean ± SD. *, **, Significantly different from control (p<0.05 and p<0.01, respectively). n = 10 Combined Subgroups; n = 5 individual Subgroups.

19. Body weights were significantly decreased in males at the high dose in Subgroup A by 17.9% (p<0.01). In Subgroup B, even though body weights were decreased by 14.2%, this did not achieve statistical significance. This could be due to the relatively high standard deviation seen in this Subgroup (see right hand column in the above table). Overall food consumption for the study was significantly decreased in males at the low- and mid-dose in Subgroup A, but not at the high dose in Subgroup A or at any dose in Subgroup B.

Summary – Nonylphenol

20. Taking into account the difference in the test substance levels at the high dose between the laboratories, the values for body weight and food consumption observations at the high dose in the two laboratories appear to be dose related for both sexes in the combined Subgroups with nonylphenol. Body weight and food consumption observations at the mid- and low doses were consistent for both sexes in the combined Subgroups of the two laboratories.

Tamoxifen

Laboratory 3 – Tamoxifen

21. No mortalities were observed during the study period. Clinical signs were limited to one female in the high dose group and consisted of reduced motor activity, thin appearance, pilo-erection and ocular discharge for a limited period between days 4 and 9. Body weight and body weight gain were significantly reduced throughout treatment at the high dose in both sexes and in females at the mid-dose. The body weight decrease in the male high dose group was 15.4% and in the female high dose group 12.9%. Food consumption was significantly reduced in both sexes at the high dose and in females at the mid-dose. Body weight means and SDs are in Table 7.

Table 7. Body Weight Means and SD's of Combined and Individual Subgroups for Tamoxifen in the Laboratory 3 Study

Group		Control	Low	Middle	High
Tamoxifen (µg/kg/day)		0	5	30	200
Body weight	Males - combined	397.0 ± 25.5	417.3 ± 31.2	390.6 ± 16.6	**336.4 ± 19.5
Body weight	Females - combined	249.3 ± 13.4	240.4 ± 13.6	*231.6 ± 16.0	**217.2 ± 8.0
Body wt	Males – Subgroup A	400.0 ± 25.8	409.8 ± 30.4	386.0 ± 17.6	**334.8 ± 23.3
Body wt	Males - Subgroup B	394.0 ± 27.9	424.8 ± 33.5	395.2 ± 16.0	**338.0 ± 17.6
Body wt	Females – Subgroup A	251.4 ± 16.6	235.8 ± 13.4	237.8 ± 18.9	**216.8 ± 7.3
Body wt	Females - Subgroup B	247.2 ± 10.9	245.0 ± 13.6	*225.4 ± 11.1	**217.6 ± 9.4

Values were expressed as mean ± SD. *, **, Significantly different from control (p<0.05 and p<0.01, respectively). n = 10 Combined Subgroups; n = 5 individual Subgroups.

22. Overall food consumption at the high dose was not significantly different in either individual Subgroup for either sex and significantly decreased only in the combined Subgroups for both sexes at the high dose. For the mid-dose females, food consumption was significantly decreased in Subgroup B and in the combined Subgroups, but not Subgroup A. Body weights were significantly decreased in both sexes at the high dose in both Subgroup A (16.3% for males and 13.8% for females) and Subgroup B (14.2% for males and 12.0% for females). Body weights were significantly decreased in mid-dose females in Subgroup B (8.8%) and in the combined Subgroup (7.1%), but were not significantly changed in Subgroup A with a decrease of 5.4%.

Laboratory 10 – Tamoxifen

23. This laboratory employed only a single group with 6 animals per sex for the study. Therefore, no Subgroup data are available, and the statistical power is not equivalent to the combined Subgroups of ten animals per sex.

24. No mortalities or clinical abnormal signs were observed during the study period. Food consumption was significantly decreased in both sexes at the high dose. The body weights were significantly decreased in both sexes at the high dose. The body weight decrease in the male high dose group was 19.4% and in the female high dose group 14.3%. Body weight means and SDs are in Table 8.

Table 8. Body Weight Means and SD's of Study Groups for Tamoxifen in the Laboratory 10 Study

Group		Control	Low	Middle	High
Tamoxifen Dose ($\mu\text{g}/\text{kg}/\text{day}$)		0	5	30	200
Body weight	Males	419.15 \pm 35.41	385.93 \pm 40.28	391.52 \pm 17.28	*338.02 \pm 11.588
Body weight	Females	259.02 \pm 13.45	259.65 \pm 14.16	242.90 \pm 22.69	*221.85 \pm 12.48

Values were expressed as mean \pm SD. *, Significantly different from control ($p < 0.05$). n = 6 per group.

Summary – Tamoxifen

25. Body weight and food consumption observations were consistent for both sexes in the combined Subgroups of laboratory 3 and the group size of 6 used by laboratory 10 at the high dose. At the intermediate dose in the females, a close examination shows that the results were also similar and consistent. Laboratory 3's decrease in body weights of 7.1% with the relatively small coefficient of variations in the groups (5.4 for female controls, 3.7 for high dose, and 6.9 at the mid-dose) was sufficient to achieve statistical significance. In comparison, in laboratory 10, the 6.2% decrease at the mid-dose with the smaller group size and CV of 9.3% did not achieve statistical significance. Note, however, that the percentage decreases were very similar.

CGS 18320B

Laboratory 8 – CGS 18320B

26. One male died due to a dosing error (mortality was not treatment related). Clinical abnormal signs observed during the study period included decreased activity, loose stool and soiled fur in the male high dose group. Body weights were significantly increased in the females at all doses (+23.6% low dose, +32.8% mid-dose, and +26.0% high dose). Food consumption was significantly increased in the females at all doses. Body weight means and SDs are in Table 9.

Table 9. Body Weight Means and SD's of Combined and Individual Subgroups for CGS 18320B in the Laboratory 8 Study

Group		Control	Low	Middle	High
CGS 18320B Dose (mg/kg/day)		0	0.3	3	30
Body weight	Males- combined	403 ± 37	397 ± 40	397 ± 23	364 ± 28
Body weight	Females- combined	250 ± 10	**309 ± 19	**332 ± 21	**315 ± 26
Body wt	Males – Subgroup A	405 ± 45	391 ± 38	391 ± 21	361 ± 26
Body wt	Males - Subgroup B	402 ± 33	403 ± 45	404 ± 24	369 ± 34
Body wt	Females – Subgroup A	251 ± 8	315 ± 11	**340 ± 11	323 ± 31
Body wt	Females - Subgroup B	250 ± 14	**302 ± 24	**325 ± 26	**307 ± 20

Values expressed as mean ± SD. *, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively). $n = 10$ Combined Subgroups; $n = 5$ individual Subgroups.

27. Body weights were significantly increased in females in Subgroup B at all doses and in Subgroup A only at the mid-dose, despite body weight increases of 20.8% at the low dose and 22.8% at the high dose. Food consumption for the study was not significantly increased in males in either Subgroup at the high dose. Food consumption for the study was significantly increased in females in both Subgroups at the mid- and high doses, but not in the low dose of either Subgroup.

Laboratory 13 – CGS 18320B

28. No mortalities or clinical abnormal signs were observed during the study period. Body weights were significantly increased in the females at all doses (34.3% low dose, 41.2% mid-dose, and 31.0% high dose). Food consumption was also significantly increased in females at all doses. Body weights and food consumption were not significantly different in males. Body weight means and SDs are in Table 10.

Table 10. Body Weight Means and SD's of Study Groups for CGS 18320B in the Laboratory 13 Study

Group		Control	Low	Middle	High
CGS 18320B Dose (mg/kg/day)		0	0.3	3	30
Body weight	Males	327.4 ± 22.7	310.6 ± 13.4	333.2 ± 18.0	319.3 ± 26.4
Body weight	Females	202.5 ± 17.5	**265.2 ± 16.1	**286.0 ± 16.6	**271.9 ± 17.7

Values expressed as mean ± SD. *, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively). $n = 10$ per group in this study.

29. The laboratory 13 study did not record the animal data into two Subgroups of five animals per sex. Therefore, no analysis can be performed on the possible effect on study findings and power by increasing the group size to 10 animals per sex.

Summary – CGS 18320B

30. Body weight and food consumption observations were consistent for both sexes in the combined Subgroups of the two laboratories with CGS 18320B, particularly, the limited impact on male body weights and the dramatic increase in female body weights at all doses used in the studies.

Methyl Testosterone

31. In both methyl testosterone studies, different doses were used for the mid- and high dose groups of males (40 and 200 mg/kg/day) and females (100 and 600 mg/kg/day).

Laboratory 3 – Methyl Testosterone

32. No mortalities were observed during the study period. Clinical signs observed included pilo-erection, salivation and nasal discharge in males at the high dose and females at mid- and high doses, and soiled fur in both sexes at the high doses (male and female doses were different, see table below). Body weights were significantly decreased in males at the high dose; the decrease was 7.8% versus the control body weights. In females, body weight and body weight gain were significantly increased at all treatment levels, although not in a clear dose-related manner (+12.6% low dose, +16.1% mid-dose, and +11.0% high dose). Overall food consumption for the study was significantly increased in males at the high dose and in females at all doses. Body weight means and SDs are in Table 11.

Table 11. Body Weight Means and SD's of Combined and Individual Subgroups for Methyl Testosterone in the Laboratory 3 Study

Group		Control	Low	Middle	High
Methyl testosterone (mg/kg/day)		0	10	40	200
Body weight	Males- combined	380.7 ± 29.1	378.5 ± 27.2	385.5 ± 22.7	*351.0 ± 19.1
Body wt	Males – Subgroup A	387.2 ± 34.4	380.4 ± 33.5	377.2 ± 21.3	362.8 ± 14.5
Body wt	Males - Subgroup B	374.2 ± 24.9	376.6 ± 23.2	393.8 ± 23.1	339.2 ± 16.1
Methyl testosterone (mg/kg/day)		0	10	100	600
Body weight	Females- combined	246.0 ± 6.3	**277.1 ± 9.6	**285.6 ± 21.0	**273.1 ± 20.6
Body wt	Females – Subgroup A	242.4 ± 3.5	**277.8 ± 6.5	**268.4 ± 11.8	260.6 ± 16.1
Body wt	Females - Subgroup B	249.6 ± 6.5	**276.4 ± 12.8	**302.8 ± 10.5	**285.6 ± 17.5

Values were expressed as mean ± SD. *, **, Significantly different from control (p<0.05 and p<0.01, respectively). n = 10 Combined Subgroups; n = 5 individual Subgroups.

33. Body weights were not significantly decreased in males at the high dose in either Subgroup. In the females, body weights were significantly increased at the low and mid-dose in both Subgroups A (+14.6% low dose and +10.7% mid-dose) and B (+10.7% low dose and +21.3% mid-dose). However, at the high dose, female body weights were significantly increased in Subgroup B (+14.4%), but not in Subgroup A (+7.5%). Overall food consumption for the study was not significantly different in males at the high dose in either Subgroup A or B. Overall food consumption for the study was not significantly different in females at the low or the high dose in either Subgroup A or B; however, at the mid-dose, Subgroup B was significantly increased, and Subgroup A was not.

Laboratory 12 – Methyl Testosterone

34. No mortalities were observed during the study period. Recorded clinical signs included some salivation in both sexes at the high dose, but this was judged to be in response to the gavage dosing. Body weights were significantly decreased in males at the mid- and high doses. The decrease in the males at the mid-dose was 10.1% and at the high dose 16.6%. In females, the body weights were significantly increased at the low and the mid-dose, but not at the high dose. Food consumption of the high dose males was statistically decreased over the first three weeks of the study period, but not for the overall study. Body weight means and SDs are in Table 12.

Table 12. Body Weight Means and SD's of Combined and Individual Subgroups for Methyl Testosterone in the Laboratory 12 Study

Group		Control	Low	Middle	High
Methyl testosterone (mg/kg/day)		0	10	40	200
Body weight	Males – Mean ± SD Adjusted Mean	374.5 ± 33.3 373.3	362.1 ± 19.9 362.3	**336.7 ± 17.0 338.3	**312.2 ± 16.0 311.6
Body wt	Males – Subgroup A	386.4 ± 37.7 384.5	*356.2 ± 19.9 356.5	**341.6 ± 17.2 344.0	**318.0 ± 15.9 317.2
Body wt	Males - Subgroup B	362.6 ± 26.9 362.4	368.0 ± 12.7 368.0	*331.8 ± 17.2 332.0	**306.4 ± 15.5 306.3
Methyl testosterone (mg/kg/day)		0	10	100	600
Body weight	Females- combined	226.0 ± 15.0 226.8	**250.6 ± 11.1 251.3	**257.2 ± 17.9 257.7	226.5 ± 10.8 224.4
Body wt	Females – Subgroup A	224.4 ± 14.9 226.1	**247.8 ± 9.7 247.4	*251.4 ± 21.8 250.2	224.2 ± 13.5 224.1
Body wt	Females - Subgroup B	227.6 ± 16.7 227.9	*253.4 ± 12.8 254.9	*263.0 ± 12.6 264.8	228.8 ± 8.1 225.2

Values were expressed as mean ± SD. *, **, Significantly different from control (p<0.05 and p<0.01, respectively). n = 10 Combined Subgroups; n = 5 individual Subgroups.

35. Body weights were significantly decreased in males at the mid- and high doses in both Subgroup A (11.6% mid-dose and 17.7% high dose) and Subgroup B (8.5% mid-dose and 15.5% high dose). Body weights were not significantly different in Subgroup B at the low dose (actually increasing by 1.5%), but were significantly decreased in Subgroup A (7.8%). Body weights were significantly increased in females at the low and mid-doses in both Subgroup A and Subgroup B, and body weights were not significantly different in either Subgroup at the high dose. As the animals were housed five per cage, no statistical analyses were performed on the food consumption of the Subgroups.

Summary – Methyl Testosterone

36. Food consumption observations were not consistent between the two studies. The direction of the changes in the body weights was consistent between the combined Subgroups of the two studies, decreasing in males and increasing in females. The dose response in males was somewhat stronger in the laboratory 12 study and in females in the laboratory 3 study.

Flutamide

Laboratory 2 – Flutamide

37. No mortalities were observed during the study period. Clinical signs were observed in both sexes at the high dose and included piloerection 8/20, reduced motility 18/20, and bright colored feces 13/20. Body weights were statistically decreased in the males at the high dose. The decrease in the male high dose group was 14.1% versus the control group. Food consumption was reduced in both sexes, but was not statistically significant. Body weight means and SDs are in Table 13.

Table 13. Body Weight Means and SD's of Combined and Individual Subgroups for Flutamide in the Laboratory 2 Study

Group		Control	Low	Middle	High
Flutamide Dose (mg/kg/day)		0	1	10	100
Body weight	Males- combined	327 ± 18.7	305 ± 25.9	316 ± 32.3	**281 ± 11.7
Body weight	Females- combined	205 ± 13.1	200 ± 10.5	203 ± 13.9	199 ± 5.8
Body weight	Males – Subgroup A	339 ± 13.9	307 ± 24.0	328 ± 35.7	**283 ± 10.9
Body weight	Males - Subgroup B	316 ± 16.7	303 ± 30.3	303 ± 26.1	279 ± 13.3
Body weight	Females – Subgroup A	205 ± 15.1	200 ± 9.1	208 ± 10.3	197 ± 5.3
Body weight	Females – Subgroup B	205 ± 12.5	199 ± 12.9	198 ± 16.5	201 ± 5.8

Values expressed as mean. Standard Deviations were not provided in the final report. *, **, Significantly different from control (p<0.05 and p<0.01, respectively). n = 10 Combined Subgroups; n = 5 individual Subgroups.

38. Overall food consumption for the study was not significantly different in either Subgroup A or Subgroup B. Body weights were significantly decreased in males at the high dose in Subgroup A. However, the absolute decrease in high dose male body weights in Subgroup B did not achieve overall statistical significance even though the mean body weight in Subgroup B versus the controls decreased by 11.7%.

Laboratory 11 – Flutamide

39. No mortalities or clinical abnormal signs were observed during the study period. A non-statistically significant decrease in the treated body weights versus control values (5.3%) was observed in males at the high dose. No significant changes in food consumption were observed. Body weight means and SDs are in Table 14.

Table 14. Body Weight Means and SD's of Combined and Individual Subgroups for Flutamide in the Laboratory 11 Study

Group		Control	Low	Middle	High
Flutamide Dose (mg/kg/day)		0	1	10	100
Body weight	Males- combined	381.9 ± 30.8	398.1 ± 31.3	380.3 ± 32.4	361.5 ± 25.5
Body weight	Females- combined	244.8 ± 15.7	249.3 ± 20.8	249.2 ± 16.6	242.6 ± 20.5
Body weight	Males – Subgroup A	393.8 ± 28.7	404.1 ± 37.5	381.1 ± 46.5	350.4 ± 17.1
Body weight	Males - Subgroup B	369.9 ± 30.8	392.1 ± 26.5	379.5 ± 14.3	372.7 ± 29.4
Body weight	Females – Subgroup A	244.4 ± 16.7	248.0 ± 25.9	249.7 ± 24.7	246.0 ± 18.0
Body weight	Females – Subgroup B	245.2 ± 16.6	250.6 ± 17.3	248.7 ± 3.3	239.2 ± 24.4

Values expressed as mean ± SD. *, Significantly different from control (p<0.05). n = 10 Combined Subgroups; n = 5 individual Subgroups.

40. Overall food consumption for the study was not significantly changed in either Subgroup A or B. There were no significant body weight changes at termination in either Subgroup A or B in either sex.

Summary – Flutamide

41. Food consumption observations were consistent. However, the degree of change in the body weights with flutamide was not consistent between the combined Subgroups of the two studies, being 14.1% in the former case and 5.3% in the latter.

p,p'-DDE

42. In both *p,p'*-DDE studies, different doses were used for males and females. Additionally, the laboratory 7 study was run after the laboratory 6 study. With mortalities in the laboratory 6 high dose

groups, the dosage for the laboratory 7 groups was reduced to 100 mg DDE/kg bw/d for males and 75 mg DDE/kg bw/d for females. These differences in administered doses should be taken into account when comparing the studies.

Laboratory 6 – *p,p'*-DDE

43. Three mortalities occurred by day 8 in the high dose males that were judged to be treatment related, and the dosage was reduced from 200 to 150 mg/kg/d. Subsequently, two additional males died in the high dose group, and three females died in their high dose group of 100 mg/kg/d. Clinical signs included hypothermia, reduced food consumption, emaciation, and anogenital staining of the fur in both sexes in their respective high dose group. Tremors were noted in one high dose male and two high dose females. However, no statistical differences were observed in the functional observational and motor activity batteries. The body weights were not significantly changed by treatment. Food consumption was significantly decreased in both sexes at their respective high doses. Body weight means and SDs are in Table 15.

Table 15. Body Weight Means and SD's of Combined and Individual Subgroups for *p,p'*-DDE in the Laboratory 6 Study.

Group		Control	Low	Middle	High
<i>p,p'</i> -DDE Dose (mg/kg/day)		0	12.5	50	200/150
Body weight	Males- combined	369.8 ± 32.2	379.1 ± 31.4	392.4 ± 25.2	364.7 ± 14.1
Body wt	Males – Subgroup A	351.0 ± 30.3	384.2 ± 23.3	*391.4 ± 22.5	360.3 ± 17.5
Body wt	Males - Subgroup B	388.6 ± 23.2	374.0 ± 40.1	393.4 ± 30.3	369.0 ± 11.5
<i>p,p'</i> -DDE Dose (mg/kg/day)		0	6.5	25	100
Body weight	Females- combined	228.8 ± 15.8	240.5 ± 21.8	231.8 ± 22.8	241.7 ± 17.3
Body wt	Females – Subgroup A	231.6 ± 9.9	233.2 ± 22.73	227.6 ± 14.0	232.7 ± 15.5
Body wt	Females - Subgroup B	226.0 ± 21.1	247.8 ± 20.5	236.0 ± 30.5	248.5 ± 17.3

Values expressed as mean ± SD. n = 10 Combined Subgroups; n = 5 individual Subgroups.

44. Body weights in Subgroup A were significantly increased in males at the mid-dose; this appears to be related to the lower mean of the vehicle control in this Subgroup and not to be of biological significance. No other significant body weight change was observed in either Subgroup for either sex at any dose. Overall food consumption for the study was not significantly changed in either Subgroup for either sex at any dose.

Laboratory 7 – *p,p'*-DDE

45. No mortalities occurred during the study period. Clinical signs in individual males included salivation, soiled fur, and loose stools. In females, three individuals in the high dose group showed severe clinical signs by the fourth week of treatment, including tremors, staggered gait, and hypoactivity. Neither body weights nor food consumption were significantly changed by treatment. Body weight means and SDs are in Table 16.

Table 16. Body Weight Means and SD's of Combined and Individual Subgroups for *p,p'*-DDE in the Laboratory 7 Study

Group		Control	Low	Middle	High
<i>p,p'</i> -DDE Dose (mg/kg/day)		0	12.5	50	100
Body weight	Males- combined	421.1 ± 26.1	414.2 ± 18.7	417.6 ± 22.4	401.4 ± 17.1
Body wt	Males – Subgroup A	423.8 ± 30.0	420.7 ± 21.8	410.7 ± 31.4	407.8 ± 16.3
Body wt	Males - Subgroup B	418.3 ± 24.8	407.8 ± 14.4	424.4 ± 5.1	395.1 ± 17.1
<i>p,p'</i> -DDE Dose (mg/kg/day)		0	6.5	25	75
Body weight	Females- combined	249.0 ± 20.4	247.4 ± 18.6	246.0 ± 20.4	240.0 ± 23.1
Body wt	Females – Subgroup A	245.1 ± 21.8	243.5 ± 26.4	248.2 ± 20.6	250.7 ± 10.6
Body wt	Females - Subgroup B	252.9 ± 20.5	251.2 ± 6.8	243.9 ± 22.4	229.2 ± 28.3

Values expressed as mean ± SD. n = 10 Combined Subgroups; n = 5 individual Subgroups.

46. Body weights were not significantly different in either sex in either Subgroup at any dose. Overall good consumption for the study was not significantly different in either sex in either Subgroup at any dose. Thus, all results were consistent between the combined Subgroups and individual Subgroups.

Summary – *p,p'*-DDE

47. The overall food consumption observations were not consistent, but this could be attributed to the higher doses employed in the laboratory 6 study in the third dose group. The body weight observations were consistent for the females in the combined Subgroups of the two laboratories with *p,p'*-DDE. In the case of the males, there was no change in statistical significance. An increase in the mean body weight (not significant) at the mid-dose of the laboratory 6 study has been interpreted as not biologically significant.

Propylthiouracil

Laboratory 1 – Propylthiouracil

48. No mortalities or clinical abnormal signs were observed during the study period. Body weights were significantly decreased in both sexes at all doses. The decrease in the male high dose group was 17.8% and in the female high dose group 12.8%. Food consumption was also significantly decreased in both sexes at the high dose. Body weight means and SDs are in Table 17.

Table 17. Body Weight Means and SD's of Combined and Individual Subgroups for PTU in the Laboratory 1 Study

Group		Control	Low	Middle	High
Propylthiouracil Dose (mg/kg/day)		0	0.1	1	10
Body weight	Males- combined	278.6 ± 17.2	291.5 ± 17.1	275.7 ± 18.5	**229.1 ± 10.0
Body weight	Females- combined	199.6 ± 10.2	197.9 ± 9.2	191.0 ± 8.4	**174.1 ± 14.8
Body wt	Males – Subgroup A	276.1 ± 5.5	293.6 ± 23.5	275.9 ± 17.9	**226.2 ± 5.3
Body wt	Males - Subgroup B	281.1 ± 24.9	289.5 ± 9.9	275.6 ± 21.2	**231.9 ± 13.2
Body wt	Females – Subgroup A	193.5 ± 6.4	196.5 ± 6.3	191.5 ± 7.2	*174.0 ± 17.6
Body wt	Females - Subgroup B	205.7 ± 10.1	199.4 ± 12.1	190.6 ± 10.3	**174.2 ± 13.6

Values expressed as mean ± SD. *, **, Significantly different from control (p<0.05 and p<0.01, respectively). n = 10 Combined Subgroups; n = 5 individual Subgroups.

49. Body weights were significantly decreased in both sexes at the high dose in both Subgroup A (18.1% for males and 10.1% for females) and Subgroup B (17.5% for males and 15.3% for females). Body weights were not significantly different in either sex in either Subgroup at the low or mid- dose. Food consumption was significantly decreased in both sexes at the high dose in both Subgroup A and Subgroup B.

Laboratory 10 – Propylthiouracil

50. This laboratory employed only a single group with 6 animals per sex for the study. So no Subgroup data are available, and the statistical power is not equivalent to the combined Subgroups of ten animals per sex.

51. No mortalities or clinical abnormal signs were observed during the study period. Body weights were significantly decreased in both sexes at the high dose. The decrease in the male high dose group was 16.8% and in the female high dose group was less than 10% decrease from the control weights (8.7%). Food consumption was significantly decreased in both sexes at the high dose. Body weight means and SDs are in Table 18.

Table 18. Body Weight Means and SD's of Study Groups for PTU in the Laboratory 10 Study

Group		Control	Low	Middle	High
Propylthiouracil Dose (mg/kg/day)		0	0.1	1	10
Body weight	Males	336.9 ± 17.2	333.72 ± 18.5	339.05 ± 27.4	*280.4 ± 87.6
Body weight	Females	221.0 ± 9.8	223.32 ± 31.9	222.67 ± 22.0	*201.7 ± 26.5

Values expressed as mean ± SD. *, Significantly different from control (p<0.05). n = per group in this study.

52. This study did not record the animal data into two Subgroups of five animals per sex. Therefore, no analysis can be performed on the possible effect on study findings and power by increasing the group size to 10 animals per sex.

Summary – Propylthiouracil

53. Food consumption and body weight decreases were highly consistent for both sexes between the two studies with propylthiouracil.

L-Thyroxine**Laboratory 9 – L-Thyroxine**

54. No mortalities or clinical abnormal signs were observed during the study period. No significant changes were observed in the body weights of either sex at any dose. Food consumption was significantly increased in both sexes at the high dose. Body weight means and SDs are in Table 19.

Table 19. Body Weight Means and SD's of Combined and Individual Subgroups for L-Thyroxine in the Laboratory 9 Study

Group		Control	Low	Middle	High
L-Thyroxine Dose (mg/kg/day)		0	0.01	0.1	1
Body weight	Males- combined	397.9 ± 23.3	400.9 ± 24.9	412.4 ± 37.7	388.2 ± 22.3
Body weight	Females- combined	278.1 ± 22.9	275.6 ± 25.1	280.9 ± 16.9	282.1 ± 13.9
Body wt	Males – Subgroup A	404.0 ± 21.6	399.2 ± 22.9	423.0 ± 52.3	391.4 ± 25.3
Body wt	Males - Subgroup B	391.8 ± 25.6	402.6 ± 29.3	401.8 ± 13.6	385.0 ± 21.2
Body wt	Females – Subgroup A	287.6 ± 8.4	263.0 ± 24.0	280.4 ± 7.2	281.6 ± 18.3
Body wt	Females - Subgroup B	268.6 ± 29.7	288.2 ± 20.9	281.4 ± 24.2	282.6 ± 10.1

Values expressed as mean ± SD. *, Significantly different from control (p<0.05). n = 10 Combined Subgroups; n = 5 individual Subgroups.

55. Body weights were not significantly different in either sex in either Subgroup at any dose. In males, overall food consumption for the study was significantly increased at the high dose in both

Subgroup A and Subgroup B. In females, overall food consumption for the study was significantly increased at the high dose in Subgroup B, but not in Subgroup A.

Laboratory 12 – L-Thyroxine

56. No mortalities were observed during the study period, and no clinical signs related to treatment were observed during the study period. There were no significant differences in body weights for males. Female body weights were significantly increased at the high dose (+6.4%). Food consumption was significantly increased in both sexes at the high dose. Body weight means and SDs are in Table 20.

**Table 20. Body Weight Means and SD's of Study Groups
for l-Thyroxine in the Laboratory 13 Study**

Group		Control	Low	Middle	High
L-Thyroxine Dose (mg/kg/day)		0	0.01	0.1	1
Body weight	Males	314.0 ± 22.7	312.7 ± 13.7	324.9 ± 13.1	319.2 ± 21.8
Body weight	Females	202.6 ± 11.0	203.2 ± 7.1	208.4 ± 9.5	*216.4 ± 11.9

Values expressed as mean ± SD. *, Significantly different from control (p<0.05). n = 10 per group in this study.

57. This study did not record the animal data into two Subgroups of five animals per sex. Therefore, no analysis can be performed on the possible effect on study findings and power by increasing the group size to 10 animals per sex.

Summary – l-Thyroxine

58. Food consumption was consistent for both sexes between the two studies with l-thyroxine. The body weights in males were consistently unchanged in either study. The body weights in females differed, unchanged in the laboratory 9 study and significantly increased in the laboratory 13 study with an increase of 6.4%.

ANNEX 4

Haematology and Clinical Chemistry Data Generated for the Updated TG 407 Validation Studies

1. The purposes of this Annex are to record for the haematology and clinical chemistry data from the updated TG 407 validation studies:

- the various analyses performed by each laboratory;
- the basic statistical findings for each sex from each Subgroups and the combined Subgroups, when these data were available from the final reports; and
- the means and standard deviations (SDs) for each sex from each Subgroup and the combined Subgroups, when these data were available from the final reports.

2. Additional purposes of this Annex are to compare the reproducibility and reliability of the experimental findings for haematology and clinical chemistry data:

- in regards to a dose response or possible random findings;
- taking into account both statistical significance and absolute trends;
- between Subgroups in the same lab;
- the added value and power of combined the Subgroups (i.e., a group size of five versus ten animals per sex); and
- between the two laboratories studying each chemical.

3. This Annex is organized in the following manner:

- each of the ten chemicals tested in the updated TG 407 validation program has its own individual section. The chemicals are roughly grouped as:
 - Estrogens: ethinyl oestradiol, genistein, and nonylphenol.
 - Antiestrogens: tamoxifen
 - Aromatase inhibitors: CGS 18320B
 - Androgens: methyl testosterone
 - Antiandrogens: flutamide and *p,p'*-DDE
 - Thyroid toxicants and hormones: propylthiouracil and l-thyroxine
- in each section, the haematology and clinical chemistry data of both laboratories testing the chemical are presented with the laboratories in alphabetical order;
- a summary table is presented with the statistically significant findings from the haematology and clinical chemistry data with the combined Subgroups in the left-hand data column, then for the two individual Subgroups, when available, in two columns to the right;
- the sex and dose groups are indicated by M for male and F for female and L for the low, I for the mid or intermediate, and H for the high dose; and
- the achievement of statistical significance, in the values, is denoted with arrows pointing up for an increase or down for a decrease, usually one arrow for $p < 0.05$ and two arrows for $p < 0.01$, as most laboratories reported the statistics in this manner.

4. Overall integration and interpretation of the haematology and clinical chemistry data with other findings are noted in the body of this report, not this or other Annexes. Likewise, discussion, recommendations, and conclusions are in the body of this report, not this or other Annexes. In a number of cases, the statistical changes recorded for haematological and clinical chemistry findings were judged by

the laboratory not to be biologically relevant. Most often, this judgment was due to the fact that the values, despite being statistically significant, fell within the range of historical control values and, thus, were within a biological range considered normal. In some other cases, the judgment was based on the absolute change being so small from the control as to have no consequence. Typically, these judgements have not been recorded in the Annex, and only base statistical significance has been considered.

Ethinyl Oestradiol

Laboratory 2 – Ethinyl oestradiol

5. Haematology results: Prothrombin times were significantly increased in both sexes at the mid- and high doses, reticulocytes values were significantly increased in both sexes at the high dose, haemoglobin and erythrocyte counts were significantly decreased in the females at the high dose, leukocytes and atypical leukocyte counts were significantly decreased in the males at the high dose, basophil counts were significantly decreased in the males at the mid- and high doses, and lymphocyte counts were significantly decreased in the males at all doses.

6. Plasma chemistry results: Cholesterol was significantly decreased in the males at all doses and in the females at the high dose, aspartate aminotransferase activity was significantly decreased in the males at high dose and the females at the mid- and high doses, triglycerides were significantly increased in the males at the mid- and the high doses, sodium values were significantly decreased in the males at all doses, albumin values were significantly decreased in the females at all doses, alkaline phosphatase was significantly increased in the females at all doses, total protein was significantly increased in females at the high dose, and total bilirubin decreased in males at the high dose.

7. The haematology and plasma chemistry results of the combined Subgroups are compared with those of the individual Subgroups A and B in Table 1.

Table 1. Statistically Significant Haematological and Clinical Chemistry Parameters for EE in the Laboratory 2 Study

	Combined Subgroups (n = 10)	Subgroup A (n = 5)	Subgroup B (n = 5)
Haematology results			
Haemoglobin concentration	↓ F (H)	↓ F (I, H)	
Reticulocytes	↑ M (H) F (I, H)		
Prothrombin quick times	↑ M (I, H) F (I, H)	↑ F (H)	↑ F (L, I, H)
Erythrocyte counts	↓ F (H)	↓ F (I, H)	
Leucocyte counts	↓ M (H)	↓ M (H)	
Atypical leu. counts	↓ M (H)	↓ M (H)	↓ M (H)
Lymphocyte counts	↓ M (L, I, H)	↓ M (H)	
Basophil counts	↓ M (I, H)	↓ M (H)	
Haematocrit		↓ F (I, H)	
Plasma chemistry results			
Total cholesterol	↓ M (L, I, H) F (H)		↓ M (L, I, H)
Triglycerides	↑ M (I, H)	↑ M (H) F (I, H)	
Albumin	↓ F (L, I, H)	↓ F (L, I, H)	↓ F (H)
Alkaline phosphatase	↑ F (L, I, H)	↑ F (H)	
Total protein	↑ F (H)		
Total bilirubin	↓ M (H)		
Aspartate aminotransferase	↓ M (H) ↓ F (I, H)	↓ F (I, H)	↓ F (H)
Sodium	↓ M (L, I, H)	↓ M (L, I, H)	
Alanine aminotransferase			↓ M (H)

M – male, F – female, H – high dose, I – intermediate or mid-dose, L – low dose, ↓ - statistically significant decrease, ↑ - statistically significant increase.

8. A comparison of the results from the combined and individual Subgroups shows some similarities and a number of differences if only statistical significance is used. In 2 haematology measures, the increase in female prothrombin quick times and the decrease in male atypical leucocyte counts. In 2 plasma chemistry measures, decreases in female albumin and aspartate aminotransferase, there was statistical significance in the combined Subgroups and in both individual Subgroups. However, in 5 other haematology and 4 plasma chemistry cases, statistical significance was achieved in the combined Subgroups and one of the individual Subgroups, but not in the remaining Subgroup. In 2 other haematology and 2 plasma chemistry cases, neither individual Subgroup achieved statistical significance compared to the combined Subgroups. In 1 haematology and 1 plasma chemistry case, statistical significance occurred in an individual Subgroup, but in neither the other Subgroup or in the combined Subgroups.

9. Several of the outcomes were judged to be spurious effects or not of biological significance. The decrease in leucocyte, atypical leucocyte, lymphocyte, and basophil values were judged to be spurious based on 1) the control means were driven by high values in one control animal in Subgroup A and 2) the treated animal values were within historical values. Decreased total cholesterol and sodium in males, and decreased albumin in females at all dose levels were considered to result from elevated control values, as there was no clear dose dependency and values of treated animals generally corresponded to that of historical control values. Slight reductions of aminotransferase activities were judged to lack biological significance.

Laboratory 5 – Ethinyl oestradiol

10. Haematology results: Haemoglobin concentration and haematocrit values were significantly decreased in males at the high dose and in females at the mid- and high doses. Erythrocyte counts were significantly decreased in females at the mid- and high doses.

11. Plasma chemistry results: The albumin-globulin ratio was significantly decreased in females in all dose groups and in males at the mid- and high doses. The alkaline phosphatase and γ -glutamyl transferase activity was significantly increased in females at the mid- and high dose and in males at the high dose. Cholinesterase and albumin were significantly decreased in females at the mid- and high dose, and the total protein was significantly increased in the females at the high dose. Total cholesterol significantly decreased in females at the high dose and was significantly decreased in males in all dose groups.

12. The haematology and plasma chemistry results of the combined are compared with those of the individual Subgroups A and B in Table 2.

Table 2. Statistically Significant Haematological and Clinical Chemistry Parameters for EE in Laboratory 5 Study

	Combined Subgroups (n = 10)	Subgroup A (n = 5)	Subgroup B (n = 5)
Haematology results			
Haemoglobin concentration	↓ M (H) F (I, H)	↓ M (H) F (H)	↓ M (H) F (I, H)
Haematocrit	↓ M (H) F (I, H)	↓ F (H)	↓ M (H) F (I, H)
Erythrocyte counts	↓ F (I, H)	↓ F (H)	↓ F (H)
White blood cell counts			↑ M (H)
Mean cell haemoglobin content			↓ F (H)
Reticulocytes			↑ F (H)
Plasma chemistry results			
Total cholesterol	↓ M (L, I, H) F (H)	↓ M (L, I, H) F (H)	↓ M (I, H)
Cholinesterase	↓ F (I, H)		↓ F (L, I, H)
Albumin	↓ F (I, H)		
Albumin/globulin ratio	↓ M (I, H) F (L, I, H)	↓ M (H) F (I, H)	↓ M (L, I, H) F (L, I, H)
Alkaline phosphatase	↑ M (H) F (I, H)	↑ F (L, I, H)	
Total protein	↑ F (H)		
γ-Glutamyl Transferase	↑ M (H) F (I, H)		↑ M (H)
Total bilirubin		↓ M (H)	
Aspartase aminotransferase	↓ F (L, I, H)	↓ F (L, I, H)	↓ M (H) F (L)
Potassium		↓ F (L, H)	
Chloride			↓ F (H)

M – male, F – female, H – high dose, I – intermediate or mid-dose, L – low dose, ↓ - statistically significant decrease, ↑ - statistically significant increase.

13. A comparison of the results from the combined and individual Subgroups shows some similarities and a number of differences if only statistical significance is used. In 4 haematology cases (decreases in male and female haemoglobin concentrations, decrease in female haematocrit, and decrease in female erythrocyte counts) and 3 plasma chemistry cases (decrease in male cholesterol and decreases in male and female albumin/globulin ratio), statistical significance was achieved in the combined Subgroups and both individual Subgroups. However, in the case of the haematocrit value in males and 4 plasma chemistry measures, the combined Subgroups and one individual Subgroup were significant, but not the other individual Subgroup. In the case of 2 plasma chemistry measures, neither individual Subgroup achieved statistical significance compared to the combined Subgroups. In the case of 3 haematology and 4 plasma chemistry measure, statistical significance occurred in an individual Subgroup, but in neither the other Subgroup or in the combined Subgroups.

Summary – EE

14. The two ethinyl estradiol studies are concordant for statistically significant increases in two haematology values, a decrease in female erythrocyte counts and haemoglobin concentrations at the high dose. The other haematology measures did not achieve significance in both studies (reticulocyte values and prothrombin quick times in the laboratory 2 study and haematocrit values in the laboratory 5 study). A review of the absolute values indicates that the laboratory 2 haematocrit values did decrease in a dose responsive fashion, but the percentage decrease was less than the laboratory 5 studies (1.3% and 5.7% for males and 5.1% and 8.6% for females, respectively). Similarly, the absolute female reticulocyte values increased by a similar percentage in females in the laboratory 5 study when compared to the laboratory 2, but they did not achieve statistical significance. The laboratory 5 male reticulocyte values did not increase and, therefore, contrast with the laboratory 2 findings. While the laboratory 5 quick or activated prothrombin times were increased in females by a similar percentage at the low and the mid-doses, they

did not achieve statistical significance. At the high dose, the laboratory 5 values were only modestly increased in contrast with the laboratory 2 findings. The laboratory 5 lab did not conduct the four white blood cell type counts that were significant in the laboratory 2 study.

15. For clinical values, the studies were concordant for significant decreases in male cholesterol levels at all doses and in females at the high dose, significantly decreased female albumin levels at the mid- and high dose, significantly increased alkaline phosphatase values in females at the mid- and the high doses, significantly increased total protein in females at the high dose, and significantly decreased female aspartase aminotransferase values at the mid- and high doses. Male triglyceride values increased in a dose responsive manner in the laboratory 2 study, and, while significantly increased at the low and mid-doses in the laboratory 5 study, the values were only moderately increased at the high dose and did not attain statistical significance. Male aspartase aminotransferase absolute values were decreased in the laboratory 5 study, but did not achieve statistical significance as in the laboratory 2 study. The significant decrease in male total bilirubin in the laboratory 2 study was not reproduced; male values increased in the laboratory 5 study, and female values increased by a similar percentage in both studies. The slight decreases in sodium values in the laboratory 2 study at all three EE doses, while statistically significant, do not appear to have been biological meaningful (0.7-1.4% decreases and within historical values). The laboratory 2 study did not perform the three clinical analyses that were significant in the laboratory 5 study.

16. In summary, comparing those values performed in both laboratories and examining trends in absolute values along with statistical significance reveals an overall concordance in the haematological and clinical values between the two studies administering EE.

Genistein

Laboratory 4 – Genistein

17. Haematology results: There were no statistically significant changes in haematology parameters.

18. Plasma chemistry results: Total protein was significantly increased in both sexes at the high dose. In females, the albumin/globulin ratio was significantly decreased at the high dose, and triglyceride levels were significantly increased at the mid- and high doses.

19. The haematology and plasma chemistry results of the combined Subgroups are compared with those of the individual Subgroups A and B in Table 3.

Table 3. Statistically Significant Haematological and Clinical Chemistry Parameters for Genistein in the Laboratory 4 Study

	Combined Subgroups (n = 10)	Subgroup A (n = 5)	Subgroup B (n = 5)
Haematology results			
Haemoglobin concentration		↓ M (H)	↑ M (H)
Haematocrit			↑ M (H)
Reticulocyte counts		↑ M (I, H)	
Plasma chemistry results			
Total protein	↑ M (H) F (H)		↑ M (H)
Albumin/globulin ratio	↓ F (H)		
Triglycerides	↑ F (I, H)	↑ F (I, H)	
Lactate dehydrogenase		↑ F (H)	
Phospholipid		↑ F (H)	
Sodium			↑ F (H)

M – male, F – female, H – high dose, I – intermediate or mid-dose, L – low dose, ↓ - statistically significant decrease, ↑ - statistically significant increase.

20. A comparison of the results from the combined and individual Subgroups shows no case of consistent statistical significance among the combined Subgroups and the individual Subgroups. None of the 3 haematology measures there were statistically significant in the individual Subgroups achieved significance in the combined Subgroups, and 4 plasma chemistry measures also were statistically significant in one individual Subgroup but not the other Subgroup or the combined Subgroups. Three plasma chemistry measures were statistically significant in the combined Subgroups and one individual Subgroup, not the other individual Subgroup. The female total protein was statistically significant only in the case of the combined Subgroups.

Laboratory 12 – Genistein

21. Haematology results: The mean prothrombin times were significantly increased in males at the high dose.

22. Plasma chemistry results: Total cholesterol concentrations were significantly decreased in males at the mid- and high dose levels and in females at all doses. Albumin, total protein, and triglyceride levels were significantly increased in females at the mid- and high doses. Chloride was significantly decreased in females at the mid- and high doses. Total bilirubin was significantly increased in females at the high dose.

23. The haematology and plasma chemistry results of the combined Subgroups are compared with those of the individual Subgroups A and B in Table 4.

Table 4. Statistically Significant Haematological and Clinical Chemistry Parameters for Genistein in the Laboratory 12 Study

	Combined Subgroups (n = 10)	Subgroup A (n = 5)	Subgroup B (n = 5)
Haematology results			
Prothrombin times	↑ M (H)		↑ M (H)
Plasma chemistry results			
Total cholesterol	↓ M (I, H) F (L, I, H)	↓ M (I, H) F (L, I, H)	↓ M (H) F (I, H)
Triglycerides	↑ F (I, H)	↑ F (I, H)	↑ F (H)
Albumin	↑ F (I, H)	↑ F (I, H)	↑ F (I, H)
Aspartase aminotransferase	↓ F (L)	↓ F (L, I, H)	
Total protein	↑ F (I, H)		↑ F (I, H)
Total bilirubin	↑ F (H)		↑ F (H)
Chloride	↓ F (I, H)		↓ F (H)
Creatinine	↓ F (H)		
Albumin/globulin ratio			↓ F (H)
Alkaline phosphatase		↑ F (L, I, H)	↑ F (H)

M – male, F – female, H – high dose, I – intermediate or mid-dose, L – low dose, ↓ - statistically significant decrease, ↑ - statistically significant increase.

24. A comparison of the results from the combined and individual Subgroups shows a number of differences. The total cholesterol decrease in both sexes, the increase in female albumin and triglyceride, and the decrease in female aspartate aminotransferase were consistently significant in the combined Subgroups and both individual Subgroups. Three other plasma chemistry measures were statistically significant in the combined Subgroups and one individual Subgroup, but not the other individual Subgroup. However, 2 haematology measures and 4 plasma chemistry measures (including the male aspartate aminotransferase) were statistically significant in one of the individual Subgroups, but did not achieve significance in the combined Subgroups or the other Subgroup.

Summary – Genistein

25. In the combined Subgroups, the only consistent findings there were statistically significant in both genistein studies were an increase in female total protein and triglyceride concentrations. The cholesterol levels that were significantly decreased in both sexes in the laboratory 12 study (-18% in males and -31% in females) also fell in absolute terms in the laboratory 4 study but to a lesser degree (-13% and -15%). The laboratory 4 female albumin levels rose more in absolute terms than the laboratory 12 study (7 vs 6%), but did not achieve statistical significance. The female chloride changes were of a similar degree and direction, and bilirubin changes were also similar in absolute terms (+46 and +49%, although the laboratory 4 study did not achieve statistical significance in either case. The male total protein values were, however, in contradictory directions, increasing at the high dose in the laboratory 4 study but decreasing in the laboratory 12 study. The same was true for the female creatinine changes that were significantly decreased in the laboratory 12 study. In conclusion, when taking the absolute trends into account, the studies were largely in agreement.

Nonylphenol

Laboratory 1 – Nonylphenol

26. Haematology results: Erythrocyte counts, haemoglobin concentration and haematocrit values were significantly decreased and white blood cell counts were significantly increased in the females at the high dose. Prothrombin times were significantly decreased in the males at the mid- and high doses.

27. Plasma chemistry results: Alanine aminotransferase activities were significantly increased, and triglycerides were significantly decreased in the males at the mid and high doses. Globulin and cholesterol were significantly increased in the females at the high dose.

28. The haematology and plasma chemistry results of the combined Subgroups are compared with those of the individual Subgroups A and B in Table 5.

Table 5. Statistically Significant Haematological and Clinical Chemistry Parameters for Nonylphenol in the Laboratory 1 Study

	Combined Subgroups (n = 10)	Subgroup A (n = 5)	Subgroup B (n = 5)
Haematology results			
Prothrombin times	↓ M (I, H)	↓ M (H)	↓ M (H)
Haematocrit	↓ F (H)		↓ F (H)
Erythrocyte counts	↓ F (H)		↓ F (H)
White blood cell counts	↑ F (H)		↑ F (H)
Haemoglobin concentration	↓ F (H)		
Plasma chemistry results			
Triglycerides	↓ M (I, H)	↓ M (H)	↓ M (H)
Alanine aminotransferase	↑ M (I, H)		↑ M (I)
Globulin	↑ F (H)		
Cholesterol	↑ F (H)	↑ M (H)	

M – male, F – female, H – high dose, I – intermediate or mid-dose, L – low dose, ↓ - statistically significant decrease, ↑ - statistically significant increase.

29. A comparison of the results from the combined and individual Subgroups shows several differences. Only one haematology measure (prothrombin times) and one plasma chemistry measure (triglycerides) were statistically significant in both Subgroups and the combined Subgroups. In 3

haematology cases and 2 clinical chemistry cases, statistical significance was achieved in the combined Subgroups and one individual Subgroup, but not the remaining individual Subgroup. In 2 cases, statistical significance was achieved only in the combined Subgroups but in neither individual Subgroup. In the case of the male cholesterol, statistical significance occurred in Subgroup A, but in neither the other individual Subgroup or in the combined Subgroups.

Laboratory 6 – Nonylphenol

30. Haematology results: Erythrocyte counts were significantly decreased in females at the high dose, and haemoglobin concentration and haematocrit were significantly decreased in females at both the mid- and high dose. Neutrophil counts were significantly decreased in males at the high dose.

31. Plasma chemistry results: Blood urea nitrogen and sodium concentrations were significantly increased in both sexes at the high dose. Triglycerides were significantly decreased in both sexes at the high dose and in males also at the mid-dose. Creatinine was significantly increased and alkaline phosphatase significantly decreased in the males at the high dose. Albumin was significantly decreased in females at the high dose, and globulin was significantly increased in females at the mid- and high doses. The albumin/globulin ratio was significantly decreased in females at both the mid- and high doses. Sodium was significantly increased in both sexes at the high dose, and chloride was significantly increased in females at the high dose.

32. The haematology and plasma chemistry results of the combined Subgroups are compared with those of the individual Subgroups A and B in Table 6.

Table 6. Statistically Significant Haematological and Clinical Chemistry Parameters for Nonylphenol in Laboratory 6 Study

	Combined Subgroups (n = 10)	Subgroup A (n = 5)	Subgroup B (n = 5)
Haematology results			
Haemoglobin concentration	↓ F (I, H)	↓ F (L, I, H)	↓ F (H)
Haematocrit	↓ F (I, H)	↓ F (I, H)	↓ F (H)
Erythrocyte counts	↓ F (H)	↓ F (H)	↓ F (H)
Mean cell volume		↓ M (H)	
Platelet counts			↑ F (L, I, H)
Plasma chemistry results			
Blood urea nitrogen	↑ M (H) F (H)	↑ F (H)	↑ F (H)
Creatinine	↑ M (H)		↑ M (H)
Triglycerides	↓ M (I, H) F (H)	↓ M (I, H)	↓ M (I, H) F (I, H)
Albumin	↓ F (H)	↑ M (H)	↓ F (H)
Globulin	↑ F (I, H)		↑ F (H)
Albumin/globulin ratio	↓ F (I, H)	↓ F (I, H)	↓ F (I, H)
Alkaline phosphatase	↓ M (H)		
Sodium	↑ M (H) F (H)		↑ F (H)
Chloride	↑ F (H)		↑ M (H)
Cholesterol			↑ F (H)

M – male, F – female, H – high dose, I – intermediate or mid-dose, L – low dose, ↓ - statistically significant decrease, ↑ - statistically significant increase.

33. A comparison of the results from the combined and individual Subgroups shows that several measures were consistently statistically significant in both Subgroups and the combined Subgroups: decreases in female erythrocyte counts, haematocrit, and haemoglobin concentrations; increases in female blood urea nitrogen, decreases in male triglycerides, and decreases in female albumin /globulin ratios. However, with 8 plasma chemistry measures, one and sometimes both of the individual Subgroups did not achieve statistical significance compared to the combined Subgroups. Further, in the case of 2 haematology and 2 plasma chemistry measures, statistical significance occurred in an individual Subgroup, but in neither the other Subgroup or in the combined Subgroups.

Summary – Nonylphenol

34. The two nonylphenol studies are concordant for a decrease in female erythrocyte counts, haemoglobin concentrations and haematocrit at the high dose, for a decrease in male triglycerides at mid- and high doses, and for an increase in female globulin concentrations at the high dose. However, the other haematology and plasma chemistry measures were not fully repeatable between the studies, although some were suggestive, e.g., an increase in female cholesterol in the combined laboratory 1 Subgroups and in one laboratory 6 Subgroup. Female albumin trends were similar, if one takes into account that the laboratory 1 high dose was slightly lower than the laboratory 6 high dose. Other parameters were in disagreement, white blood cell counts increased by 48% at the high dose in the laboratory 1 study and fell 27% in the laboratory 6 study; female triglycerides rose by 22% in the laboratory 1 study and fell 57% in the laboratory 6 study; urea nitrogen levels rose very modestly in the laboratory 1 study, but sharply in the laboratory 6 study; and male creatinine changes in the laboratory 6 study were not seen in the laboratory 1 study. The differences in the high dose of NP between the studies does not appear to account for these different observations.

Tamoxifen

Laboratory 3 – Tamoxifen

35. Haematology results: The mean prothrombin times were significantly increased in males at the high dose, and mean cell haemoglobin was significantly decreased in females at the high dose. The latter was considered of doubtful toxicological relevance as the magnitude of change was low and no differences were noted in the measured parameters on which this *calculated* parameter is based.

36. Plasma chemistry results: Total cholesterol concentrations were significantly decreased in both sexes at the high dose and in males at the mid-dose. Triglyceride concentrations were significantly increased in males at all dose levels, and alkaline phosphatase activity was significantly increased in males at the high dose. Chloride and sodium concentrations were significantly increased in females at the high dose, and calcium and inorganic phosphorus were significantly decreased in females at the high dose. The changes in mean chloride, sodium, calcium and inorganic phosphorus concentrations in females at the high dose were considered incidental and not toxicologically relevant, due to variations in individual values and/or their low degree of change.

37. The haematology and plasma chemistry results of the combined Subgroups are compared with those of the individual Subgroups A and B in Table 7.

Table 7. Statistically Significant Haematological and Clinical Chemistry Parameters for Tamoxifen in the Laboratory 3 Study

	Combined Subgroups (n = 10)	Subgroup A (n = 5)	Subgroup B (n = 5)
Haematology results			
Prothrombin times	↑ M (H ⁸)	↑ M (H ⁴)	
Mean cell haemoglobin conc.	↓ F (H ⁷)	↓ F (H ⁴)	
Plasma chemistry results			
Total cholesterol	↓ M (I ⁹ , H ⁸) F (H ⁷)	↓ M (I ⁴ , H ⁴) F (H ⁴)	↓ M (I ⁴ , H ⁴) F (H ³)
Triglycerides	↑ M (L ⁹ , I ⁹ , H ⁸)		↑ M (L ⁴ , H ⁴)
Alkaline phosphatase	↑ M (H ⁸)	↑ M (H ⁴)	
Sodium	↑ F (H ⁷)	↓ F (I ⁴), ↑ F (H ⁴)	
Calcium	↓ F (H ⁷)	↓ M (I ⁴ , H ⁴) F (I ⁴ , H ⁴)	↑ M (I ⁴)
Phosphorus	↓ F (H ⁷)	↓ F (H ⁴)	
Chloride	↑ F (H ⁷)		
Blood urea nitrogen		↓ F (H ⁴)	

M – male, F – female, H – high dose, I – intermediate or mid-dose, L – low dose, ↓ - statistically significant decrease, ↑ - statistically significant increase. Superscript indicates number of animals, if not 10 for combined Subgroups or 5 for individual Subgroups.

38. A comparison of the results from the combined and individual Subgroups shows a number of differences. Only the decrease in male and female cholesterol was significant in the combined Subgroups and both individual Subgroups. In 2 haematology cases and five plasma chemistry cases, statistical significance was achieved in the combined Subgroups and one individual Subgroup, but not the other individual Subgroup. The increase in female chloride values was significant only in the combined Subgroups. The changes in male calcium values seen in the individual Subgroups, were not observed in the combined Subgroups.

Laboratory 10 – Tamoxifen

39. Haematology results: White blood cell counts were significantly decreased in both sexes at the high dose.

40. Plasma chemistry results: Cholesterol was significantly decreased in males at the mid- and high doses. Potassium was significantly decreased in the males at the high dose.

41. The laboratory 10 tamoxifen study did not record the animal data into two Subgroups of five animals per sex. Therefore, no analysis can be performed on the possible effect on study findings and power by increasing the group size to 10 animals per sex.

Summary – Tamoxifen

42. Only the decrease in male cholesterol levels consistently achieved statistical significance in both tamoxifen studies, and the absolute changes were similar (at the high dose, -61% in the laboratory 3 study and -51% in the laboratory 10 study). The female mean cell haemoglobin concentrations and the female calcium changes were similar in absolute terms in both studies. Female cholesterol levels were in disagreement (at the high dose, -41% in the laboratory 3 study and +2% in the laboratory 10 study). However, the reduced power of six animals in the laboratory 10 study should be noted and taken into account when observing that none of the other statistically significant measures in one study were reproduced in the other study.

CGS 18320B

Laboratory 8 – CGS 18320B

43. Haematology results: The haematocrit, haemoglobin concentration and erythrocyte count were significantly decreased in both sexes in the high dose group. Prothrombin times were significantly increased in females at the low and high doses; the mid-dose was increased but did not achieve statistical significance. Reticulocytes, leucocytes, and lymphocytes were significantly increased in females at all doses.

44. Plasma chemistry results: Total protein, albumin, albumin/globulin ratio, and chloride were significantly decreased in females at all doses, and creatinine was significantly decreased in females at the mid- and high doses. Potassium was significantly decreased in both sexes at the high dose. Aspartate aminotransferase activity was significantly decreased in females at the high dose. Phosphorus was significantly increased in females at all doses, and sodium was significantly increased in females at the high dose.

45. The haematology and plasma chemistry results of the combined Subgroups are compared with those of the individual Subgroups A and B in Table 8.

Table 8. Statistically Significant Haematological and Clinical Chemistry Parameters for CGS 18320B in the Laboratory 8 Study

	Combined Subgroups (n = 10)	Subgroup A (n = 5)	Subgroup B (n = 5)
Haematology results			
Haematocrit	↓ M (H) F (H)	↓ M (H) F (H)	↓ M (H) F (H)
Erythrocyte counts	↓ M (H) F (H)	↓ M (H) F (H)	
Haemoglobin concentration	↓ M (H) F (H)	↓ M (H) F (H)	↓ M (H) F (H)
Reticulocyte count	↑ F (L, I, H)	↑ F (I, H)	↑ F (I, H)
Leucocyte count	↑ F (L, I, H)	↑ M (H)	↑ F (L, I, H)
Lymphocyte count	↑ F (L, I, H)	↑ M (H)	↑ F (L, I, H)
Segmented neutrophil count	↑ F (I)	↑ M (H)	
Prothrombin times	↑ F (L, H)		
Plasma chemistry results			
Total protein	↓ F (L, I, H)	↓ F (L, I, H)	↓ F (L, I, H)
Albumin	↓ F (L, I, H)	↓ F (L, I, H)	↓ F (L, I, H)
Albumin/globulin ratio	↓ F (L, I, H)	↓ F (L, I, H)	↓ F (L, I, H)
Creatinine	↓ F (I, H)	↓ F (I, H)	↓ F (H)
Chloride	↓ F (L, I, H)	↓ F (I, H)	↓ F (I, H)
Sodium	↑ F (H)	↑ F (H)	
Phosphorus	↑ F (L, I, H)	↑ F (L, I, H)	
Alanine aminotransferase	↓ F (H)		
Potassium	↓ M (H) F (H)		

M – male, F – female, H – high dose, I – intermediate or mid-dose, L – low dose, ↓ - statistically significant decrease, ↑ - statistically significant increase.

46. A comparison of the results from the combined and individual Subgroups shows a number of similarities differences. In 5 cases, the haematology measures (decreases in male and female haematocrits, decreases in male and female haemoglobin concentrations, and increases in female reticulocyte values) were significant in the combined Subgroups and both individual Subgroups. In 5 cases, the plasma chemistry measures (decreases in female total protein, albumin, albumin/globulin ratio, creatinine levels, and chloride) were significant in the combined Subgroups and both individual Subgroups. In 1 cases, the haematology measures and, in 2 cases, the plasma chemistry measures were significant only in the combined Subgroups. In 2 cases, haematology measures and, in 2 cases, the plasma chemistry measures were significant in the combined Subgroup and one of the individual Subgroups, but not the other. In three cases involving male leucocyte, lymphocyte or segmented neutrophil counts, only a single individual Subgroup was significant.

Laboratory 13 – CGS 18320B

47. Haematology results: The white blood cell count was significantly increased in the females at the mid- and high doses, and the leucocyte count was significantly increased in the females at the high dose.

48. Plasma chemistry results: The triglyceride and sodium values were significantly increased in males at the high dose and the chloride values were significantly decreased in males at the high dose. Globulin and alanine aminotransferase values were significantly increased in females at the mid- and high doses, chloride values were significantly decreased in females at the mid- and high doses, sodium values were significantly increased in females at the high dose, and potassium values were values were significantly decreased in females at the high dose. Alkaline phosphatase was significantly increased in females at the low and high doses, but, although elevated, the mid-dose group did not achieve statistical significance.

49. The laboratory '3 CGS 18320B study did not record the animal data into two Subgroups of five animals per sex. Therefore, no analysis can be performed on the possible effect on study findings and power by increasing the group size to 10 animals per sex.

Summary - CGS 18320B

50. The increases in female leucocyte counts, increases in female sodium values, and decreases in female chloride and potassium values were reproduced in both studies. However, none of the other statistically significant plasma chemistry results in one study achieved statistical significance in the other study. A review of the absolute trends indicates that for the following parameters the trends are similar and could be judged concordant: male erythrocyte counts, aspartase aminotransferase and alkaline phosphatase, and sodium and chloride values. The other parameters where one study achieved statistical significance do not appear to agree when comparing the absolute trends: male and female haematocrits, female erythrocyte counts, male and female haemoglobin concentrations, female total protein, albumin and globulin changes, and alanine aminotransferase.

Methyl Testosterone

Laboratory 3 – Methyl Testosterone

51. Haematology results: The mean prothrombin times were statistically higher in males at the mid- and high doses. Erythrocyte counts were significantly increased in females at the mid- and high doses. Mean cell haemoglobin was significantly decreased in females at the high dose. Neutrophil and lymphocyte percentages were significantly different in females at the high dose.

52. Plasma chemistry results: Total cholesterol concentrations were significantly decreased in males at all dose levels and in females at the mid- and high doses. Triglyceride concentrations were significantly increased in both sexes at the mid- and high dose levels. Alkaline phosphatase activity was significantly increased in females at all doses. Glucose, urea and potassium concentrations were significantly decreased in females at the high dose, and inorganic phosphorus was significantly increased in females at the mid- and high doses.

53. The haematology and plasma chemistry results of the combined Subgroups are compared with those of the individual Subgroups A and B in Table 9.

Table 9. Statistically Significant Haematological and Clinical Chemistry Parameters for Methyl Testosterone in the Laboratory 3 Study

	Combined Subgroups (n = 10)	Subgroup A (n = 5)	Subgroup B (n = 5)
Haematology results			
Prothrombin time	↑ M (I ⁹ , H)	↑ M (H)	↑ M (I, H)
Erythrocyte counts	↑ F (I ⁹ , H ⁹)	↑ F (I)	
Mean cell haemoglobin	↓ F (H ⁹)		
% neutrophils	↑ F (H ⁹)		
% lymphocytes	↓ F (H ⁹)		
Mean corpuscular cell vol.		↓ M (H)	
Plasma chemistry results			
Total cholesterol	↓ M (L, I, H) F (I ⁸ , H)	↓ M (L, I, H) F (L, I, H)	↓ M (I, H) F (I ³ , H)
Triglycerides	↑ M (I, H) F (I ⁸ , H)	↑ F (I)	↑ M (I) F (I ³)
Albumin	↓ M (H) F (H)	↓ M (H)	↓ F (H)
Alkaline phosphatase	↑ F (L, I ⁸ , H)	↑ F (L)	↑ F (H)
Blood urea nitrogen	↓ F (H)	↓ F (H)	↓ F (H)
Glucose	↓ F (H)	↓ F (H)	
Chloride	↓ M (H) ↑ F (L)	↓ F (H)	↑ F (L)
Phosphorus	↑ F (I ⁸ , H)	↑ F (I, H)	↑ F (I ³)
Aspartate aminotransferase	↓ F (H)		
Potassium	↓ F (H)		

M – male, F – female, H – high dose, I – intermediate or mid-dose, L – low dose, ↓ - statistically significant decrease, ↑ - statistically significant increase. Superscript indicates number of animals, if not 10 for combined Subgroups or 5 for individual Subgroups

54. A comparison of the results from the combined and individual Subgroups shows that an increase in male prothrombin times at the high dose, decreases in cholesterol in both sexes at the mid- and high doses, an increase in female triglyceride levels and alkaline phosphatase, and a decrease in female blood urea nitrogen were consistently statistically significant in both Subgroups and the combined Subgroups. However, 1 haematology and 4 plasma chemistry measures achieved statistical significance in the combined Subgroups and one individual Subgroup, but not in the other individual Subgroup. Three haematology and 2 plasma chemistry measures achieved statistical significance only in the combined Subgroups. One haematology measure achieved statistical significance only in an individual Subgroup.

Laboratory 12 – Methyl Testosterone

55. Haematology results: The haemoglobin concentration was significantly decreased in both sexes at the respective high doses, and mean prothrombin times were significantly increased in both sexes at the respective mid- and high doses. In males, monocyte and large unstained cell counts were significantly decreased at all doses; erythrocyte, total white cell, eosinophil counts and mean cell haemoglobin concentration were significantly decreased at the high dose; and platelet counts were significantly increased at the high dose. In females, the total white blood cell counts were significantly decreased at the high and also at the low doses. Lymphocyte, eosinophil, basophil and large unstained cell counts were significantly decreased in the high dose group.

56. Plasma chemistry results: The total cholesterol was significantly decreased in both sexes at all doses, triglycerides were significantly increased in both sexes at the respective mid- and high doses, and creatinine and glucose levels were significantly decreased in both sexes at the respective high doses. In males, alkaline phosphatase was significantly increased at the high dose, aspartate aminotransferase and creatinine kinase were significantly decreased at the high dose, and plasma phosphorus levels were

significantly increased at the high dose. In females, γ -glutamyl transferase was significantly increased at the high dose, alkaline phosphatase was significantly increased at the mid- and high doses, although the absolute levels were higher at the mid- than the high dose, and plasma phosphorus levels were increased at all treatment doses.

57. The haematology and plasma chemistry results of the combined Subgroups are compared with those of the individual Subgroups A and B in Table 10.

Table 10. Statistically Significant Haematological and Clinical Chemistry Parameters for Methyl Testosterone in the Laboratory 12 Study

	Combined Subgroups (n = 10)	Subgroup A (n = 5)	Subgroup B (n = 5)
Haematology results			
Haemoglobin concentration	↓ M (H) F (H)	↓ M (H) F (H)	
Prothrombin times	↑ M (I, H) F (I, H)	↑ M (I, H) F (I, H)	↑ M (H) F (H)
Large unstained cells	↓ M (L, I, H) F (H)	↓ M (H)	↓ F (H)
Mean cell haemoglobin	↓ M (H)		↓ F (H)
White blood cell count	↓ M (H) F (L, H)		↓ M (I, H) F (H)
Lymphocyte count	↓ F (H)	↓ F (H)	↓ M (I, H) F (H)
Monocyte count	↓ M (L, I, H)		↓ M (L, I, H)
Eosinophil count	↓ M (H) F (H)	↓ F (H)	↓ M (I, H) F (H)
Basophil count	↓ F (H)	↓ F (L, I, H)	↓ F (H)
Plasma chemistry results			
Total cholesterol	↓ M (L, I, H) F (L, I, H)	↓ M (I, H) F (L, I, H)	↓ M (L, I, H) F (L, I, H)
Triglycerides	↑ M (I, H) F (I, H)	↑ M (I, H)	↑ M (H) F (I, H)
γ -Glutamyl transferase	↑ F (H)	↑ F (H)	↑ F (H)
Aspartate aminotransferase	↓ M (H)	↓ M (H)	
Alkaline phosphatase	↑ M (H) F (I, H)	↑ M (H)	
Creatinine	↓ M (H) F (H)	↓ M (H) F (H)	
Phosphorus	↑ M (H) F (L, I, H)	F (I, H)	↑ M (L, I, H) F (L, I, H)
Creatine kinase	↓ M (H) F (H)		
Glucose	↓ M (H) F (H)		
Total bilirubin		↑ F (H)	

M – male, F – female, H – high dose, I – intermediate or mid-dose, L – low dose, ↓ - statistically significant decrease, ↑ - statistically significant increase.

58. A comparison of the results from the combined and individual Subgroups shows both similarities and differences. An increase in prothrombin times in both sexes; decreases in female lymphocyte, eosinophil, and basophil counts; decreases in cholesterol in both sexes, an increase in male triglyceride levels, an increase in female γ -glutamyl transferase, and an increase in female phosphorus were consistently statistically significant in both Subgroups and the combined Subgroups. In 11 cases with haematology measures and 7 cases with plasma chemistry measures, statistical significance was observed in the combined Subgroups and one individual Subgroup, but not the other individual Subgroup. In 2 cases with haematology measures and four cases with plasma chemistry measures, statistical significance was observed only with the combined Subgroups. With mean cell haemoglobin and total bilirubin in females, statistical significance was observed in an individual Subgroup, but not in the other individual Subgroup or the combined Subgroups.

Summary - Methyl Testosterone

59. Increases in prothrombin times in males, decreases in cholesterol levels in both sexes, increases in triglycerides in both sexes, and increases in female alkaline phosphatase and phosphorus were consistent across the two studies. Erythrocyte counts were inconsistent, increasing in females in the laboratory 3 study and decreasing in males in the laboratory 12 study. None of the other measures achieving statistical significance in one study were significant in the other study.

Flutamide

Laboratory 2 – Flutamide

60. Haematology results: Haemoglobin was significantly decreased in both sexes at the high dose, reticulocyte values were significantly increased in both sexes at the high dose, haematocrit was significantly decreased in males at the high dose, and erythrocyte counts and mean cell haemoglobin concentration were significantly decreased in females at the high dose.

61. Plasma chemistry results: Albumin and cholesterol were significantly increased in both sexes at the high dose; cholesterol was significantly increased in the males at the mid- and high doses and in the females at the high dose; phosphorus values were significantly decreased in males at all doses; bilirubin, alanine aminotransferase, and urea were significantly increased in females at the high dose; potassium and alkaline phosphatase were significantly decreased in males at the high dose; and triglycerides were significantly decreased in both sexes at the high dose.

62. The haematology and plasma chemistry results of the combined Subgroups are compared with those of the individual Subgroups A and B in Table 11.

Table 11. Statistically Significant Haematological and Clinical Chemistry Parameters for Flutamide in the Laboratory 2 Study

	Combined Subgroups (n = 10)	Subgroup A (n = 5)	Subgroup B (n = 5)
Haematology results			
Haemoglobin concentration	↓ M (H) F (H)	↓ F (H)	↓ M (H)
Haematocrit	↓ M (H)		↓ M (H)
Mean cell haemoglobin conc.	↓ F (H)	↓ F (H)	↓ M (H)
Erythrocyte counts	↓ F (H)		
Reticulocytes	↑ M (H) F (H)	↑ F (H)	
Mean cell haemoglobin			↓ M (H)
Atypical leucocytes			↓ M (I, H)
Eosinophils			↓ M (I, H)
Plasma chemistry results			
Albumin	↑ M (H) F (H)	↑ M (H) F (H)	↑ M (H)
Alkaline phosphatase	↓ M (H)	↓ M (H)	
Blood urea nitrogen	↑ F (H)	↑ F (H)	↑ F (H)
Total bilirubin	↑ F (H)	↑ F (H)	↑ M (H) F (H)
Creatinine	↓ M (H)		↓ M (L, I, H)
Phosphorus	↓ M (L, I, H)	↓ M (L, I, H)	↓ M (I, H)
Total cholesterol	↑ M (H) F (H)		
Triglycerides	↓ M (H) F (H)		
Alanine aminotransferase	↑ F (H)		
Potassium	↓ M (H)		

M – male, F – female, H – high dose, I – intermediate or mid-dose, L – low dose, ↓ - statistically significant decrease, ↑ - statistically significant increase.

63. None of the haematology measures were statistically significant in the combined Subgroups and both individual Subgroups. In 3 cases, statistical significance in an individual Subgroup was not seen in the other Subgroup or the combined Subgroups. In 5 cases, statistical significance in the combined Subgroups was seen in only one of the individual Subgroups. With reticulocytes, the increase in both sexes at the high dose in the combined Subgroups was not seen in either sex in any individual Subgroup.

64. The increase in male albumin, the increase in female blood urea nitrogen and total bilirubin, and the decrease in male phosphorus were seen in both the combined Subgroups and the both individual Subgroups. Six measures were statistically significant only in the combined Subgroups. A significant increase in male bilirubin was seen in only one Subgroup.

Laboratory 11 – Flutamide

65. Urinalysis results: Potassium excretion was significantly decreased in both sexes at the high dose, and urine specific gravity was significantly higher in the females at the high dose.

66. Haematology results: Erythrocyte counts, haemoglobin, and haematocrit were significantly decreased in both sexes at the high dose, and reticulocyte values were significantly increased in both sexes at the high dose.

67. Plasma chemistry results: Albumin, albumin-globulin ratio, γ -GTP, total cholesterol, and phospholipids were all significantly increased in both sexes at the high dose. In males, total protein was significantly increased at the high dose, triglycerides and alkaline phosphatase were significantly decreased at the high dose, and aspartate aminotransferase was significantly decreased at all doses. In females, BUN and calcium were significantly increased at the high dose, and glucose was significantly decreased at the high dose.

68. The haematology and plasma chemistry results of the combined Subgroups are compared with those of the individual Subgroups A and B in Table 12.

Table 12. Statistically Significant Haematological and Clinical Chemistry Parameters for Flutamide in the Laboratory 11 Study

	Combined Subgroups (n = 10)	Subgroup A (n = 5)	Subgroup B (n = 5)
Haematology results			
Haemoglobin concentration	↓ M (H) F (H)	↓ F (H)	↓ M (H) F (H)
Haematocrit	↓ M (H) F (H)		↓ M (H)
Erythrocyte counts	↓ M (H) F (H)		↓ M (H)
Reticulocytes	↑ M (H) F (H)	↑ F (H)	
Plasma chemistry results			
Total cholesterol	↑ M (H) F (H)	↑ M (H) F (H)	↑ M (H) F (H)
Phospholipids	↑ M (H) F (H)	↑ M (H) F (H)	↑ M (H)
Triglycerides	↓ M (H)	↓ M (H)	
Total protein	↑ M (H)		↑ M (H)
Albumin	↑ M (H) F (H)	↑ M (H) F (H)	↑ M (H)
Albumin/globulin ratio	↑ M (H) F (H)	↑ M (H)	↑ M (H) F (H)
Blood urea nitrogen	↑ F (H)		↑ F (H)
Calcium	↑ F (H)		↑ M (H)
Glucose	↓ F (H)		
Aspartate aminotransferase	↓ M (L, I, H)		
γ-Glutamyl transferase	↑ M (H) F (H)	↑ M (H)	
Alkaline phosphatase	↓ M (H)		

M – male, F – female, H – high dose, I – intermediate or mid-dose, L – low dose, ↓ - statistically significant decrease, ↑ - statistically significant increase.

69. Only the decrease in female haemoglobin concentration was significant in the combined Subgroups and both individual Subgroups. In 4 cases, a significant change was seen in the combined Subgroups and one individual Subgroup, but not the other individual Subgroup. In 3 cases, statistical significance was achieved in the combined Subgroups, but in neither individual Subgroup.

70. In the plasma chemistry results, significant total cholesterol increases in both sexes, significant phospholipid increases in males, and significant albumin and albumin ratio increases in males were seen in the combined Subgroups and in both individual Subgroups. In seven other cases, statistical significance was seen in the combined Subgroups and one individual Subgroup, but not the other individual Subgroup. In four cases, statistical significance was seen only in the combined Subgroups and neither individual Subgroup.

Summary – Flutamide

71. Decreases in male haematocrit, the haemoglobin concentration in both sexes, female erythrocyte counts, and increases in reticulocyte in both sexes were consistent across the two studies. The other measures achieving statistical significance in one study were not reproduced in the other study. Increases in albumin levels in both sexes, a decrease in male alkaline phosphatase, an increase in female blood urea nitrogen, and a decrease in male triglycerides were consistent across the two studies. The other measures achieving statistical significance in one study, however, often had similar absolute trends in the other study.

p,p'*-DDE*Laboratory 6 –*p,p'*-DDE**

73. Haematology results: Platelet counts were significantly increased in males at the mid- and high doses and in females at the high dose. Activated plasma thromboplastin times were significantly increased in males at the high dose, and similar, but not statistically significant changes, occurred in females at their respective high dose. Erythrocyte count, haemoglobin, haematocrit, and mean cell haemoglobin were significantly decreased in females at their respective high dose. Mean erythrocyte volume was significantly decreased in males at the mid- and high dose, and in females at their respective high dose.

74. Plasma chemistry results: Total protein was significantly increased in both sexes at the respective mid- and high doses, globulin was significantly increased in males at all doses and in females at their respective high dose, and albumin and cholesterol were significantly increased in males at the mid- and high dose and in females at their respective high dose. Blood urea nitrogen and calcium were significantly increased in males at the mid- and high dose, and the albumin/globulin ratio was significantly decreased at all doses. Chloride was significantly decreased and calcium and phosphorus significantly increased in females at the high dose.

75. The haematology and plasma chemistry results of the combined Subgroups are compared with those of the individual Subgroups A and B in Table 13.

Table 13. Statistically Significant Haematological and Clinical Chemistry Parameters for *p,p'*-DDE in the Laboratory 6 Study

	Combined Subgroups (n = 10)	Subgroup A (n = 5)	Subgroup B (n = 5)
Haematology results			
Act. partial thromboplastin time	↑ M (H)	↑ M (H)	
Haemoglobin concentration	↓ F (H)		↓ M (H) F (H)
Haematocrit	↓ F (H)		↓ F (H)
Erythrocyte counts	↓ F (H)		↓ M (I, H) F (H)
Mean cell haemoglobin conc.	↓ M (H) F (H)	↓ F (H)	
Mean erythrocyte cell vol	↓ M (I, H)		
Platelet count	↑ M (I, H) F (H)		
Prothrombin times			↓ M (I, H) F (H)
Plasma chemistry results			
Cholesterol	↑ M (I, H) F (H)	↑ M (I, H) F (H)	↑ M (H) F (H)
Total protein	↑ M (I, H) F (I, H)	↑ M (I, H) F (H)	↑ M (I, H) F (H)
Globulin	↑ M (L, I, H) F (I, H)	↑ M (L, I, H) F (I, H)	↑ M (H) F (H)
Albumin	↑ M (I, H) F (H)	↑ M (I, H)	↑ M (H)
Albumin/globulin ratio	↓ M (I, H)	↓ M (L, I, H)	
Blood urea nitrogen	↑ M (I, H)		↑ M (H)
Calcium	↑ M (I, H) F (H)	↑ M (I, H)	↑ F (H)
Phosphorus	↑ F (H)		↑ F (H)
Chloride	↓ F (H)		↓ F (H)
Triglycerides	↓ M (H)		

M – male, F – female, H – high dose, I – intermediate or mid-dose, L – low dose, ↓ - statistically significant decrease, ↑ - statistically significant increase.

76. None of the 8 haematology measures were statistically significant in the combined Subgroups and in both individual Subgroups. In 5 cases, a measure for one sex was significant in the combined

Subgroups and one individual Subgroup, but not the other individual Subgroup. In 4 cases, a measure was significant in only one of the individual Subgroups. For the plasma chemistry measures, in 7 cases a measure for one sex was significant in the combined Subgroups and in both individual Subgroups (increases in male and female cholesterol, total protein, and globulin and increases in male albumin). In 6 cases, a plasma chemistry measure was significant in the combined Subgroups and one individual Subgroup, but not the other individual Subgroup. There was one case where a plasma chemistry measure was significant in the combined Subgroups and in neither of the individual Subgroups.

Laboratory 7 – *p,p'*-DDE

77. Haematology results: The platelet count and the prothrombin and activated thromboplastin times were significantly increased on both sexes at the high doses used in this study.

78. Plasma chemistry results: Total protein was significantly increased in both sexes at the respective high doses, the albumin/globulin ratio was significantly decreased in males at the mid- and high doses and in females at all doses, glucose was significantly decreased in both sexes at the high dose, triglycerides were significantly decreased in males at the mid- and high doses and in females at the respective high dose, and total cholesterol was significantly increased in males at the mid- and high doses and in females at the respective high dose. In males, calcium, sodium, and γ -glutamyl transferase activity were significantly increased at the high dose and aspartate aminotransferase was significantly decreased. In females, blood urea nitrogen and alanine amino transferase were significantly decreased at the mid- and high doses, and alkaline phosphatase was significantly decreased at the high dose.

79. The haematology and plasma chemistry results of the combined Subgroups are compared with those of the individual Subgroups A and B in Table 14.

Table 14. Statistically Significant Haematological and Clinical Chemistry Parameters for *p,p'*-DDE in the Laboratory 7 Study

	Combined Subgroups (n = 10)	Subgroup A (n = 5)	Subgroup B (n = 5)
Haematology results			
Act. thromboplastin time	↑ M (I, H) F (H)	↑ M (I, H)	↑ M (H) F (H)
Prothrombin time	↑ M (H) F (H) ↓ F (I)	↓ F (I, H)	
Platelet count	↑ M (H) F (H)	↑ F (H)	
Plasma chemistry results			
Cholesterol	↑ M (I, H) F (H)	↑ M (H) F (H)	↑ M (I, H) F (H)
Triglycerides	↓ M (I, H) F (H)	↓ M (I, H) F (H)	↓ F (H)
Total protein	↑ M (H) F (H)	↑ F (H)	↑ M (H) F (I, H)
γ -Glutamyl transferase	↑ M (H)	↑ M (H)	
Alkaline phosphatase	↓ F (H)	↓ F (H)	
Alanine aminotransferase	↓ F (I, H)	↓ F (I, H)	↑ M (H)
Aspartate aminotransferase	↓ M (H)	↓ M (L, I, H) F (H)	
Albumin/globulin ratio	↓ M (I, H) F (L, I, H)	↓ M (I, H) F (H)	↓ M (I) F (L, I, H)
Blood urea nitrogen	↓ F (I, H)		↑ M (I) ↓ F (I, H)
Glucose	↓ M (H) F (H)	↓ F (H)	↓ M (I, H)
Calcium	↑ M (H)		
Sodium	↑ M (H)		

M – male, F – female, H – high dose, I – intermediate or mid-dose, L – low dose, ↓ - statistically significant decrease, ↑ - statistically significant increase.

80. One of the haematology measures was statistically significant in the combined Subgroups and in both individual Subgroups (an increase in male activated thromboplastin times). In 3 cases, a measure was significant in the combined Subgroups and one individual Subgroup, but not the other individual Subgroup. In 2 cases, a measure was significant in the combined Subgroups only. For the plasma chemistry measures, in 6 cases, a measure for one sex was significant in the combined Subgroups and in both individual Subgroups (increases in male and female cholesterol levels and total protein, decreases in female triglycerides, decreases in female albumin/globulin ratio). In 7 cases, a plasma chemistry measure was significant in the combined Subgroups and one individual Subgroup, but not the other individual Subgroup. In 2 cases, a plasma chemistry measure was significant in the combined Subgroups and in neither of the individual Subgroups. In another two cases, a plasma chemistry measure was significant in only one of the individual Subgroups.

Summary - *p,p'*-DDE

81. The DDE studies were the most consistent overall. The increase in platelet counts in both sexes, the decrease in male triglycerides, the increase in cholesterol in both sexes, the increase in total protein in both sexes, the decrease in the male albumin/globulin ratio, and the increase in male calcium values were significant in both studies. Thus, about 50% of the statistically significant measures were consistent between the two studies in this case, and there were no observed conflicts. The other measures achieving statistical significance in one study, however, often had similar absolute trends in the other study.

Propylthiouracil

Laboratory 1 – Propylthiouracil

82. Haematology results: Prothrombin time was significantly increased in both sexes at the high dose. Platelet counts were significantly decreased in males at the mid- and high doses. Erythrocyte, haemoglobin, and haematocrit values were significantly decreased and mean cell haemoglobin concentration and reticulocyte values were significantly increased in females at the high dose.

83. Plasma chemistry results: Alanine aminotransferase was significantly decreased in both sexes at the high dose, and alkaline phosphatase values were significantly decreased in males at the high dose. Potassium and triglyceride values were significantly decreased and urea and creatinine values were significantly increased in both sexes at the high dose. Sodium values were significantly increased in females at the mid- and high doses. Phosphorus values were significantly decreased in males at the high dose. Albumin was significantly increased in males at the high dose.

84. The haematology and plasma chemistry results of the combined Subgroups are compared with those of the individual Subgroups A and B in Table 15.

Table 15. Statistically Significant Haematological and Clinical Chemistry Parameters for PTU in the Laboratory 1 Study

	Combined Subgroups (n = 10)	Subgroup A (n = 5)	Subgroup B (n = 5)
Haematology results			
Haemoglobin concentration	↓ F (H)		
Haematocrit	↓ F (H)		
Prothrombin times	↑ M (H) F (H)		↑ M (H)
Platelet counts	↓ M (I, H)		
Erythrocyte counts	↓ F (H)		
Mean cell haemoglobin conc.	↑ F (H)		
Reticulocytes	↓ F (H)	↓ F (H)	↓ F (H)
Plasma chemistry results			
Triglycerides	↓ M (H) F (H)	↓ M (H) F (H)	↓ M (H)
Blood urea nitrogen	↑ M (H) F (H)	↑ F (H)	
Creatinine	↑ M (H) F (H)	↑ M (H) F(H)	↑ F (H)
Phosphorus	↓ M (H)	↓ M (H)	
Sodium	↑ F (I, H)		
Potassium	↓ M (H) F (H)		
Alanine aminotransferase	↓ M (H) F (H)		
Alkaline phosphatase	↓ M (H)		
Albumin	↑ M (H)		
Total protein		↑ M (H)	

M – male, F – female, H – high dose, I – intermediate or mid-dose, L – low dose, ↓ - statistically significant decrease, ↑ - statistically significant increase.

85. A comparison of the results from the combined and individual Subgroups shows several differences. Of the eight cases of statistical significance in the combined Subgroups in the haematology results, two also had statistical significance in one or both of the individual Subgroups.

86. In the plasma chemistry results, the decrease in male triglycerides and increase in female creatinine were the only measures having statistical significance in the combined Subgroups and both individual Subgroups. In 2 cases, statistical significance in the combined Subgroups was also found in one of the individual Subgroups. In the case of male total protein, an individual Subgroup achieved statistical significance that was not seen in the other individual Subgroup or in the combined Subgroups. In 5 cases, the combined Subgroups were significant, when neither Subgroup achieved statistical significance, suggesting the combined Subgroups had greater power.

Laboratory 10 – Propylthiouracil

87. Haematology results: Platelet counts were significantly decreased and neutrophil counts were significantly increased in females at the high dose.

88. Plasma chemistry results: Activated partial thromboplastin time (APTT), total protein, and cholesterol were significantly increased in males at the high dose. Blood urea nitrogen (BUN) and chloride were significantly increased in females at the high dose.

89. The laboratory 10 propylthiouracil study did not record the animal data into two Subgroups of five animals per sex. Therefore, no analysis can be performed on the possible effect on study findings and power by increasing the group size to 10 animals per sex.

Summary – Propylthiouracil

90. There were no consistent statistically significant results in both propylthiouracil studies. However, the reduced power of six animals in the laboratory 10 study should be noted and taken into account when observing that none of the other statistically significant measures in one study were reproduced in the other study. The other measures achieving statistical significance in one study, however, often had similar absolute trends in the other study.

L-Thyroxine

Laboratory 9 – L-Thyroxine

91. Haematology results: The reticulocyte value was significantly increased and the mean cell haemoglobin concentration and activated partial thromboplastin time were significantly decreased in both sexes of the high group. The erythrocyte count was significantly increased, and the mean cell haemoglobin and prothrombin time were significantly decreased in the male high dose group. The haematocrit was significantly increased in the female high dose group.

92. Plasma chemistry results: The inorganic phosphorus was significantly increased in both sexes in the high dose groups; the alanine aminotransferase activity was significantly increased and the total cholesterol and triglyceride was significantly decreased in the male high dose group, and the urea nitrogen, total protein, and albumin was significantly decreased in the female high dose group.

93. The haematology and plasma chemistry results of the combined Subgroups are compared with those of the individual Subgroups A and B in Table 16.

Table 16. Statistically Significant Haematological and Clinical Chemistry Parameters for L-Thyroxine in the Laboratory 9 Study

	Combined Subgroups (n = 10)	Subgroup A (n = 5)	Subgroup B (n = 5)
Haematology results			
Prothrombin times	↓ M (H)	↓ M (H)	↓ M (H)
Mean cell haemoglobin conc.	↓ M (H) F (H)	↓ M (H)	↓ M (H)
Reticulocytes	↑ M (H) F (H)	↑ M (H) F (H)	↑ M (H)
Act. partial thromboplastin time	↓ M (H) F (H)	↓ M (H) F (H)	↓ M (H)
Haematocrit	↑ F (H)		
Erythrocyte counts	↑ M (H)		
Mean cell haemoglobin	↓ M (H)		
Mean erythrocyte cell vol		↑ F (H)	
Plasma chemistry results			
Triglycerides	↓ M (H)		↓ M (H)
Alanine aminotransferase	↑ M (H)	↑ M (H)	
Total protein	↓ F (H)	↓ F (H)	↓ F (H)
Albumin	↓ F (H)	↓ F (H)	↓ F (H)
Blood urea nitrogen	↓ F (H)	↓ F (H)	↓ F (H)
Phosphorus	↑ M (H) F (H)	↑ M (H)	↑ M (H) F (H)
Cholesterol	↓ M (H)		
Sodium		↑ F (H)	

M – male, F – female, H – high dose, I – intermediate or mid-dose, L – low dose, ↓ - statistically significant decrease, ↑ - statistically significant increase.

94. A comparison of the results from the combined and individual Subgroups shows a number of similarities and also differences. Four haematology parameters, male prothrombin times, male reticulocyte values, male activate partial thromboplastin times, and mean cell haemoglobin concentrations, were statistically significant in the combined Subgroups and both individual Subgroups. In 2 cases, measures were significant in the combined Subgroups and one individual Subgroup, but not the other individual Subgroup. In 4 four cases, measures were significant only in the combined Subgroups and neither individual Subgroup. The mean erythrocyte cell volume was significantly increased in females in one individual Subgroup, but was not significant in the combined Subgroups or the other individual Subgroup.

95. Four plasma chemistry parameters, female total protein, female albumin, female blood urea nitrogen, phosphorus levels, were statistically significant in the combined Subgroups and both individual Subgroups. In 3 cases, statistical significance was seen in the combined Subgroups and one individual Subgroup and not the remaining individual Subgroup. Male cholesterol was significantly decreased only in the combined Subgroups, and female sodium was significantly increased only in of the individual Subgroups.

Laboratory 13 – L-Thyroxine

96. Haematology results: The haemoglobin in males, the mean cell haemoglobin content in males, the mean cell haemoglobin in females, and lymphocyte counts in males were significantly decreased at the high dose. The eosinophil count in males, the prothrombin time in males, and monocyte and leukocyte counts in females were significantly increased at the high dose.

97. Plasma chemistry results: The glucose, creatinine, total protein, globulin, and calcium values were significantly decreased in males at the high dose. Urea, total protein, albumin, and creatinine were significantly decreased in females at the high dose, and creatinine was significantly decreased in females at the mid- and high doses. Bilirubin, potassium, phosphorus, and alkaline phosphatase were significantly increased in males at the high dose. Bilirubin, phosphorus, alanine aminotransferase and aspartate aminotransferase were significantly increased in females at the high dose.

98. The laboratory 13 l-thyroxine study did not record the animal data into two Subgroups of five animals per sex. Therefore, no analysis can be performed on the possible effect on study findings and power by increasing the group size to 10 animals per sex.

Summary - l-Thyroxine

99. The male mean cell haemoglobin and the female mean cell haemoglobin concentration significantly decreased in both studies. The male prothrombin times were in conflict, increasing in the laboratory 13 study and decreasing in the laboratory 9 study. None of the other statistically significant haematology results in one study were reproduced in the other study.

100. The decreases in female blood urea nitrogen, total protein, and albumin and the increases in the phosphorus of both sexes were reproduced in both studies. However, none of the other statistically significant plasma chemistry results in one study were reproduced in the other study. The other measures achieving statistical significance in one study, however, often had similar absolute trends in the other study.

ANNEX 5

Absolute and Relative Organ and Tissue Weight Data Generated for the Updated TG 407 Validation Studies

1. The purposes of this Annex are to record for the absolute and relative tissue weights:
 - the basic statistical findings for each sex from each Subgroups and the combined Subgroups for the absolute and relative tissue weights, when these data were available from the final reports; and
 - the means and standard deviations for each sex from each Subgroup and the combined Subgroups, when these data were available from the final reports.
2. Additional purposes of this Annex are to compare the reproducibility and reliability of the findings for the absolute and relative tissue weights:
 - in regards to a dose response or possible random findings;
 - between Subgroups in the same lab;
 - the added value and power of combined the Subgroups (i.e., a group size of five versus ten animals per sex); and
 - between the two laboratories studying each chemical.
3. This Annex is organized in the following manner:
 - each of the ten chemicals tested in the updated TG 407 validation program has its own individual section. The chemicals are roughly grouped as:
 - Estrogens: ethinyl oestradiol, genistein, and nonylphenol.
 - Antiestrogens: tamoxifen
 - Aromatase inhibitors: CGS 18320B
 - Androgens: methyl testosterone
 - Antiandrogens: flutamide and *p,p'*-DDE
 - Thyroid toxicants and hormones: propylthiouracil and l-thyroxine
 - in each section, the absolute and relative tissue weight data of both laboratories testing the chemical are presented with the laboratories in alphabetical order;
 - there are two tables for each laboratory with the male data in the top half and the female data in the bottom half of the table; the first table contains the absolute tissue weights that were statistically significant or of interest and the second table contains the relative tissue weights that were statistically significant or of interest ;
 - for each tissue, the data for the combined Subgroups is presented first, then for the two individual Subgroups, where available;
 - the dose groups left to right are the vehicle control, the low dose, the mid-dose, and the high dose; and
 - the achievement of statistical significance, either an increase or a decrease in the values, is denoted with one or two asterisks depending upon the level of statistical significance reported by the laboratory.
4. Overall integration and interpretation of the tissue weight data with other findings are noted in the body of this report, and only occasionally this or other Annexes. Likewise, discussion, recommendations,

and conclusions are in the body of this report, except for the clear detection of possible endocrine effects by the measured absolute or calculated relative weights.

Ethinyl Oestradiol

Laboratory 2 – Ethinyl oestradiol

5. The gross pathology observations related to treatment in this study were atrophy of the seminal vesicles in males at the high dose 200 µg/kg EE.

6. The absolute weights discussed for this study are shown in Table 1A and the relative weights in Table 1B. The absolute liver weights were significantly increased in females at the mid- and high doses, and the relative weights were significantly increased at all doses. The absolute adrenal weights were significantly increased in males at the high dose, and the relative weights of the adrenals were significantly increased at the mid- and high doses. The relative weights of the brain, pituitary, thyroid, and liver were significantly increased in males at the high dose, but the weights of these organs and tissues tend to be conserved and were attributed to be the result of decreasing male body weights at the high dose (not all data are included in the tables). The absolute kidney weights were significantly decreased in males at the high dose, but the relative weights were not significantly decreased; therefore, this change was also attributed to be the result of decreasing body weights.

7. In the male reproductive tract, the absolute and the relative weights of the ventral prostate, dorsolateral prostate, coagulating glands, and seminal vesicles were significantly decreased in males at the high dose.

8. In the female reproductive tract, the relative weight of the uterus was significantly increased at the high dose. Although the absolute uterine weight increased by over 50%, it did not achieve statistical significance.

Table 1A. Absolute Organ Weights with EE in the Laboratory 2 study

Ethinyl oestradiol Dose (µg/kg/day)	Control	Low	Middle	High
	0	10	50	200
Male Body Wt Δ 28d		+2.9%	-5.1%	↓↓ -12.5%
Male Body Wt Δ Necropsy		+3.5%	-4.8%	↓↓ -12.5%
Kidney (mg)	2376 ± 273.4	2418 ± 252.9	2262 ± 322.0	*1989 ± 270.8
Subgroup A	2454 ± 324.2	2367 ± 139.0	2244 ± 162.1	*2020 ± 282.3
Subgroup B	2297 ± 218.4	2469 ± 343.6	2281 ± 454.0	1958 ± 288.0
Adrenal (mg)	50 ± 6.3 ⁹	53 ± 8.0 ⁹	59 ± 7.4	**67 ± 10.8
Subgroup A	49 ± 8.1	51 ± 7.7	57 ± 7.2	**66 ± 5.5
Subgroup B	48 ± 5.5 ⁴	57 ± 7.9 ⁴	60 ± 8.0	*68 ± 15.1
Ventral prostate (mg)	412 ± 74.3	467 ± 91.6	341 ± 63.9 ⁹	**187 ± 68.9
Subgroup A	404 ± 55.9	452 ± 111.2	330 ± 72.1	**191 ± 94.1
Subgroup B	425 ± 88.6	481 ± 77.3	355 ± 59.2 ⁴	**182 ± 42.0
Dorsolateral prostate (mg)	490 ± 75.9	485 ± 76.1	422 ± 122.0 ⁹	**254 ± 89.5
Subgroup A	485 ± 61.7	493 ± 91.7	402 ± 130.8	*282 ± 122.0
Subgroup B	495 ± 95.4	476 ± 66.5	447 ± 124.2 ⁴	**227 ± 35.5
Seminal vesicles (mg)	796 ± 154.6	788 ± 130.2	646 ± 117.5 ⁹	**307 ± 100.0
Subgroup A	738 ± 121.7	727 ± 130.1	598 ± 65.0	**288 ± 118.9
Subgroup B	833 ± 205.3	849 ± 109.3	705 ± 151.0 ⁴	**326 ± 86.3
Coagulating glands (mg)	149 ± 34.8	147 ± 38.8	116 ± 28.5 ⁹	**50 ± 26.1
Subgroup A	148 ± 31.0	126 ± 26.2	109 ± 15.2	**48 ± 31.8
Subgroup B	149 ± 42.0	169 ± 39.1	125 ± 40.9 ⁴	**51 ± 22.6
Female Body Wt Δ 28d		+3.5%	+3.5%	+1.0%
Female Body Wt Δ Necropsy		+3.0%	+3.5%	-0.5%
Liver (mg)	7889 ± 745.4	8976 ± 1103.2	**9702 ± 1278.4	**9779 ± 1276.4
Subgroup A	7852 ± 275.6	8732 ± 1365.1	*10133 ± 964.8	9656 ± 1696.5
Subgroup B	7926 ± 1082.1	9220 ± 851.9	9271 ± 1510.5	*9901 ± 866.0
Spleen (mg)	420 ± 76.3	442 ± 69.1	473 ± 92.2	484 ± 107.1
Subgroup A	396 ± 46.4	416 ± 60.4	445 ± 38.8	*521 ± 114.0
Subgroup B	445 ± 97.2	469 ± 73.3	501 ± 125.2	447 ± 96.8

Values were expressed as mean ± SD. Superscript indicates number of animals, if not 10 in combined Subgroups or 5 in individual Subgroups. *, **, Significantly different from control (p<0.05 and p<0.01, respectively).

Table 1B. Relative Organ Weights with EE in the Laboratory 2 Study

Ethinyl oestradiol Dose (µg/kg/day)	Control	Low	Middle	High
	0	10	50	200
Male Body Wt Δ 28d		+2.9%	-5.1%	↓↓ -12.5%
Male Body Wt Δ Necropsy		+3.5%	-4.8%	↓↓ -12.5%
Liver	4.28% ⁹	4.52%	4.68%	*4.73%
Subgroup A	^d 4.21% ⁴	4.51%	4.46%	4.74%
Subgroup B	4.34%	4.50%	*4.89%	4.72%
Kidney	0.76%	0.75%	0.76%	0.73%
Subgroup A	0.76%	0.76%	0.77%	0.73%
Subgroup B	0.76%	0.73%	0.76%	0.73%
Brain	0.60%	0.58%	0.62%	*0.67%
Subgroup A	0.60%	0.60%	0.62%	*0.68%
Subgroup B	0.60%	0.56%	0.63%	0.66%
Adrenal	0.0160% ⁹	0.0164% ⁹	**0.0199%	**0.0245%
Subgroup A	0.0153%	0.0164%	*0.0195%	*0.0238%
Subgroup B	0.0158% ⁴	^d 0.0170% ⁴	^d 0.0199%	^d 0.0253%
Thyroid	0.0051%	0.0050%	0.0054%	*0.0062%
Subgroup A	0.0050%	0.0051%	0.0058%	*0.0065%
Subgroup B	0.0053%	0.0048%	0.0050%	0.0063%
Pituitary	0.0032%	0.0034%	0.0034%	*0.0040%
Subgroup A	0.0028%	0.0032%	0.0031%	0.0036%
Subgroup B	0.0033%	0.0033%	0.0037%	0.0048%
Ventral prostate	0.132%	0.145%	0.115% ⁹	**0.068%
Subgroup A	0.126%	0.145%	0.113%	*0.069%
Subgroup B	0.140%	0.143%	^d 0.118% ⁴	**0.068%
Dorsolateral prostate	0.157%	0.150%	0.142% ⁹	**0.093%
Subgroup A	0.151%	0.159%	0.137%	0.102%
Subgroup B	0.163%	0.142%	^d 0.149% ⁴	**0.084%
Seminal vesicles	0.252%	0.244%	0.218% ⁹	**0.112%
Subgroup A	0.230%	0.234%	0.204%	**0.104%
Subgroup B	0.274%	0.253%	^d 0.234% ⁴	**0.121%
Coagulating glands	0.048%	0.046%	0.039% ⁹	**0.018%
Subgroup A	0.046%	0.040%	0.040%	*0.018%
Subgroup B	0.049%	0.050%	^d 0.042% ⁴	**0.019%
Female Body Wt Δ 28d		+3.5%	+3.5%	+1.0%
Female Body Wt Δ Necropsy		+3.0%	+3.5%	-0.5%
Livere	3.98%	*4.40%	**4.73%	**4.96%
Subgroup A	3.99%	4.32%	**4.90%	**4.78%
Subgroup B	4.02%	4.48%	4.57%	**5.13%
Spleen	0.21%	0.22%	0.23%	0.26%
Subgroup A	0.20%	0.21%	0.21%	**0.26%
Subgroup B	0.23%	0.23%	0.25%	0.23%
Thymus	0.21%	0.20%	0.21%	0.22%
Subgroup A	0.22%	0.19%	0.21%	0.22%
Subgroup B	0.21%	0.21%	0.21%	0.21%
Ovaries	0.060%	0.057%	0.057%	0.055%
Subgroup A	0.060%	0.056%	0.052%	0.057%
Subgroup B	0.386%	0.361%	0.394%	0.554%
Uterus	0.35%	0.45%	0.40%	*0.54%
Subgroup A	0.31%	0.55%	0.42%	0.53%
Subgroup B	0.39%	0.36%	0.39%	0.55%

Values were expressed as mean \pm SD. Superscript indicates number of animals, if not 10 in combined or 5 in Subgroups. *, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively).

^d statistics were not calculated for this value and Subgroup, where a control or test group size was $n \leq 4$; under these conditions the power of the statistics was judged to be too low to provide meaningful information.

9. The combined Subgroups and the individual Subgroups were consistent in detecting changes in the male adrenal weights and several tissues of the male reproductive tract (ventral and dorsolateral prostate and both seminal vesicles and coagulating glands) on an absolute basis. No essential advantage for the combined Subgroups is evident to detect EE activity as it is suggested by these data that individual Subgroups of five animals per sex would be sufficient to detect a compound as potent as EE.

10. After the issue of detection, a second concern is the LOEL that a study determines. In the laboratory 2 study, the use of the combined Subgroups would potentially change the LOEL by detecting the relative change in the female liver at 10 rather than 50 $\mu\text{g}/\text{kg}/\text{d}$.

11. A final issue is the number of endpoints that might achieve statistical significance that are affected by a chemical substance. Accounting for the decreased male body weights, the Subgroup A dorsolateral prostate relative weight did not achieve statistical significance; and, the relative weights of the pituitary were not significantly increased in either Subgroup and the brain and thyroid were not significantly increased in Subgroup B. The combined Subgroups were then more effective in detecting significant increases in absolute and relative female liver weights than the individual Subgroups, and detected a significant increase in relative uterine weights that was not detected in either Subgroup. Apparently spurious significant increases in the female absolute and relative spleen weights in Subgroup A at the high dose were not seen in either Subgroup B or the combined Subgroups. These data would then lend support that statistical power is modestly improved with the combined Subgroups of ten animals per sex.

Laboratory 5 – Ethinyl oestradiol

12. The gross pathology observations were atrophy of the prostates and seminal vesicle in the mid- and high dose groups (50 and 200 $\mu\text{g}/\text{kg}$ EE); hypertrophy of the liver in females at the high dose; and atrophy of the ovaries at the high dose.

13. The absolute weights discussed for this study are shown in Table 2A and the relative weights in Table 2B. The absolute heart and kidney weights were significantly decreased in males at the high dose. The relative male heart and kidney weights were not significantly different at the high dose, indicating the absolute weight decreases were related to a similar decrease in body weights. The absolute and relative adrenal weights were significantly increased in males at the high dose. In females, the absolute liver weights were significantly increased in the mid- and high dose groups. When expressed on a relative basis, the liver weights in the males were significantly increased at the mid- and the high dose, and the female liver weights were significantly increased at all doses. Similarly, the relative weights of the brain, pituitary, and testes were significantly increased in males at the high dose, but the weights of these organs and tissues tend to be conserved and were attributed to be the result of decreasing male body weights at the high dose (and are not included in the absolute table).

14. In the male reproductive tract, the absolute total prostate, ventral prostate, dorsolateral prostate and seminal vesicle weights were all significantly decreased in both the mid- and high dose groups, and the absolute epididymal weights were significantly decreased at the high dose. When expressed on a relative basis, the epididymal weight decreases were no longer significant; the relative prostate, dorsolateral prostate, and seminal vesicle weights were significantly decreased at the mid- and high doses; and the relative ventral prostate weights were significantly decreased at the high dose.

15. In the female reproductive tract, both the absolute and relative uterine weights were significantly increased and both the absolute and relative ovary weights significantly decreased at the high dose.

Table 2A.– Absolute Organ Weights with EE in the Laboratory 5 Study

Ethinyl oestradiol Dose (µg/kg/day)	Control 0	Low 10	Middle 50	High 200
Male Body Wt Δ 28d		-1.0%	-3.0%	↓↓ -14.0%
Male Body Wt Δ Necropsy		-1.3%	-3.3%	↓↓ -14.5%
Heart (g)	1.15 ± 0.11	1.17 ± 0.06	1.09 ± 0.08	*1.03 ± 0.10
Subgroup A	1.21 ± 0.09	1.20 ± 0.06	1.14 ± 0.05	*1.06 ± 0.12
Subgroup B	1.08 ± 0.08	1.13 ± 0.04	1.04 ± 0.07	1.00 ± 0.06
Kidney (g)	2.57 ± 0.33	2.41 ± 0.14	2.43 ± 0.18	*2.24 ± 0.18
Subgroup A	2.63 ± 0.08	2.42 ± 0.14	2.44 ± 0.18	**2.30 ± 0.16
Subgroup B	2.52 ± 0.49	2.40 ± 0.16	2.41 ± 0.20	2.18 ± 0.19
Adrenals (mg)	51.8 ± 5.8 ⁹	50.4 ± 7.5 ⁹	62.8 ± 14.2	*67.6 ± 12.5
Subgroup A	50.4 ± 5.4	50.1 ± 6.6	59.5 ± 8.7	**71.1 ± 14.7
Subgroup B	53.2 ± 6.5 ⁴	50.7 ± 9.0 ⁴	66.2 ± 18.7	64.1 ± 10.1
Epididymes (mg)	941.9 ± 52.2	911.5 ± 59.0	903.6 ± 98.2	*843.2 ± 71.4
Subgroup A	956.1 ± 70.6	929.3 ± 64.8	972.6 ± 82.2	862.5 ± 62.9
Subgroup B	927.6 ± 25.0	893.8 ± 53.3	834.6 ± 55.1	*823.9 ± 81.1
Prostate (mg)	961.6 ± 132.3	862.1 ± 184.8	*723.5 ± 215.2	**480.3 ± 197.7
Subgroup A	976.4 ± 127.8	912.3 ± 206.8	762.9 ± 111.9	**438.7 ± 228.2
Subgroup B	946.8 ± 150.0	812.0 ± 166.7	684.1 ± 296.3	*521.8 ± 177.5
Ventral prostate (mg)	588.9 ± 94.8	543.6 ± 157.5	*444.3 ± 137.7	**316.3 ± 129.3
Subgroup A	592.8 ± 117.6	581.3 ± 175.0	467.9 ± 112.5	**291.9 ± 121.0
Subgroup B	585.0 ± 79.6	505.8 ± 147.1	420.7 ± 169.2	*340.8 ± 146.6
Dorsolateral prostate (mg)	440.2 ± 65.3	407.6 ± 77.1	**301.7 ± 85.4	**186.3 ± 104.9
Subgroup A	456.7 ± 69.4	418.0 ± 98.5	334.6 ± 49.1	**150.1 ± 127.4
Subgroup B	423.8 ± 63.9	397.2 ± 58.3	*268.7 ± 106.2	**222.5 ± 72.6
Seminal vesicle (mg)	1416.7 ± 118.5	1271.5 ± 212.1	*997.4 ± 356.8	**638.9 ± 328.9
Subgroup A	1443.1 ± 120.4	1299.9 ± 266.4	1187.5 ± 188.0	**680.9 ± 260.3
Subgroup B	1390.3 ± 123.9	1243.1 ± 168.0	*807.3 ± 401.0	**597.0 ± 413.9
Female Body Wt Δ at 28 d		-0.6%	-0.8%	-6.9%
Female Body Wt Δ at Necropsy		-0.1%	-1.3%	-7.3%
Liver (g)	9.33 ± 0.52	10.22 ± 1.42	*10.65 ± 1.23	*10.95 ± 1.33
Subgroup A	9.36 ± 0.55	10.69 ± 1.87	10.62 ± 1.51	10.99 ± 0.96
Subgroup B	9.29 ± 0.55	9.74 ± 0.67	10.68 ± 1.05	10.91 ± 1.74
Ovary (mg)	82.9 ± 21.3	78.7 ± 15.8	77.3 ± 11.0 ⁹	**49.9 ± 11.6
Subgroup A	69.3 ± 6.2	82.2 ± 21.4	78.0 ± 9.3	47.9 ± 10.5
Subgroup B	96.6 ± 22.8	75.1 ± 8.5	76.4 ± 14.4 ⁴	**51.9 ± 13.5
Uterus (mg)	396.6 ± 58.7	420.3 ± 47.8	400.3 ± 30.2 ⁹	**477.4 ± 60.4
Subgroup A	421.3 ± 41.6	387.4.3 ± 43.0	401.6 ± 33.2	*521.9 ± 43.6
Subgroup B	371.8 ± 67.0	*453.1 ± 24.4	398.6 ± 30.8 ⁴	432.9 ± 36.8

Values were expressed as mean ± SD. Superscript indicates number of animals, if not 10 in combined Subgroups or 5 in the individual Subgroups. *, **, Significantly different from control (p<0.05 and p<0.01, respectively).

Table 2B. Relative Organ Weights with EE in the Laboratory 5 Study

Ethinyl oestradiol Dose (µg/kg/day)	Control	Low	Middle	High
	0	10	50	200
Male Body Wt Δ 28d		-1.0%	-3.0%	↓↓ -14.0%
Male Body Wt Δ Necropsy		-1.3%	-3.3%	↓↓ -14.5%
Liver	3.85%	4.07%	**4.29%	**4.70%
Subgroup A	3.88%	4.04%	**4.32%	**4.85%
Subgroup B	4.07%	4.15%	4.53%	**5.23%
Heart	0.30%	0.31%	0.30%	0.32%
Subgroup A	0.31%	0.32%	0.30%	0.32%
Subgroup B	0.29%	0.31%	0.29%	0.32%
Kidney	0.68%	0.65%	0.67%	0.69%
Subgroup A	0.68%	0.64%	0.65%	0.69%
Subgroup B	0.68%	0.65%	0.68%	0.70%
Brain	0.51%	0.52%	0.54%	**0.59%
Subgroup A	0.50%	0.52%	0.53%	*0.57%
Subgroup B	0.52%	0.53%	0.53%	*0.60%
Adrenals	0.0137%	0.0135%	0.0172%	**0.0209%
Subgroup A	0.0130%	0.0133%	0.0159%	**0.0212%
Subgroup B	0.0144%	0.0138%	0.0186%	0.0206%
Pituitary	0.0029%	0.0030%	**0.0038%	**0.0039%
Subgroup A	0.0027%	0.0030%	**0.0038%	**0.0041%
Subgroup B	0.0031%	0.0030%	*0.0037%	0.0036%
Testes	0.83%	0.83%	0.83%	*0.95%
Subgroup A	0.81%	0.84%	0.81%	0.92%
Subgroup B	0.85%	0.82%	0.85%	0.98%
Epididymes	0.250%	0.245%	0.248%	0.261%
Subgroup A	0.244%	0.241%	0.242%	0.252%
Subgroup B	0.252%	0.243%	0.234%	0.265%
Prostate	0.255%	0.231%	*0.198%	**0.149%
Subgroup A	0.253%	0.242%	0.204%	**0.131%
Subgroup B	0.257%	0.221%	0.192%	0.168%
Ventral prostate	0.156%	0.146%	0.122%	**0.098%
Subgroup A	0.153%	0.154%	0.125%	*0.087%
Subgroup B	0.159%	0.138%	0.118%	0.110%
Dorsolateral prostate	0.117%	0.109%	**0.083%	**0.058%
Subgroup A	0.118%	0.111%	0.090%	**0.045%
Subgroup B	0.115%	0.108%	*0.075%	*0.072%
Seminal vesicle	0.375%	0.341%	*0.273%	**0.198%
Subgroup A	0.373%	0.371%	0.318%	**0.203%
Subgroup B	0.377%	0.338%	0.227%	0.192%

Table 2B continued. Relative Organ Weights with EE in the Laboratory 5 Study.

Ethinyl oestradiol Dose (µg/kg/day)	Control	Low	Middle	High
	0	10	50	200
Female Body Wt Δ at 28 d		-0.6%	-0.8%	-6.9%
Female Body Wt Δ at Necropsy		-0.1%	-1.3%	-7.3%
Liver	3.63%	*3.98%	**4.20%	**4.60%
Subgroup A	3.59%	4.06%	**4.22%	**4.63%
Subgroup B	3.71%	4.28%	*4.16%	**4.62%
Kidney	0.65%	0.68%	0.67%	0.72%
Subgroup A	0.63%	0.67%	0.69%	*0.72%
Subgroup B	0.67%	0.68%	0.65%	0.73%
Ovary	0.032%	0.031%	0.030%	**0.021%
Subgroup A	0.027%	0.031%	0.031%	0.020%
Subgroup B	0.038%	0.030%	0.030%	**0.022%
Uterus	0.154%	0.164%	0.158%	**0.201%
Subgroup A	0.162%	0.147%	0.160%	**0.220%
Subgroup B	0.147%	0.181%	0.156%	0.182%

Values were expressed as mean ± SD. Superscript indicates number of animals, if not 10 in combined Subgroups or 5 in the individual Subgroups. *, **, Significantly different from control (p<0.05 and p<0.01, respectively).

16. The combined Subgroups often achieved statistical significance when only one (absolute male heart, kidney and adrenal weights; absolute female ovarian and uterine weights) or even neither of the individual Subgroups (absolute female liver weights) achieved statistical significance. In the male reproductive tract, the combined Subgroups also sometimes achieved significance at a lower dose than either individual Subgroup, e.g., absolute total prostate and ventral prostate weights and relative seminal vesicle weights. The conclusion that this is due to improved statistical power is further supported by similar the absolute trends and percentage changes in the individual Subgroup(s) not achieving statistical significance when compared to the combined Subgroups.

17. In the laboratory 5 study, the use of the combined Subgroups would potentially change the LOEL by detecting or clearly detecting the absolute changes in the prostate tissues and seminal glands at 50 µg/kg/d and the relative change in the female liver at 10 rather than 50 µg/kg/d.

Summary – Ethinyl oestradiol

18. The organ and tissue weight observations, both absolute and relative, between the two ethinyl estradiol studies are consistent. When decreases in male organ and tissue weights sensitive to body weight changes are taken into account, both studies suggest a modest increase in the male liver at the high dose and in female liver at all doses. Male adrenal weights were increased in both studies. In the male reproductive tract, the prostate and the seminal vesicles and coagulating glands were decreased in both studies at the high dose. In the female reproductive tract, the decrease in ovarian weight and increase in uterine weight were consistent with estrogen mechanisms and significant in the laboratory 5 study. Although the mean absolute uterine weight increase in the laboratory 2 study was > 50% and did not achieve statistical significance, the relative uterine weights were statistically significant in concordance with the laboratory 5 study. The ovarian weights decreased a modest 10%, and did not achieve statistical significance in absolute or relative weights.

19. Both studies clearly identify significant changes in the male and the female at the EE doses of 200 µg/kg/d that would lead to suspicion of endocrine activity. These doses compare to the minimal effective dose for a uterine tissue increase in the uterotrophic assay via oral gavage of 1 µg/kg/d (Kanno et al 2001; OECD 2001). They are also consistent with the similar pattern of male and female tissue weights that were

seen at 150 µg/kg/d 17β-estradiol in a one-generation study (Beigel *et al.*, 1998; Cook *et al.*, 1998). Therefore, with these added or enhanced tissue weight endpoints, the updated TG 407 was successful in identifying the possible endocrine activity of EE.

Genistein

Laboratory 4 – Genistein

20. There were no gross pathology observations that were judged to be treatment related.

21. The absolute weights discussed for this study are shown in Table 3A and the relative weights in Table 3B. In males, the relative liver weights were significantly increased at the mid- and high dose. Statistical significance was observed only in the combined Subgroups. No other organ weight in either sex in either absolute or relative weights achieved statistical significance. No trends were evident for a decrease in the thymus of either sex; the female adrenals were about 10% lower than controls at the high dose only (but not significantly different). Male reproductive tract tissues such as the ventral and dorsolateral prostate and the seminal vesicles were fundamentally unchanged. In the female, the uterine absolute and relative weights declined by about 5-6% at the high dose and the ovaries by 4-5% at the high dose. These trends, while somewhat suggestive of estrogenic activity, do not provide clear evidence as statistical significance was not observed.

Table 3A. Absolute Organ Weights with Genistein in the Laboratory 4 Study

Genistein Dose (mg/kg/day)	Control	Low	Middle	High
	0	120	400	1000
Male Body Wt Δ 28d		-0.2%	+1.5%	+0.2%
Male Body Wt Δ Necropsy		-0.2%	+2.2%	+1.2%
Liver (g)	14.77 ± 1.47	15.25 ± 1.28	16.28 ± 2.39	16.12 ± 1.67
Subgroup A	15.21 ± 1.34	15.29 ± 1.46	16.79 ± 1.94	16.14 ± 2.00
Subgroup B	14.34 ± 1.61	15.21 ± 1.24	15.77 ± 2.91	16.10 ± 1.51
Female Body Wt Δ at 28 d		-2.4%	-2.8%	0.0%
Female Body Wt Δ at Necropsy		-3.5%	-3.1%	-1.6%
Liver (g)	9.45 ± 0.92	9.49 ± 1.14	9.96 ± 1.12	10.11 ± 1.06
Subgroup A	8.88 ± 0.24	9.98 ± 1.04	9.64 ± 1.31	10.07 ± 1.00
Subgroup B	10.01 ± 1.03	9.01 ± 1.12	10.28 ± 0.90	10.15 ± 1.24

Values were expressed as mean ± SD. *, **, Significantly different from control (p<0.05 and p<0.01, respectively). n = 10 Combined Subgroups; n = 5 individual Subgroups.

Table 3B. Relative Organ Weights with Genistein in the Laboratory 4 Study

Genistein Dose (mg/kg/day)	Control	Low	Middle	High
	0	120	400	1000
Male Body Wt Δ 28d		-0.2%	+1.5%	+0.2%
Male Body Wt Δ Necropsy		-0.2%	+2.2%	+1.2%
Liver	3.58%	3.71%	*3.87%	*3.87%
Subgroup A	3.64%	3.73%	3.96%	3.95%
Subgroup B	3.53%	3.68%	3.77%	3.80%
Epididymes	0.233%	0.233%	0.228%	0.229%
Subgroup A	0.234%	0.235%	0.222%	0.225%
Subgroup B	0.232%	0.230%	0.235%	0.233%
Ventral prostate	0.129%	0.122%	0.116%	0.110%
Subgroup A	0.117%	0.137%	0.116%	0.115%
Subgroup B	0.140%	0.107%	0.117%	0.104%
Dorsolateral prostate	0.078%	0.088%	0.097%	0.091%
Subgroup A	0.077%	0.090%	0.094%	0.103%
Subgroup B	0.076%	0.082%	0.098%	0.080%
Seminal vesicle	0.325%	0.319%	0.311%	0.312%
Subgroup A	0.342%	0.327%	0.316%	0.333%
Subgroup B	0.308%	0.310%	0.304%	0.292%
Female Body Wt Δ at 28 d		-2.4%	-2.8%	0.0%
Female Body Wt Δ at Necropsy		-3.5%	-3.1%	-1.6%
Liver	3.67%	3.82%	3.99%	4.00%
Subgroup A	3.53%	*3.92%	*3.93%	*4.16%
Subgroup B	3.81%	3.72%	4.06%	3.84%
Ovary	0.035%	0.033%	0.035%	0.034%
Subgroup A	0.035%	0.032%	0.035%	0.034%
Subgroup B	0.035%	0.033%	0.035%	0.033%
Uterus	0.177%	0.162%	0.158%	0.168%
Subgroup A	0.175%	0.167%	0.153%	0.151%
Subgroup B	0.178%	0.156%	0.163%	0.182%

Values were expressed as mean ± SD. *, **, Significantly different from control (p<0.05 and p<0.01, respectively). n = 10 Combined Subgroups; n = 5 individual Subgroups.

22. Although the combined Subgroups and the individual Subgroups were largely in agreement, there were no remarkable changes to challenge the need for statistical power. In the case of the increase in male relative liver weights, only the combined Subgroups achieved statistical significance. In the laboratory laboratory 4 study, the use of the combined Subgroups would be essential to lowering and establishing the LOEL of at 400 mg/kg/d by detecting relative changes in the male liver.

Laboratory 12 – Genistein

23. There were no gross pathology observations that were judged to be treatment related.

24. The absolute weights discussed for this study are shown in Table 4A and the relative weights in Table 4B. Absolute and adjusted adrenal weights were significantly increased in both sexes at the high dose, and the adjusted weights were also increased in the females also at the mid-dose. Absolute and adjusted thymus weights were significantly decreased in males at the mid- and high doses. In females, these values were lower, but achieved statistical significance only at the mid-dose (absolute weight). Absolute and adjusted liver weights were significantly increased in females at the high dose. Absolute and adjusted thyroid weight values were increased in females at all doses, but the absolute weights achieved statistical significance only at the low and mid-dose. These thyroid results were considered equivocal.

Unlike the other laboratories in the validation effort, laboratory 12 uses a ANCOVA procedure for adjusted weights which is indicated to be slightly more sensitive.

25. In the male reproductive tract, there were no significant changes, and the only trend towards lower absolute values was in the ventral prostate (16.7% decrease at the high dose). In the female reproductive tract, the absolute uterus plus cervix weights were significantly increased at the high dose, but this was dependent upon the removal of a high value individual data point at the mid-dose (see table and footnotes).

Table 4A. Absolute Organ Weights with Genistein in the Laboratory 12 Study

Genistein Dose (mg/kg/day)	Control	Low	Middle	High
	0	120	400	1000
Male Body Wt Δ 28d		+2.6%	-3.2%	-2.3%
Male Body Wt Δ Necropsy		+2.5%	-3.3%	-2.4%
Adrenal (g)	0.053 ± 0.006	0.056 ± 0.007	0.051 ± 0.007	**0.061 ± 0.005
Subgroup A	0.052 ± 0.008	0.053 ± 0.008	0.052 ± 0.009	0.061 ± 0.005
Subgroup B	0.055 ± 0.002	0.060 ± 0.002	0.051 ± 0.004	**0.062 ± 0.005
Thymus (g)	0.588 ± 0.099	0.597 ± 0.102	*0.487 ± 0.088	**0.484 ± 0.044
Subgroup A	0.624 ± 0.101	0.610 ± 0.144	0.542 ± 0.056	*0.485 ± 0.042
Subgroup B	0.551 ± 0.091	0.583 ± 0.049	*0.432 ± 0.083	0.484 ± 0.052
Female Body Wt Δ at 28 d		+0.6%	-2.1%	-1.7%
Female Body Wt Δ at Necropsy		+0.5%	-2.1%	-0.04%
Liver (g)	9.1 ± 1.5	9.7 ± 0.8	9.7 ± 0.7	*10.7 ± 1.4
Subgroup A	8.9 ± 1.5	10.1 ± 0.8	9.9 ± 0.8	*10.7 ± 1.3
Subgroup B	9.3 ± 1.6	9.3 ± 0.5	9.6 ± 0.7	10.7 ± 1.7
Adrenal (g)	0.070 ± 0.008	0.078 ± 0.012	0.079 ± 0.011	*0.083 ± 0.016
Subgroup A	0.071 ± 0.009	0.079 ± 0.005	0.079 ± 0.004	*0.084 ± 0.009
Subgroup B	0.068 ± 0.008	0.077 ± 0.017	0.080 ± 0.016	0.083 ± 0.022
Thymus (g)	0.499 ± 0.080	0.448 ± 0.052	*0.437 ± 0.072	0.446 ± 0.062
Subgroup A	0.517 ± 0.076	0.447 ± 0.070	0.439 ± 0.076	0.476 ± 0.058
Subgroup B	0.480 ± 0.089	0.449 ± 0.035	0.435 ± 0.050	0.416 ± 0.056
Thyroid (g)	0.012 ± 0.003	*0.015 ± 0.002	**0.016 ± 0.003	0.015 ± 0.002
Subgroup A	0.013 ± 0.003	0.015 ± 0.003	*0.017 ± 0.004	0.015 ± 0.003
Subgroup B	0.012 ± 0.004	0.016 ± 0.002	0.015 ± 0.003	0.014 ± 0.002
Uterus + cervix (g)	0.367 ± 0.071	0.397 ± 0.053	*0.451 ± 0.111	0.431 ± 0.055
Uterus + cervix (g)^a	0.367 ± 0.071	0.397 ± 0.053	0.423 ± 0.070	*0.431 ± 0.055
Subgroup A	0.367 ± 0.093	0.395 ± 0.061	*0.502 ± 0.131	0.440 ± 0.066
Subgroup B	0.367 ± 0.050	0.398 ± 0.050	0.400 ± 0.066	0.421 ± 0.046

^a This line has the one high-value data point from the mid-dose group excluded, which results in the high-dose group becoming significant

Values were expressed as mean ± SD. Superscript indicates number of animals, if not 10 in combined Subgroups or 5 in individual Subgroups. *, **, Significantly different from control (p<0.05 and p<0.01, respectively).

Table 4B. Relative Organ Weights with Genistein in the Laboratory 12 Study^a

Genistein Dose (mg/kg/day)	Control	Low	Middle	High
	0	120	400	1000
Male Body Wt Δ 28d		+2.6%	-3.2%	-2.3%
Male Body Wt Δ Necropsy		+2.5%	-3.3%	-2.4%
Adrenal	0.0146%	0.0150%	0.0145%	**0.0172%
Subgroup A	0.0140%	0.0143%	0.0145%	*0.0174%
Subgroup B	0.0154%	0.0160%	0.0148%	**0.0173%
Kidney	0.72%	*0.77%	**0.79%	*0.77%
Subgroup A	0.73%	*0.78%	**0.79%	0.75%
Subgroup B	0.72%	0.76%	*0.79%	*0.79%
Thymus	0.16%	0.16%	0.14%	0.14%
Subgroup A	0.17%	0.16%	0.15%	0.14%
Subgroup B	0.15%	0.16%	0.13%	0.13%
Epididymes	0.221%	0.227%	0.227%	0.226%
Subgroup A	0.213%	0.220%	0.221%	0.228%
Subgroup B	0.230%	0.234%	0.233%	0.223%
Ventral prostate	0.069%	0.064%	0.065%	0.059%
Subgroup A	0.066%	0.070%	0.063%	0.059%
Subgroup B	0.072%	0.058%	0.066%	*0.059%
Dorsolateral prostate	0.055%	0.059%	0.067%	0.061%
Subgroup A	0.055%	0.060%	0.070%	0.061%
Subgroup B	0.055%	0.059%	0.064%	0.062%
Seminal vesicle	0.231%	0.250%	0.273%	0.231%
Subgroup A	0.229%	0.270%	0.243%	0.214%
Subgroup B	0.236%	0.227%	0.304%	0.242%
Female Body Wt Δ at 28 d		+0.6%	-2.1%	-1.7%
Female Body Wt Δ at Necropsy		+0.5%	-2.1%	-0.04%
Liver	3.96%	4.20%	**4.31%	**4.66%
Subgroup A	3.89%	**4.40%	**4.42%	**4.65%
Subgroup B	4.04%	4.02%	4.26%	**4.67%
Adrenal	0.0305%	0.0338%	*0.0351%	*0.0362%
Subgroup A	0.0310%	0.0344%	0.0352%	*0.0365%
Subgroup B	0.0295%	0.0332%	0.0355%	0.0362%
Thymus	0.22%	*0.19%	0.19%	0.19%
Subgroup A	0.23%	0.19%	0.20%	0.21%
Subgroup B	0.21%	0.19%	0.19%	*0.18%
Thyroid	0.0052%	*0.0065%	**0.0071%	0.0065%
Subgroup A	0.0057%	0.0065%	0.0076%	0.0065%
Subgroup B	0.0052%	0.0069%	0.0066%	0.0061%
Ovary	0.041%	0.045%	0.045%	0.041%
Subgroup A	0.041%	0.046%	0.046%	0.042%
Subgroup B	0.042%	0.044%	0.044%	0.041%
Uterus + cervix	0.160%	0.172%	0.188%	^b *0.188%
Subgroup A	0.160%	0.172%	0.224%	0.191%
Subgroup B	0.159%	0.172%	0.177%	0.184%

^a Laboratory 12 uses an ANCOVA procedure adjusting organ weights with body weight as a covariate, a slightly different procedure than in most labs. Thus, the statistical significance here is not strictly comparable to the results of other laboratories. These are relative weights in the table, which were calculated by the Secretariat.

^b This line has the one high-value data point from the mid-dose group excluded, which results in the high-dose group becoming significant

Values were expressed as mean \pm SD. Superscript indicates number of animals, if not 10 in combined Subgroups or 5 in individual Subgroups. *, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively).

26. Where statistical differences were observed in the combined Subgroups in either sex, there was typically only one of the individual Subgroups that achieved statistical significance. This supports the increase in statistical power with the combined Subgroups. There was also a decrease in the adjusted ventral prostate weight that was statistically significant in Subgroup B, but not in Subgroup A or in the combined Subgroups so that the male data did not detect any endocrine activity for genistein.

27. In the laboratory 12 study, the use of the combined Subgroups would potentially be essential to the detection of possible endocrine activity with the uterine weight change at 1000 mg/kg/d, bearing in mind the need to eliminate a high value individual in the mid-dose range in order to achieve statistical significance in this dose group. The dose detecting adjusted adrenal weight changes in the females was also lowered with the combined Subgroups from 1000 to 400 mg/kg/d.

Summary Genistein

28. Neither study unequivocally identified genistein as having estrogenic activity based solely on relative organ and tissue weights. The male reproductive tract tissues that had clearly responded to EE in other studies did not respond with a pattern of decreases in the prostate, seminal vesicles, or other tissue weights in either GN study. In the laboratory 12 study, there was attainment of statistical significance in the uterine weight, but there was no corresponding decrease in ovarian weights. The laboratory 4 study was negative for statistically significant changes in female reproductive tract tissues.

29. The tissue weights of the genistein-dosed animals then provide modest evidence of possible endocrine activity based on the 12 study. The results are consistent with other GN studies, considering the 28-day length of the updated 407 studies and their use of sexually mature young adults. In a one-generation reproduction study in rats (Delclos et al. 2001) at doses up to 240 mg/kg/d in the male pups, the only significant change in relative tissue weights was the ventral prostate at the high dose. No changes in the male reproductive tract were observed in a multi-generation study with GN at doses up to 80 mg/kg/d (Delclos et al. 2003). No changes in uterine weights were observed in sexually mature females in either of these studies. However, in the more sensitive immature female rat, GN has increased the uterine weights in developmental studies (Casanova et al. 1999; Lewis et al. 2003). In the mouse, reproductive studies conducted in the 1950s at higher GN doses did result in reproductive impairment (East 1955, 1956), but the data for changes in organ weights of the reproductive tract were limited to the testes. In the uterotrophic assay, activity was evident in all four laboratories administering GN by oral gavage at 60 mg/kg/d (OECD 2003).

Nonylphenol

Laboratory 1 – Nonylphenol

30. The gross pathology observations indicated that the lesions were of low incidence and equally distributed among control and treated animals, so they were judged not to be treatment related.

31. The absolute weights discussed for this study are shown in Table 5A and the relative weights in Table 5B. Absolute kidney weights were significantly increased in both sexes at the high dose, absolute weights of the heart and adrenal glands were significantly decreased in females at the high dose, and absolute weights of the liver were significantly increased in males at the high dose. Relative weights of the liver and kidneys were significantly increased in both sexes at the mid- and high doses, relative weights of the thyroid were significantly increased in females at the high dose, and relative weights of the brain were significantly increased in females at the mid- and high doses.

32. No significant absolute or relative weight changes were observed in the reproductive tract of either sex.

Table 5A. Absolute Organ Weights with Nonylphenol in the Laboratory 1 study

Nonylphenol Dose (mg/kg/day)	Control	Low	Middle	High
	0	20	80	200/150
Male Body Wt Δ 28d		-1.4%	-2.5%	-1.9%
Male Body Wt Δ Necropsy		-1.2%	-4.2%	-3.1%
Liver (g)	10.54 \pm 1.06	10.30 \pm 0.93	10.82 \pm 0.68	*12.12 \pm 1.43
Subgroup A	10.53 \pm 0.95	9.96 \pm 0.95	11.08 \pm 0.62	12.61 \pm 1.60
Subgroup B	10.55 \pm 1.27	10.65 \pm 0.88	10.56 \pm 0.71	11.63 \pm 1.20
Kidneys (g)	2.113 \pm 0.228	2.147 \pm 0.145	2.240 \pm 0.130	**2.461 \pm 0.234
Subgroup A	2.214 \pm 0.203	2.098 \pm 0.091	2.212 \pm 0.106	2.52 \pm 0.324
Subgroup B	2.012 \pm 0.225	2.196 \pm 0.181	2.268 \pm 0.158	*2.402 \pm 0.098
Female Body Wt Δ at 28 d		+5.2%	-4.1%	-1.5%
Female Body Wt Δ at Necropsy		+3.8%	-7.2%	-6.9%
Liver (g)	6.983 \pm 0.632	7.53 \pm 0.770	7.025 \pm 0.826	7.618 \pm 0.962
Subgroup A	7.118 \pm 0.806	7.12 \pm 0.507	6.678 \pm 0.762	6.843 \pm 0.390
Subgroup B	6.848 \pm 0.45	*7.94 \pm 0.811	7.372 \pm 0.808	*8.238 \pm 0.810
Kidneys (g)	1.386 \pm 0.124	1.539 \pm 0.191	1.410 \pm 0.135	**1.967 \pm 0.325
Subgroup A	1.364 \pm 0.169	1.428 \pm 0.126	1.35 \pm 0.163	1.905 \pm 0.442
Subgroup B	1.408 \pm 0.070	*1.65 \pm 0.188	1.47 \pm 0.073	**2.016 \pm 0.241
Heart (g)	0.715 \pm 0.066	0.742 \pm 0.081	0.669 \pm 0.050	*0.653 \pm 0.063
Subgroup A	0.706 \pm 0.073	0.732 \pm 0.079	0.636 \pm 0.044	0.640 \pm 0.036
Subgroup B	0.724 \pm 0.066	0.752 \pm 0.091	0.702 \pm 0.031	0.664 \pm 0.082
Adrenals (mg)	78.6 \pm 5.4	84.5 \pm 9.13	75.6 \pm 7.72	*71.78 \pm 6.83
Subgroup A	77.2 \pm 5.63	81.2 \pm 4.82	73.6 \pm 9.42	70.0 \pm 6.38
Subgroup B	80.0 \pm 5.39	87.8 \pm 11.71	77.6 \pm 5.94	73.2 \pm 7.56

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

Table 5B. Relative Organ Weights with Nonylphenol in the Laboratory 1 Study

Nonylphenol Dose (mg/kg/day)	Control	Low	Middle	High
	0	20	80	200/150
Male Body Wt Δ 28d		-1.4%	-2.5%	-1.9%
Male Body Wt Δ Necropsy		-1.2%	-4.2%	-3.1%
Liver	3.52%	3.48%	*3.77%	**4.17%
Subgroup A	3.45%	3.44%	*3.84%	**4.18%
Subgroup B	3.58%	3.51%	3.69%	*4.16%
Kidneys	0.70%	0.72%	**0.78%	**0.85%
Subgroup A	0.73%	0.73%	0.77%	*0.83%
Subgroup B	0.68%	0.73%	*0.79%	**0.86%
Epididymes	0.267%	0.276%	0.272%	0.257%
Subgroup A	0.254%	0.283%	0.282%	0.256%
Subgroup B	0.281%	0.270%	0.262%	0.258%
Whole prostate	0.198%	0.226%	0.205%	0.182%
Subgroup A	0.193%	0.222%	0.218%	0.179%
Subgroup B	0.203%	0.230%	0.191%	0.185%
Seminal vesicle	0.252%	0.269%	0.260%	0.214%
Subgroup A	0.251%	0.272%	0.284%	0.207%
Subgroup B	0.255%	0.266%	0.236%	0.221%
Female Body Wt Δ at 28 d		+5.2%	-4.1%	-1.5%
Female Body Wt Δ at Necropsy		+3.8%	-7.2%	-6.9%
Liver	3.55%	3.69%	*3.85%	**4.16%
Subgroup A	3.63%	3.65%	3.70%	3.88%
Subgroup B	3.47%	3.73%	*4.00%	**4.37%
Kidneys	0.70%	0.75%	*0.77%	*1.07%
Subgroup A	0.70%	0.73%	0.75%	1.08%
Subgroup B	0.71%	0.78%	*0.80%	**1.07%
Heart	0.36%	0.36%	0.37%	0.36%
Subgroup A	0.36%	0.38%	0.35%	0.36%
Subgroup B	0.37%	0.35%	0.38%	0.35%
Adrenals	0.040%	0.041%	0.041%	0.039%
Subgroup A	0.039%	0.042%	0.041%	0.040%
Subgroup B	0.041%	0.041%	0.042%	0.039%
Brain	0.909%	0.883%	*0.965%	**0.982%
Subgroup A	0.906%	0.923%	0.977%	1.023%
Subgroup B	0.911%	0.844%	0.953%	0.950%
Spleen	0.269%	0.238%	0.261%	0.261%
Subgroup A	0.279%	*0.223%	0.272%	0.247%
Subgroup B	0.258%	0.254%	0.251%	0.273%
Thyroid	0.007%	0.007%	0.008%	*0.008%
Subgroup A	0.007%	0.007%	0.008%	0.007%
Subgroup B	0.007%	0.007%	0.008%	0.009%
Ovary	0.040%	0.041%	0.045%	0.040%
Subgroup A	0.041%	0.041%	0.041%	0.038%
Subgroup B	0.039%	0.041%	0.048%	0.042%
Uterus + cervix	0.261%	0.276%	0.234%	0.230%
Subgroup A	0.233%	0.268%	0.224%	0.235%
Subgroup B	0.286%	0.284%	0.242%	0.228%

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

33. The combined Subgroups alone detected significant changes in the male liver, female heart, and female adrenal absolute weights. In several instances, such as the relative male liver and kidney changes at

the mid-dose and female relative liver and kidney changes, only one individual Subgroup achieved statistical significance. The LOEL, based on liver and kidney relative weight changes, would not have been decreased by using the combined Subgroups compared to Subgroup B, but would have been decreased relative to Subgroup A. In this study, clear evidence of endocrine activity was not detected from the organ weights.

Laboratory 6 – Nonylphenol

34. The gross pathology observations were: small prostate and/or seminal vesicles in five males at the high dose (300/250-mg/kg/day); and large and/or discoloured kidneys in the males at the high dose. Other observations were low in incidence and were not considered to be treatment related.

35. The absolute weights discussed for this study are shown in Table 6A and the relative weights in Table 6B. Absolute and relative kidney weights were significantly increased in both sexes at the high dose. Absolute and relative liver weights were significantly increased in females at the high dose, and relative liver weights were significantly increased in males at the high dose. Adrenal weights were significantly increased in both sexes at the high dose, and thymus weights were significantly decreased in males at all doses and in females at the high dose. Heart and spleen weights were significantly decreased in males at the high dose. The significant decrease in body weight appears related to the increase in relative weights of the brain, pituitary and testes, and the reduced intensity of the relative differences versus absolute differences in several other tissues (e.g., spleen and heart).

36. In the male reproductive tract, absolute seminal vesicle, prostate, and dorsolateral prostate weights were significantly decreased at the high dose. When the body weight loss is considered, while the relative weight values do decrease with dose from 15 to 21%, none of the tissues achieved statistical significance.

37. In the female reproductive tract, there were no increases in the uterine absolute or relative weights or decreases in the ovarian absolute or relative weights.

Table 6A. Absolute Organ Weights with Nonylphenol in the Laboratory 6 Study

Nonylphenol Dose (mg/kg/day)	Control	Low	Middle	High
	0	20	80	300/250
Male Body Wt Δ 28d		-6.4%	-7.6%	↓↓ -16.1%
Male Body Wt Δ Necropsy		-4.3%	-5.5%	↓↓ -16.1%
Kidneys (g)	3.00 ± 0.20	2.94 ± 0.19	2.98 ± 0.20	**4.13 ± 0.99
Subgroup A	3.08 ± 0.15	2.82 ± 0.14	2.92 ± 0.22	3.82 ± 0.69
Subgroup B	2.92 ± 0.23	3.06 ± 0.16	3.04 ± 0.17	**4.45 ± 1.22
Heart (g)	1.40 ± 0.11	1.35 ± 0.10	1.33 ± 0.09	**1.12 ± 0.12
Subgroup A	1.44 ± 0.05	1.29 ± 0.09	1.38 ± 0.05	**1.11 ± 0.11
Subgroup B	1.35 ± 0.14	1.41 ± 0.07	1.29 ± 0.11	*1.13 ± 0.14
Spleen (g)	0.76 ± 0.09	0.74 ± 0.13	0.68 ± 0.05	**0.57 ± 0.11
Subgroup A	0.76 ± 0.08	0.68 ± 0.10	0.68 ± 0.04	**0.53 ± 0.10
Subgroup B	0.75 ± 0.10	0.81 ± 0.13	0.68 ± 0.06	0.60 ± 0.12
Adrenals (g)	0.055 ± 0.003	0.058 ± 0.007	0.057 ± 0.007	**0.080 ± 0.011
Subgroup A	0.055 ± 0.004	0.056 ± 0.007	0.061 ± 0.006	**0.076 ± 0.005
Subgroup B	0.054 ± 0.001	0.059 ± 0.008	0.053 ± 0.005	*0.080 ± 0.011
Thymus (g)	0.680 ± 0.146	*0.524 ± 0.124	**0.508 ± 0.089	**0.344 ± 0.105
Subgroup A	0.652 ± 0.062	**0.446 ± 0.108	0.524 ± 0.087	**0.310 ± 0.068
Subgroup B	0.708 ± 0.205	0.603 ± 0.087	0.491 ± 0.098	*0.378 ± 0.132
Seminal Vesicle (g)	1.368 ± 0.153	1.409 ± 0.311	1.285 ± 0.261	**0.898 ± 0.331
Subgroup A	1.474 ± 0.126	1.511 ± 0.220	1.134 ± 0.198	**0.972 ± 0.326
Subgroup B	1.263 ± 0.094	1.308 ± 0.378	1.437 ± 0.237	0.824 ± 0.356
Prostate (g) - whole	0.402 ± 0.084	0.401 ± 0.078	0.397 ± 0.113	*0.266 ± 0.123
Subgroup A	0.391 ± 0.104	0.385 ± 0.076	0.404 ± 0.067	0.322 ± 0.119
Subgroup B	0.414 ± 0.069	0.418 ± 0.085	0.390 ± 0.156	*0.210 ± 0.109
Ventral Prostate (g)	0.0396 ± 0.016	0.0368 ± 0.019	0.0419 ± 0.021	0.0280 ± 0.011
Subgroup A	0.0347 ± 0.010	0.0317 ± 0.009	0.0531 ± 0.025	0.0303 ± 0.015
Subgroup B	0.0444 ± 0.020	0.0419 ± 0.026	0.0307 ± 0.009	0.0256 ± 0.005
Dorsolateral Prostate (g)	0.340 ± 0.096	0.339 ± 0.099	0.350 ± 0.090	*0.223 ± 0.100
Subgroup A	0.369 ± 0.125	0.337 ± 0.102	0.353 ± 0.083	0.257 ± 0.061
Subgroup B	0.311 ± 0.056	0.340 ± 0.107	0.347 ± 0.106	0.190 ± 0.126
Female Body Wt Δ at 28 d		+2.9%	-0.5%	+1.3%
Female Body Wt Δ at Necropsy		+1.8%	-1.4%	-0.2%
Liver (g)	8.80 ± 0.67	9.11 ± 0.46	9.31 ± 0.80	**10.74 ± 1.54
Subgroup A	8.70 ± 0.47	9.201 ± 0.35	9.72 ± 0.72	**10.50 ± 1.13
Subgroup B	8.89 ± 0.87	9.01 ± 0.57	8.91 ± 0.73	*10.93 ± 1.91
Kidneys (g)	1.74 ± 0.11	1.98 ± 0.17	1.74 ± 0.15	**2.21 ± 0.26
Subgroup A	1.77 ± 0.08	1.90 ± 0.16	1.81 ± 0.11	*2.12 ± 0.24
Subgroup B	1.70 ± 0.14	1.90 ± 0.20	1.66 ± 0.14	**2.28 ± 0.29
Adrenals (g)	0.064 ± 0.013	0.067 ± 0.011	0.069 ± 0.007	*0.085 ± 0.014
Subgroup A	0.062 ± 0.009	0.066 ± 0.012	0.071 ± 0.007	0.072 ± 0.015
Subgroup B	0.065 ± 0.017	0.068 ± 0.011	0.067 ± 0.007	*0.087 ± 0.011
Thymus (g)	0.476 ± 0.077	0.514 ± 0.073	0.422 ± 0.100	*0.370 ± 0.049
Subgroup A	0.493 ± 0.065	0.492 ± 0.078	0.448 ± 0.050	0.381 ± 0.042
Subgroup B	0.459 ± 0.091	0.537 ± 0.068	0.395 ± 0.134	0.361 ± 0.058

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

Table 6B. Relative Organ Weights with Nonylphenol in the Laboratory 6 Study

Nonylphenol Dose (mg/kg/day)	Control	Low	Middle	High
	0	20	80	300/250
Male Body Wt Δ 28d		-6.4%	-7.6%	↓↓ -16.1%
Male Body Wt Δ Necropsy		-4.3%	-5.5%	↓↓ -16.1%
Liver	4.02%	3.95%	3.90%	*4.44%
Subgroup A	4.01%	3.87%	3.88%	4.65%
Subgroup B	4.02%	4.03%	3.93%	4.23%
Kidneys	0.75%	0.77%	0.79%	**1.23%
Subgroup A	0.75%	0.76%	0.74%	*1.13%
Subgroup B	0.75%	0.77%	0.83%	**1.33%
Heart	0.35%	0.35%	0.35%	0.33%
Subgroup A	0.35%	0.35%	0.35%	0.33%
Subgroup B	0.35%	0.36%	0.35%	0.34%
Brain	0.48%	0.49%	0.50%	*0.55%
Subgroup A	0.46%	0.49%	0.49%	0.53%
Subgroup B	0.49%	0.48%	0.51%	0.56%
Spleen	0.19%	0.19%	0.18%	0.17%
Subgroup A	0.19%	0.18%	0.17%	0.16%
Subgroup B	0.19%	0.20%	0.19%	0.18%
Adrenals	0.0137%	0.0151%	0.0150%	**0.0238%
Subgroup A	0.0134%	0.0151%	0.0155%	**0.0225%
Subgroup B	0.0138%	0.0149%	0.0145%	*0.0253%
Pituitary	0.0022%	0.0021%	0.0024%	0.0027%
Subgroup A	0.0022%	0.0024%	0.0023%	0.0030%
Subgroup B	0.0023%	0.0020%	0.0022%	0.0027%
Thymus	0.17%	0.14%	*0.13%	**0.10%
Subgroup A	0.16%	*0.12%	0.13%	**0.09%
Subgroup B	0.18%	0.15%	0.13%	*0.11%
Testes	0.84%	0.79%	0.89%	*0.96%
Subgroup A	0.79%	0.87%	0.87%	**1.00%
Subgroup B	0.88%	0.71%	0.90%	0.92%
Seminal Vesicle	0.341%	0.367%	0.339%	0.267%
Subgroup A	0.359%	0.406%	0.289%	0.288%
Subgroup B	0.323%	0.331%	0.394%	0.245%
Prostate – Whole, Fixed	0.100%	0.105%	0.105%	0.079%
Subgroup A	0.095%	0.104%	0.103%	0.095%
Subgroup B	0.011%	0.106%	0.107%	0.062%
Ventral Prostate	0.010%	0.010%	0.011%	0.008%
Subgroup A	0.008%	0.009%	0.014%	0.009%
Subgroup B	0.011%	0.011%	0.008%	0.008%
Dorsolateral Prostate	0.085%	0.088%	0.092%	0.066%
Subgroup A	0.090%	0.091%	0.090%	0.076%
Subgroup B	0.079%	0.086%	0.095%	0.057%
Female Body Wt Δ at 28 d		+2.9%	-0.5%	+1.3%
Female Body Wt Δ at Necropsy		+1.8%	-1.4%	-0.2%
Liver	3.70%	3.77%	*3.97%	**4.53%
Subgroup A	3.77%	3.76%	*3.86%	**4.57%
Subgroup B	3.67%	3.73%	3.92%	**4.58%
Kidneys	0.73%	0.79%	0.74%	**0.93%
Subgroup A	0.76%	0.79%	0.75%	**0.90%
Subgroup B	0.70%	0.79%	0.73%	**0.95%

Table 6B continued. Relative Organ Weights with Nonylphenol in the Laboratory 6 Study

Nonylphenol Dose (mg/kg/day)	Control	Low	Middle	High
	0	20	80	300/250
Adrenals	0.0269%	0.0277%	0.0294%	**0.0342%
Subgroup A	0.0266%	0.0273%	0.0294%	*0.0306%
Subgroup B	0.0269%	0.0281%	0.0295%	*0.0364%
Thymus	0.20%	0.21%	0.18%	*0.16%
Subgroup A	0.21%	0.20%	0.19%	*0.16%
Subgroup B	0.19%	0.22%	0.42%	0.15%
Ovary	0.039%	0.039%	0.036%	0.038%
Subgroup A	0.039%	0.038%	0.037%	0.032%
Subgroup B	0.039%	0.040%	0.035%	0.042%
Uterus + cervix	0.223%	0.208%	0.202%	0.229%
Subgroup A	0.247%	0.211%	0.203%	0.235%
Subgroup B	0.219%	0.208%	0.208%	0.227%

*, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively).

38. In the cases of the male heart, adrenals, and thymus and the female liver and kidneys, absolute changes were significant in the combined Subgroups and both individual Subgroups. Taking the decreases in body weights into account, relative changes in male tissues were typically seen in the combined Subgroups and only one individual Subgroup. In the male reproductive tract, absolute changes were seen in the combined Subgroups and one individual Subgroup (seminal vesicles, whole prostate, and ventral prostate) or only the combined Subgroups. When decreases in male body weight were taken into account, none of these male reproductive tract tissues in the combined Subgroups or any of the individual Subgroups achieved statistical significance.

39. The combined Subgroups would provide a dose responsive significant change in the absolute weights of the male thymus beginning at 20 mg/kg/d and in relative weights of the male thymus at 80 mg/kg/d, that would not have been unequivocal or detected, respectively, in the case of the individual Subgroups.

Summary – Nonylphenol

40. As the two studies employed different doses of nonylphenol at the high dose, a possible dose responsive result needs to be considered as well as the significant decreases in body weights. The laboratory 6 study, with the higher doses, did observe statistically significant absolute decreases in several estrogen-sensitive tissues in the male reproductive, whereas the laboratory 1 study did not. This might be interpreted as evidence for a borderline estrogenic effect. However, none of the estrogen-sensitive tissues in the male and the female reproductive tracts were changed on a relative basis.

41. Previous studies have consistently noted changes in the kidney weights, particularly for males, administered NP. Other studies have not recorded significant changes in the weights of any tissues of the male reproductive tract, including updated 407 studies (Aso et al. 2000), 90-day studies (Cunny et al. 1997), or reproductive studies with NP (Chapin et al. 1999; Nagao et al. 2001) or the related octylphenol (Tyl et al. 1999). Similarly, other studies have recorded significant differences in the adult females only in decreased ovarian weights with extended exposure of the animals (Chapin et al. 1999; Nagao et al. 2001). Changes in uterine weights have not been observed in adult animals, but only in the more sensitive immature females (Chapin et al. 1999). Thus, the updated 407 study results with NP are consistent with the available studies in all respects.

Tamoxifen

Laboratory 3 – Tamoxifen

42. Gross pathology observations included decreased sizes of the prostate and seminal vesicles in the males at the high dose and decreased sizes of the uterus in females at the high dose.

43. The absolute weights discussed for this study are shown in Table 7A and the relative weights in Table 7B. Absolute brain weights were significantly decreased in males at the high dose. Absolute adrenal, heart, kidney, and liver weights were significantly decreased in females at the high dose. When expressed relative to body weight, the adrenals, brain, heart, and kidneys were increased in males at the high dose, and the brain was increased in females at the high dose.

44. In the male reproductive tract, the absolute weights of the accessory sex organs (ventral and dorsolateral prostate, epididymis, and seminal vesicles with coagulating glands) and, in the HPV axis, the pituitary were all significantly decreased at the high dose, and the epididymis also at the mid-dose (most changes were $p < 0.01$, see Table 7). For the relative weights, the testes were significantly increased at the high dose; the pituitary, epididymis, and dorsolateral prostate were not significant at the high dose, and the ventral prostate and seminal vesicles were significantly decreased at the high dose.

45. In the female reproductive tract, the absolute weights of pituitary, ovaries, and uterus with cervix were significantly decreased at the high dose. The uterus with cervix was also significantly decreased at the mid-dose, but significantly increased at the low dose. For the relative weights, the pituitary and ovary were not significantly decreased at the high dose, although ovarian relative weights were clearly in a downward trend. The uterus and cervix relative weights were significantly decreased at the high dose.

Table 7A. Absolute Organ Weights with Tamoxifen in the Laboratory 3 Study

Tamoxifen Dose ($\mu\text{g}/\text{kg}/\text{day}$)	Control	Low	Middle	High
	0	5	30	200
Male Body Wt Δ 28d		+4.9%	-1.8%	$\downarrow\downarrow$ -15.4%
Male Body Wt Δ Necropsy		5.4%	-2.7%	$\downarrow\downarrow$ -15.5%
Brain (g)	2.016 \pm 0.069	2.009 \pm 0.051	2.002 \pm 0.070	*1.945 \pm 0.062
Subgroup A	2.010 \pm 0.060	1.992 \pm 0.047	2.010 \pm 0.048	1.966 \pm 0.061
Subgroup B	2.022 \pm 0.084	2.026 \pm 0.055	1.994 \pm 0.092	1.924 \pm 0.061
Pituitary (g)	0.009 \pm 0.002	0.009 \pm 0.002	0.009 \pm 0.002	*0.006 \pm 0.001
Subgroup A	0.009 \pm 0.001	0.009 \pm 0.001	0.010 \pm 0.000	**0.006 \pm 0.001
Subgroup B	0.008 \pm 0.002	0.009 \pm 0.002	0.009 \pm 0.002	0.007 \pm 0.001
Epididymis (g)	0.551 \pm 0.050	0.523 \pm 0.037	**0.492 \pm 0.023	**0.461 \pm 0.032
Subgroup A	0.542 \pm 0.067	0.524 \pm 0.038	0.490 \pm 0.020	*0.462 \pm 0.036
Subgroup B	0.560 \pm 0.029	0.522 \pm 0.041	*0.494 \pm 0.029	**0.460 \pm 0.033
Ventral prostate (g)	0.431 \pm 0.118	0.457 \pm 0.056	0.410 \pm 0.092	**0.227 \pm 0.050
Subgroup A	0.480 \pm 0.110	0.436 \pm 0.017	0.396 \pm 0.058	**0.224 \pm 0.047
Subgroup B	0.382 \pm 0.114	0.478 \pm 0.076	0.424 \pm 0.123	0.230 \pm 0.058
Dorsolateral prostate (g)	0.285 \pm 0.123	0.233 \pm 0.066	0.222 \pm 0.072	**0.168 \pm 0.054
Subgroup A	0.276 \pm 0.075	0.196 \pm 0.062	0.232 \pm 0.098	0.198 \pm 0.051
Subgroup B	0.294 \pm 0.169	0.270 \pm 0.049	0.212 \pm 0.043	*0.138 \pm 0.042
Seminal vesicle (g)	1.049 \pm 0.213 ⁷	1.110 \pm 0.104	0.870 \pm 0.215 ⁸	**0.613 \pm 0.088
Subgroup A	0.970 \pm 0.248 ³	1.128 \pm 0.123	0.790 \pm 0.286 ⁴	*0.604 \pm 0.061
Subgroup B	1.153 \pm 0.121 ⁴	1.092 \pm 0.091	0.950 \pm 0.094 ⁴	**0.622 \pm 0.116

Table 7A (continued). Absolute Organ Weights with Tamoxifen in the Laboratory 3 Study

Female Body Wt Δ at 28 d		-3.6%	\downarrow -7.1%	$\downarrow\downarrow$ -12.9%
Female Body Wt Δ at necropsy		-4.4%	\downarrow -7.5%	$\downarrow\downarrow$ -13.0%
Liver (g)	9.078 \pm 0.845 ⁹	*8.250 \pm 0.532	8.367 \pm 0.721 ⁹	**7.789 \pm 0.481 ⁹
Subgroup A	8.800 \pm 0.566 ⁴	8.180 \pm 0.683	8.925 \pm 0.670 ⁴	*7.550 \pm 0.520 ⁴
Subgroup B	9.300 \pm 1.025	8.320 \pm 0.396	**7.920 \pm 0.377	**7.980 \pm 0.396
Heart (g)	0.918 \pm 0.067 ⁹	0.944 \pm 0.087	0.881 \pm 0.045 ⁹	**0.796 \pm 0.042 ⁹
Subgroup A	0.935 \pm 0.052 ⁴	0.908 \pm 0.087	0.913 \pm 0.017 ⁴	*0.838 \pm 0.010 ⁴
Subgroup B	0.904 \pm 0.080	0.980 \pm 0.079	0.856 \pm 0.046	**0.762 \pm 0.018
Kidney (g)	1.898 \pm 0.148 ⁹	1.834 \pm 0.131	1.812 \pm 0.134 ⁹	**1.678 \pm 0.099 ⁹
Subgroup A	1.808 \pm 0.038 ⁴	1.768 \pm 0.153	1.820 \pm 0.145 ⁴	1.663 \pm 0.087 ⁴
Subgroup B	1.970 \pm 0.167	1.900 \pm 0.068	1.816 \pm 0.141	**1.690 \pm 0.116
Adrenals (g)	0.069 \pm 0.008 ⁹	0.063 \pm 0.006	0.062 \pm 0.007 ⁹	**0.056 \pm 0.007 ⁹
Subgroup A	0.068 \pm 0.006 ⁴	0.061 \pm 0.006	0.062 \pm 0.002 ⁴	0.059 \pm 0.008 ⁴
Subgroup B	0.070 \pm 0.010	0.065 \pm 0.007	0.063 \pm 0.009	*0.054 \pm 0.006
Pituitary (g)	0.009 \pm 0.001 ⁸	0.009 \pm 0.002	0.008 \pm 0.001 ⁹	*0.007 \pm 0.002 ⁹
Subgroup A	0.010 \pm 0.001 ⁴	0.009 \pm 0.002	0.009 \pm 0.001 ⁴	0.007 \pm 0.001 ⁴
Subgroup B	0.009 \pm 0.002 ⁴	0.009 \pm 0.002	0.007 \pm 0.001	0.007 \pm 0.002
Ovary (g)	0.093 \pm 0.031 ⁹	0.077 \pm 0.013	0.077 \pm 0.008 ⁹	**0.059 \pm 0.011 ⁸
Subgroup A	0.107 \pm 0.039 ⁴	*0.069 \pm 0.012	0.079 \pm 0.011 ⁴	**0.061 \pm 0.007 ⁴
Subgroup B	0.082 \pm 0.019	0.084 \pm 0.009	0.076 \pm 0.007	*0.058 \pm 0.015 ⁴
Uterus (g)	0.379 \pm 0.053 ⁹	**0.509 \pm 0.197	*0.316 \pm 0.046 ⁹	**0.243 \pm 0.033 ⁹
Subgroup A	0.360 \pm 0.037 ⁴	*0.512 \pm 0.212	0.305 \pm 0.031 ⁴	*0.233 \pm 0.015 ⁴
Subgroup B	0.394 \pm 0.063	0.506 \pm 0.205	0.324 \pm 0.057	*0.252 \pm 0.042

Values were expressed as mean \pm SD. Superscript indicates number of animals, if not 10 for the combined Subgroups or 5 for the individual Subgroups. *, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively).

Table 7B. Relative Organ Weights with Tamoxifen in the Laboratory 3 Study

Tamoxifen Dose ($\mu\text{g}/\text{kg}/\text{day}$)	Control	Low	Middle	High
	0	5	30	200
Male Body Wt Δ 28d		+4.9%	-1.8%	$\downarrow\downarrow$ -15.4%
Male Body Wt Δ Necropsy		+5.4%	-2.7%	$\downarrow\downarrow$ -15.5%
Kidney	0.68%	*0.64%	0.68%	**0.75%
Subgroup A	0.67%	0.64%	0.68%	*0.75%
Subgroup B	0.69%	*0.64%	0.68%	0.74%
Adrenal	0.0126%	0.0124%	0.0145%	**0.0176%
Subgroup A	0.0130%	0.0123%	0.0150%	0.0167%
Subgroup B	0.0125%	0.0124%	0.0137%	**0.0184%
Heart	0.30%	0.31%	*0.32%	**0.34%
Subgroup A	0.30%	0.31%	0.32%	0.33%
Subgroup B	0.30%	0.31%	0.32%	0.35%
Brain	0.49%	0.46%	0.50%	**0.56%
Subgroup A	0.49%	0.47%	0.51%	**0.57%
Subgroup B	0.50%	0.46%	0.49%	*0.55%
Pituitary	0.0022%	0.0021%	0.0022%	0.0017%
Subgroup A	0.0022%	0.0021%	0.0025%	0.0017%
Subgroup B	0.0020%	0.0020%	0.0022%	0.0020%
Testes	0.86%	0.81%	0.87%	*0.95%
Subgroup A	0.82%	0.85%	0.89%	*0.97%
Subgroup B	0.90%	0.78%	0.85%	0.93%

Table 7B continued. Relative Organ Weights with Tamoxifen in the Laboratory 3 Study

Tamoxifen Dose ($\mu\text{g}/\text{kg}/\text{day}$)	Control	Low	Middle	High
	0	5	30	200
Epididymis	0.134%	**0.121%	0.123%	0.133%
Subgroup A	0.131%	0.124%	0.125%	0.133%
Subgroup B	0.137%	*0.117%	0.121%	0.132%
Ventral prostate	0.105%	0.105%	0.102%	**0.065%
Subgroup A	0.116%	0.103%	0.101%	**0.065%
Subgroup B	0.094%	0.107%	0.104%	0.066%
Dorsolateral prostate	0.069%	0.054%	0.055%	0.048%
Subgroup A	0.067%	0.046%	0.059%	0.057%
Subgroup B	0.072%	0.061%	0.052%	0.040%
Seminal vesicle	0.255% ⁷	0.256%	0.217% ⁸	**0.176%
Subgroup A	0.234% ³	0.267%	0.201% ⁴	0.174%
Subgroup B	0.283% ⁴	0.245%	0.233% ⁴	**0.179%
Female Body Wt Δ at 28 d		-3.6%	\downarrow -7.1%	$\downarrow\downarrow$ -12.9%
Female Body Wt Δ at Necropsy		-4.4%	\downarrow -7.5%	$\downarrow\downarrow$ -13.0%
Liver	3.52% ⁹	3.35%	3.51% ⁹	3.47% ⁹
Subgroup A	3.36%	3.37%	3.59%	3.37%
Subgroup B	3.66% ⁴	3.33%	3.44% ⁴	3.55% ⁴
Kidney	0.74% ⁹	0.74%	0.76% ⁹	0.75% ⁹
Subgroup A	0.69%	0.73%	0.73%	0.74%
Subgroup B	0.78% ⁴	0.76%	0.78% ⁴	0.75% ⁴
Heart	0.36% ⁹	0.38%	0.37% ⁹	0.36% ⁹
Subgroup A	0.36%	0.37%	0.37%	0.37%
Subgroup B	0.36% ⁴	0.39%	0.37% ⁴	0.34% ⁴
Brain	0.73% ⁹	0.75%	0.79% ⁹	**0.83% ⁹
Subgroup A	0.71%	0.75%	0.75%	0.83%
Subgroup B	0.74% ⁴	0.75%	*0.82% ⁴	*0.82% ⁴
Adrenals	0.0268% ⁹	0.0256%	0.0260% ⁹	0.0250% ⁹
Subgroup A	0.0260%	0.0251%	0.0249%	0.0264%
Subgroup B	0.0275% ⁴	0.0260%	0.0274% ⁴	0.0240% ⁴
Thymus	0.23% ⁹	0.25%	0.24% ⁹	*0.29% ⁹
Subgroup A	0.23%	0.25%	0.23%	0.33%
Subgroup B	0.23%	0.24%	0.26%	0.27%
Pituitary	0.0035% ⁸	0.0037%	0.0034% ⁹	0.0031% ⁹
Subgroup A	0.0038% ⁴	0.0037%	0.0036%	0.0031%
Subgroup B	0.0038% ⁴	0.0037%	0.0036% ⁴	0.0031% ⁴
Ovary	0.036% ⁹	0.031%	0.032% ⁹	0.026% ⁸
Subgroup A	0.041% ⁴	0.028%	0.032% ⁴	0.027% ⁴
Subgroup B	0.032%	0.034%	0.033%	0.026% ⁴
Uterus	0.147% ⁹	*0.207%	0.133% ⁹	**0.108% ⁹
Subgroup A	0.137% ⁴	**0.211%	0.123% ⁴	0.104% ⁴
Subgroup B	0.155%	0.202%	0.141%	*0.108%

Values were expressed as mean \pm SD. Superscript indicates number of animals, if not 10 for the combined Subgroups or 5 for the individual Groups. *, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively).

46. The laboratory 3 final report notes that the statistically significant uterine weights at 5 $\mu\text{g}/\text{kg}/\text{d}$ dose in the combined Subgroups and Subgroup A depended upon values from two individuals that were sacrificed during proestrous and not diestrous. Therefore, these results cannot be considered to be a treatment-related effect under the study protocol.

47. Significant body weight losses at the high doses need to be taken into account in both sexes by assessing both absolute and relative weights. In males, the kidneys and adrenals appear to have increased relative to body weights as did organs that tend to be conserved such as brain and testes. In females, significant absolute organ weight changes were not apparent in relative weights, except for the increases in the brain. In both sexes, the significant relative changes in combined Subgroups were often not present in one of the individual Subgroups, although trends in the values were evident.

48. In the male reproductive tract, significant relative decreases in the ventral prostate and seminal vesicles in the combined Subgroups were not present in one of the individual Subgroups. In the female reproductive tract, the partial agonist/antagonist activity of tamoxifen may have contributed to a significant rise in relative weights of the uterus at the low dose and a significant decrease at the high dose. This statistical significance in the combined Subgroups was again not seen in one of the individual Subgroups. Therefore, the combined Subgroups do appear to increase the statistical power of the study in several important respects.

49. Compared to the individual Subgroups, the combined Subgroups might lower the LOEL to 30 µg/kg/d versus Subgroup A due to absolute epididymal weight, would give a more consistent pattern of relative weight changes in the male accessory reproductive tissues, and would detect relative uterine changes at 200 µg/mg/d, where Subgroup A would not, and at a higher degree of significance than Subgroup B. Therefore, the combined Subgroups again appear to increase the statistical power of the study.

Laboratory 10 – Tamoxifen

50. No gross pathology observations or findings were recorded in the final report.

51. The absolute weights discussed for this study are shown in Table 8A and the relative weights in Table 8B. The absolute and relative prostate weights decreased significantly at all doses. Both the right ovary and left ovary weights decreased significantly at the high dose, and the uterine weights decreased significantly at all doses. The relative weights of several organs and tissues whose size are typically conserved (e.g., brain) were increased in males at the high dose, where the body weights had decreased. In the male reproductive tract, the relative testes and both right and left epididymis weights increased at high dose. However, seminal vesicle and coagulating gland and thyroid weights were not recorded in the final report received.

Table 8A. Absolute Organ Weights with Tamoxifen in the Laboratory 10 Study

Tamoxifen Dose (µg/kg/day)	Control	Low	Middle	High
	0	5	30	200
Male Body Wt Δ at Necropsy		-7.9%	-6.6%	↓ -19.4%
Prostate (g)	1.06 ± 0.12	*0.73 ± 0.09	*0.61 ± 0.21	*0.47 ± 0.04
Female Body Wt Δ at Necropsy		+0.3%	-6.2%	↓ -14.3%
Ovary (left) (g)	0.062 ± 0.012	0.053 ± 0.005	0.050 ± 0.014	*0.045 ± 0.005
Ovary (right) (g)	0.080 ± 0.017	*0.057 ± 0.005	*0.063 ± 0.005	*0.042 ± 0.012
Uterus (g)	0.38 ± 0.09	0.39 ± 0.12	0.34 ± 0.08	*0.20 ± 0.02

Values expressed as mean ± SD. *, Significantly different from control (p<0.05) – p<0.01 was not analysed

Table 8B. Relative Organ Weights with Tamoxifen in the Laboratory 10 Study

Tamoxifen Dose ($\mu\text{g}/\text{kg}/\text{day}$)	Control	Low	Middle	High
	0	5	30	200
Male Body Wt Δ at Necropsy		-7.9%	-6.6%	\downarrow -19.4%
Heart	0.30%	0.39%	0.32%	*0.34%
Testes	0.69%	0.79%	0.74%	*0.89%
Epididymes	0.217%	0.262%	0.250%	*0.272%
Prostate	0.25%	*0.19%	*0.16%	*0.14%
Female Body Wt Δ at Necropsy		+0.3%	-6.2%	\downarrow -14.3%
Ovary (combined)	0.055%	0.042%	0.047%	*0.039%
Uterus	0.015%	0.015%	0.014%	*0.009%

Values expressed as mean \pm SD. *, Significantly different from control ($p < 0.05$) – $p < 0.01$ was not analysed.

52. The laboratory 10 studies used only a single group size of 6 animals and did not study the impact of Subgroup combination on power.

Summary – Tamoxifen

53. The patterns observed from the administration of tamoxifen in the two studies were similar. Trends in major organs were similar, taking body weight losses into account. In males, although the prostate dissection was apparently not the same, absolute and relative weights of prostate tissues declined in both studies. The ventral prostate relative declines were significant in the laboratory 3 study, and the whole prostate relative declines were significant in the laboratory 10 study. In females, the absolute and relative uterine weights were significantly decreased at the high dose in both studies. Ovarian weights were significantly decreased at the high dose in the laboratory 10 study, while decreased absolute weights did not achieve statistical significance in the laboratory 3 study.

54. Both studies clearly identify significant tissue weight changes in the male and the female reproductive tracts at tamoxifen doses of 200 $\mu\text{g}/\text{kg}/\text{d}$ that would lead to suspicion of endocrine activity. Therefore, with these tissue weight endpoints, the updated 407 was successful in identifying the possible activity of tamoxifen.

CGS 18320B

Laboratory 8 – CGS 18320B

55. Gross pathology observations, attributable to treatment and dose related, included males with enlarged adrenals at the high dose and females with enlarged adrenals at the high dose, cystic ovaries at all doses, and apparent atrophy of the uterus at the high dose.

56. The absolute weights discussed for this study are shown in Table 9A and the relative weights in Table 9B. Absolute adrenal weights were increased, but not significantly in males at the high dose. Relative weights of brain, liver, testes, adrenals, and thyroid were significantly higher in males at the high dose where the mean body weight was decreased about 10%. The liver, heart, kidney, spleen and thymus weights were significantly increased in females at the mid- and high doses. With 25-33% increases in body weights, the relative weights of these same organs were significantly increased in females at the high dose.

57. In the reproductive tracts, the absolute weights of seminal vesicles, whole prostate, and ventral prostate were significantly increased at the high dose, the mid- and high dose, and all doses, respectively. Absolute

and relative ovarian weights were significantly increased at all doses. Absolute and relative uterine weights significantly decreased at the mid- and high doses.

Table 9A. Absolute Organ Weights with CBS 18320B in the Laboratory 8 Study

CGS 18320B Dose (mg/kg/day)	Control	Low	Middle	High
	0	0.3	3	30
Male Body Wt Δ 28d		-2.0%	-2.0%	-10.1%
Male Body Wt Δ Necropsy		-1.7%	-2.0%	-9.9%
Adrenals (mg)	52.5 \pm 8.0	47.2 \pm 7.8	48.2 \pm 6.2	78.9 \pm 21.7
Subgroup A	49.0 \pm 5.9	44.1 \pm 5.6	45.3 \pm 6.5	88.0 \pm 18.3
Subgroup B	56.1 \pm 8.8	50.2 \pm 9.1	51.1 \pm 4.6	67.5 \pm 22.3
Seminal vesicles (mg)	1634 \pm 218	1461 \pm 300	1421 \pm 238	*1303 \pm 158
Subgroup A	1784 \pm 196	1428 \pm 415	1396 \pm 296	1289 \pm 132
Subgroup B	1485 \pm 111	1493 \pm 165	1445 \pm 195	1321 \pm 206
Whole prostate (mg)	797.3 \pm 98.2	714.1 \pm 157.1	*658.2 \pm 122.6	**617.3 \pm 79.7
Subgroup A	790.0 \pm 124.1	695.3 \pm 208.0	650.3 \pm 56.7	640.7 \pm 56.1
Subgroup B	804.6 \pm 78.4	733.0 \pm 106.6	666.1 \pm 174.5	*588.0 \pm 103.4
Ventral prostate (mg)	639.9 \pm 123.9	*498.0 \pm 106.3	*504.9 \pm 122.9	*490.8 \pm 69.2
Subgroup A	590.5 \pm 125.4	452.5 \pm 125.1	483.1 \pm 35.5	506.4 \pm 84.6
Subgroup B	689.4 \pm 112.6	543.5 \pm 67.9	526.8 \pm 177.5	471.4 \pm 48.2
Female Body Wt Δ at 28 d		$\uparrow\uparrow$ +23.6%	$\uparrow\uparrow$ +32.8%	$\uparrow\uparrow$ +26.0%
Female Body Wt Δ at Necropsy		$\uparrow\uparrow$ +23.1%	$\uparrow\uparrow$ +32.3%	$\uparrow\uparrow$ +25.5%
Liver (g)	8.75 \pm 0.50	10.75 \pm 1.00	**12.75 \pm 1.27	**13.20 \pm 1.86
Subgroup A	8.77 \pm 0.50	11.07 \pm 0.84	**12.75 \pm 0.75	**14.00 \pm 2.28
Subgroup B	8.73 \pm 0.55	10.43 \pm 1.13	**12.74 \pm 1.76	**12.40 \pm 1.00
Heart (mg)	811 \pm 36	967 \pm 76	**1078 \pm 120	**1178 \pm 107
Subgroup A	815 \pm 46	*994 \pm 81	**1121 \pm 104	**1226 \pm 130
Subgroup B	807 \pm 28	941 \pm 68	*1036 \pm 132	**1129 \pm 53
Kidney (mg)	1703 \pm 101	1980 \pm 154	**2272 \pm 180	**2375 \pm 314
Subgroup A	1728 \pm 71	2061 \pm 123	**2310 \pm 139	**2485 \pm 388
Subgroup B	1677 \pm 127	1899 \pm 149	**2233 \pm 224	**2265 \pm 201
Adrenals (mg)	65.7 \pm 7.2	53.5 \pm 7.3	73.8 \pm 14.6	80.6 \pm 18.2
Subgroup A	68.2 \pm 8.0	52.7 \pm 6.2	81.1 \pm 16.5	85.7 \pm 17.7
Subgroup B	63.1 \pm 6.0	54.3 \pm 8.9	66.5 \pm 8.7	75.5 \pm 19.1
Spleen (mg)	507 \pm 117	604 \pm 95	**767 \pm 130	**783 \pm 12.5
Subgroup A	536 \pm 162	643 \pm 113	*804 \pm 165	*846 \pm 142
Subgroup B	477 \pm 50	564 \pm 62	**731 \pm 87	**720 \pm 73
Thymus (mg)	459 \pm 107	496 \pm 118	**710 \pm 178	**783 \pm 206
Subgroup A	454 \pm 122	515 \pm 138	727 \pm 250	*834 \pm 260
Subgroup B	464 \pm 105	477 \pm 108	**693 \pm 88	*642 \pm 69
Ovaries (mg)	81.6 \pm 9.1	**123.2 \pm 19.2	**135.6 \pm 10.0	**142.9 \pm 24.4
Subgroup A	82.2 \pm 6.3	124.2 \pm 19.3	**141.8 \pm 8.4	*141.5 \pm 27.2
Subgroup B	81.0 \pm 12.0	**122.2 \pm 21.4	**129.5 \pm 7.7	**144.3 \pm 24.5
Uterus + cervix (mg)	386 \pm 36	292 \pm 56	**192 \pm 23	**173 \pm 19
Subgroup A	389 \pm 48	317 \pm 69	*199 \pm 16	**171 \pm 16
Subgroup B	384 \pm 24	**266 \pm 25	**184 \pm 29	**176 \pm 22

Values were expressed as mean \pm SD.

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

Table 9B. Relative Organ Weights with CBS 18320B in the Laboratory 8 Study

CGS 18320B Dose (mg/kg/day)	Control	Low	Middle	High
	0	0.3	3	30
Male Body Wt Δ 28d		-2.0%	-2.0%	-10.1%
Male Body Wt Δ Necropsy		-1.7%	-2.0%	-9.9%
Liver	3.57%	3.48%	3.52%	**3.91%
Subgroup A	3.62%	3.50%	3.50%	*3.93%
Subgroup B	3.52%	3.47%	3.53%	3.86%
Kidney	0.66%	0.64%	0.63%	0.72%
Subgroup A	0.66%	0.65%	0.61%	0.71%
Subgroup B	0.66%	0.63%	0.65%	*0.72%
Brain	0.48%	0.52%	0.51%	**0.56%
Subgroup A	0.48%	0.52%	0.52%	*0.57%
Subgroup B	0.48%	0.51%	0.50%	0.55%
Adrenals	0.0130%	0.0119%	0.0121%	*0.0216%
Subgroup A	0.0121%	0.0113%	0.0116%	*0.0244%
Subgroup B	0.0139%	0.0124%	0.0126%	0.0181%
Thyroid	0.0059%	0.0061%	0.0060%	*0.0075%
Subgroup A	0.0060%	0.0055%	0.0055%	*0.0078%
Subgroup B	0.0058%	0.0065%	0.0066%	0.0072%
Testes	0.73%	0.78%	0.78%	**0.84%
Subgroup A	0.73%	0.76%	0.76%	0.85%
Subgroup B	0.72%	0.80%	0.80%	0.82%
Seminal vesicles	0.403%	0.367%	0.359%	0.357%
Subgroup A	0.440%	0.365%	0.357%	0.357%
Subgroup B	0.367%	0.369%	0.358%	0.355%
Ventral prostate	0.158%	*0.125%	*0.127%	0.134%
Subgroup A	0.146%	0.116%	0.124%	0.140%
Subgroup B	0.170%	0.134%	0.130%	0.127%

Table 9B continued. Relative Organ Weights with CBS 18320B in the Laboratory 8 Study

CGS 18320B Dose (mg/kg/day)	Control	Low	Middle	High
	0	0.3	3	30
Female Body Wt Δ at 28 d		↑↑ +23.6%	↑↑ +32.8%	↑↑ +26.0%
Female Body Wt Δ at Necropsy		↑↑ +23.1%	↑↑ +32.3%	↑↑ +25.5%
Liver	3.49%	3.48%	**3.84%	**4.19%
Subgroup A	3.49%	3.51%	3.75%	**4.33%
Subgroup B	3.46%	3.45%	**3.92%	**4.04%
Kidney	0.68%	0.64%	0.68%	*0.75%
Subgroup A	0.69%	0.65%	0.68%	*0.77%
Subgroup B	0.67%	0.63%	0.69%	**0.74%
Heart	0.32%	0.31%	0.32%	**0.37%
Subgroup A	0.32%	0.32%	0.33%	**0.38%
Subgroup B	0.32%	0.31%	0.32%	**0.37%
Adrenals	0.0262%	**0.0173%	0.0222%	0.0256%
Subgroup A	0.0272%	**0.0167%	0.0239%	0.0265%
Subgroup B	0.0250%	*0.0180%	0.0205%	0.0246%
Brain	0.76%	**0.62%	**0.60%	**0.64%
Subgroup A	0.77%	**0.62%	**0.59%	**0.63%
Subgroup B	0.75%	**0.63%	**0.61%	**0.65%
Spleen	0.20%	0.20%	0.23%	*0.25%
Subgroup A	0.21%	0.20%	0.24%	0.26%
Subgroup B	0.19%	0.19%	0.22%	0.23%
Pituitary	0.0064%	**0.0043%	**0.0043%	**0.0045%
Subgroup A	0.0064%	**0.0043%	**0.0043%	**0.0044%
Subgroup B	0.0063%	**0.0042%	**0.0043%	**0.0046%
Thymus	0.18%	0.16%	0.23%	*0.25%
Subgroup A	0.18%	0.16%	0.21%	0.26%
Subgroup B	0.18%	0.16%	0.21%	0.21%
Thyroid	0.0086%	*0.0068%	*0.0068%	0.0072%
Subgroup A	0.0079%	0.0065%	0.0062%	0.0065%
Subgroup B	0.0092%	0.0073%	0.0075%	0.0079%
Ovaries	0.033%	*0.040%	**0.041%	**0.045%
Subgroup A	0.033%	0.039%	0.042%	0.044%
Subgroup B	0.032%	0.040%	0.040%	*0.047%
Uterus + cervix	0.154%	0.094%	**0.058%	**0.055%
Subgroup A	0.155%	0.101%	**0.059%	**0.053%
Subgroup B	0.152%	**0.088%	**0.057%	**0.057%

Values were expressed as mean ± SD.

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

59. In males, the statistically significant effects of CGS 18320B were seen only in the combined Subgroups where significant decreases occurred in absolute weights of the seminal vesicles and ventral prostate and in relative weights of the testes. Other significant increases in relative weights were observed in the combined Subgroups and one of the two individual Subgroups in the whole prostate, liver, adrenals, and thyroid.

60. In females, statistically significant increases occurred in the absolute weights of the liver, kidney, heart, spleen, thymus, ovaries and uterus at both the mid- and high dose in the combined Subgroups and both individual Subgroups. The same was true of relative decreases in the brain and pituitary weights at all doses, the uterus weights at the mid- and high doses, the adrenal weights at the low dose, and the liver, kidney, and heart weights at the high dose. However, as the intensity of the effects decreased at lower doses. There the additional statistical power of the combined Subgroups was evident in several cases: the

relative liver weights at the mid-dose, the relative ovary weights at all doses, and the relative thyroid weights at the low and mid-dose.

Laboratory 13 – CGS 18320B

61. Gross pathology observations included some males with enlarged kidneys at the high dose and females with apparent atrophy of the uterus at both the mid- and the high dose.

62. The absolute weights discussed for this study are shown in Table 10A and the relative weights in Table 10B. Absolute adrenal weights were significantly increased in males at the mid- and high doses and in females at the high dose; relative adrenal weights were significantly increased only in the high dose males. Absolute heart weights were significantly increased in females at all doses, but relative weights were significantly increased only at the high dose in relation to the 30-40% increases in female body weights. Absolute and relative brain weights were significantly increased in females at all doses. Absolute kidney weights were significantly increased in females at all doses, and the relative weights again only at the high dose. Absolute liver weights were significantly increased in females at all doses, and the relative weights again only at the mid- and high dose. Absolute, but not relative, spleen weights were significantly increased in females at the high dose.

63. In the reproductive tracts, the absolute prostate weights were significantly decreased at all doses, and the relative weights at the mid- and high doses. The absolute and relative uterine weights were significantly decreased at all doses. The absolute ovarian weights were significantly increased at all doses, but the relative weights at no dose.

Table 10A. Absolute Organ Weights with CGS 18320B in the Laboratory 13 Study

CGS 18320B Dose (mg/kg/day)	Control	Low	Middle	High
	0	0.3	3	30
Male Body Wt Δ 28d		-5.1%	+1.8%	-2.5%
Male Body Wt Δ Necropsy		-5.3%	+1.7%	-2.6%
Adrenals (g)	0.054 \pm 0.08	0.059 \pm 0.007	*0.067 \pm 0.008	**0.093 \pm 0.017
Prostate (g)	0.381 \pm 0.076	*0.320 \pm 0.056	**0.303 \pm 0.040	**0.284 \pm 0.041
Female Body Wt Δ at 28 d		$\uparrow\uparrow$ +31.0%	$\uparrow\uparrow$ +41.2%	$\uparrow\uparrow$ +34.3%
Female Body Wt Δ at Necropsy		$\uparrow\uparrow$ +20.20%	$\uparrow\uparrow$ +28.42%	$\uparrow\uparrow$ +21.60%
Liver (g)	8.25 \pm 1.23	**10.41 \pm 0.82	**11.68 \pm 0.89	**12.09 \pm 0.87
Adrenals (g)	0.077 \pm 0.010	0.073 \pm 0.016	0.092 \pm 0.018	*0.957 \pm 0.150
Brain (g)	1.914 \pm 0.076	2.004 \pm 0.062	**2.067 \pm 0.066	**2.044 \pm 0.111
Heart (g)	0.804 \pm 0.078	*0.910 \pm 0.081	**0.975 \pm 0.107	**1.102 \pm 0.079
Kidneys (g)	1.724 \pm 0.219	*1.963 \pm 0.213	**2.225 \pm 0.157	**2.511 \pm 0.251
Spleen (g)	0.615 \pm 0.126	0.642 \pm 0.0578	0.713 \pm 0.119	**0.769 \pm 0.065
Ovaries (g)	0.184 \pm 0.028	*0.218 \pm 0.041	*0.222 \pm 0.025	0.212 \pm 0.023
Uterus plus cervix (g)	0.599 \pm 0.046	*0.490 \pm 0.125	**0.425 \pm 0.083	**0.421 \pm 0.077

Values were expressed as mean \pm SD.

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

Table 10B. Relative Organ Weights with CGS 18320B in the Laboratory 13 Study

CGS 18320B Dose (mg/kg/day)	Control	Low	Middle	High
	0	0.3	3	30
Male Body Wt Δ 28d		-5.1%	+1.8%	-2.5%
Male Body Wt Δ Necropsy		-5.3%	+1.7%	-2.6%
Liver	4.07%	4.09%	4.12%	**4.41%
Heart	0.32%	0.31%	0.32%	*0.35%
Adrenals	0.0171%	0.0196%	0.0208%	**0.0301%
Prostate	0.120%	0.106%	**0.094%	**0.092%
Female Body Wt Δ at 28 d		↑↑ +31.0%	↑↑ +41.2%	↑↑ +34.3%
Female Body Wt Δ at Necropsy		↑↑ +20.20%	↑↑ +28.42%	↑↑ +21.60%
Liver	3.86%	4.05%	**4.25%	**4.65%
Adrenals	0.0361%	*0.0282%	0.0334%	0.0368%
Brain	0.89%	**0.78%	**0.75%	**0.79%
Heart	0.38%	0.35%	0.35%	**0.42%
Kidneys	0.81%	0.76%	0.81%	*0.97%
Spleen	0.29%	0.25%	0.26%	0.30%
Ovaries	0.086%	0.085%	0.081%	0.082%
Uterus plus cervix	0.280%	**0.191%	**0.155%	**0.162%

Values were expressed as mean ± SD.

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

64. As this study did not divide the animals into two Subgroups, no observations can be made on the issue of the possible advantage of increased statistical power using combined Subgroups.

Summary – CGS 18320B

65. The findings of the two CGS18320B studies were similar in most respects. In males, similar changes in liver and adrenal weights occurred. In females, the dramatic increases in body weights at all doses need to be taken into account. Relative liver and kidney changes were similar in both studies.

66. The reproductive tracts of both sexes were affected by CGS18320B administration. Whole relative prostate weights were significantly decreased in the laboratory 13 study, but, in the laboratory 8 study, decreases of 13-14% in the ventral and the dorsolateral prostates did not achieve statistical significance. Relative uterine weights dropped sharply (nearly 70% at the high dose in the laboratory 8 study and 45% in the laboratory 13 study). The increases in absolute ovarian weights were significant in both studies. However, the relative ovarian weights were increased significantly in the laboratory 8 study, but declined only slightly in the laboratory 13 study.

66. Both studies indicated significant tissue weight changes in the male and female reproductive tract at CGS 18320B doses of 3-30 mg/kg/d that would lead to suspicion of endocrine activity. This evidence includes the significant sexually dimorphic differences in male and female body weight changes reported in Annex 1. Therefore, with these tissue weight endpoints, the updated 407 was successful in identifying the possible activity of CGS 18320B.

Methyl Testosterone

Laboratory 3 – Methyl Testosterone

68. Gross pathology findings included decreased size of testes in some individuals, enlarged livers in both sexes, and decreased size of the thymus in females.

69. The absolute weights discussed for this study are shown in Table 11A and the relative weights in Table 11B. Absolute and relative kidney weights were significantly increased in males at the mid- and high dose; absolute but not relative weights of the brain were significantly decreased at the high dose, and both absolute and relative thymus weights were significantly decreased at the high dose. In females, the significant increases in body weights need to be taken into account. Absolute weights of the heart and kidneys were increased at all doses, absolute liver weights were increased at the mid- and high doses, absolute adrenal weights were increased at the high dose, and absolute thymus weights were decreased at the high dose. When expressed relative to body weight, the kidney and liver were significantly increased at the mid- and high doses in both sexes, the thymus was significantly decreased in males at the high dose and in females at both the mid- and high doses, and the adrenals were significantly increased in males at the high dose.

70. In the male reproductive tract, the absolute and relative testicular weights were significantly decreased at the mid- and high doses. For other male reproductive tract tissues, the absolute and relative ventral and dorsolateral prostate weights were significantly increased at the high dose. The absolute and relative seminal vesicles were significantly decreased at the mid-dose, but both were significantly increased at the high dose, while the body weights were slightly higher at the mid-dose and significantly decreased at the high dose.

71. In females, the absolute ovarian weights were significantly decreased at the mid- and high doses, and the relative weights at significantly decreased at all doses, while the body weights were significantly increased at all doses.

Table 11A. Absolute Organ Weights with Methyl Testosterone in the Laboratory 3 Study

Methyl Testosterone Dose (mg/kg/day)	Control	Low	Middle	High
	0	10	40	200
Male Body Wt Δ 28d		-0.6%	+1.3%	\downarrow -7.8%
Male Body Wt Δ Necropsy		-1.1%	+1.4%	\downarrow -7.4%
Brain (g)	2.010 \pm 0.067 ⁹	1.937 \pm 0.119	2.014 \pm 0.032	*1.944 \pm 0.044
Subgroup A	2.012 \pm 0.088	2.018 \pm 0.089	2.020 \pm 0.043	1.932 \pm 0.048
Subgroup B	2.008 \pm 0.040 ⁴	**1.856 \pm 0.088	2.008 \pm 0.018	1.956 \pm 0.040
Kidney (g)	2.659 \pm 0.157	2.658 \pm 0.292	**3.046 \pm 0.241	**3.422 \pm 0.302
Subgroup A	2.638 \pm 0.142	2.638 \pm 0.400	2.934 \pm 0.268	**3.356 \pm 0.359
Subgroup B	2.680 \pm 0.185	2.678 \pm 0.173	**3.158 \pm 0.166	**3.488 \pm 0.257
Thymus (g)	0.734 \pm 0.119	0.648 \pm 0.093	0.705 \pm 0.127	**0.384 \pm 0.116
Subgroup A	0.752 \pm 0.131	0.652 \pm 0.122	0.700 \pm 0.179	**0.437 \pm 0.113
Subgroup B	0.716 \pm 0.118	0.644 \pm 0.067	0.711 \pm 0.066	**0.331 \pm 0.102
Testes (g)	3.512 \pm 0.187	3.421 \pm 0.255	**2.536 \pm 0.467	**2.237 \pm 0.610
Subgroup A	3.560 \pm 0.191	3.590 \pm 0.215	**2.588 \pm 0.478	**2.156 \pm 0.485
Subgroup B	3.464 \pm 0.192	3.252 \pm 0.169	**2.484 \pm 0.506	**2.318 \pm 0.766
Ventral prostate (g)	0.397 \pm 0.084	0.389 \pm 0.057	0.422 \pm 0.130	**0.628 \pm 0.098
Subgroup A	0.412 \pm 0.087	0.402 \pm 0.074	0.338 \pm 0.096	*0.580 \pm 0.107
Subgroup B	0.382 \pm 0.088	0.376 \pm 0.039	0.506 \pm 0.107	**0.676 \pm 0.066

Table 11A continued. Absolute Organ Weights with Methyl Testosterone in the Laboratory 3 Study.

Methyl testosterone (mg/kg/day)	Control	Low	Middle	High
	0	10	100	600
Dorsolateral prostate (g)	0.199 ± 0.077	0.203 ± 0.046	0.195 ± 0.058	**0.371 ± 0.067
Subgroup A	0.218 ± 0.073	0.182 ± 0.026	0.182 ± 0.050	**0.370 ± 0.082
Subgroup B	0.180 ± 0.084	0.224 ± 0.055	0.208 ± 0.068	**0.372 ± 0.057
Seminal vesicle (g)	0.989 ± 0.208 ⁸	0.846 ± 0.177 ⁸	*0.635 ± 0.329 ⁸	**1.504 ± 0.288 ⁸
Subgroup A	1.000 ± 0.161 ⁴	0.900 ± 0.189 ⁴	0.576 ± 0.383 ⁴	**1.575 ± 0.378 ⁴
Subgroup B	0.978 ± 0.273 ⁴	0.793 ± 0.172 ⁴	0.733 ± 0.248 ⁴	**1.433 ± 0.193 ⁴
Female Body Wt Δ at 28 d		↑ +12.6%	↑↑ +16.1%	↑↑ +11.0%
Female Body Wt Δ at Necropsy		↑↑ +14.2%	↑↑ +17.0%	↑↑ +12.9%
Liver (g)	8.690 ± 0.874	*10.28 ± 0.885	**12.88 ± 2.222 ⁹	**15.73 ± 2.366
Subgroup A	8.260 ± 0.611	10.56 ± 0.891	**12.46 ± 2.797	**15.14 ± 2.257
Subgroup B	9.120 ± 0.939	10.00 ± 0.880	**13.40 ± 1.445 ⁴	**16.32 ± 2.576
Heart (g)	0.910 ± 0.069	**1.024 ± 0.050	*1.026 ± 0.124 ⁹	**1.074 ± 0.137
Subgroup A	0.922 ± 0.069	*1.042 ± 0.040	1.010 ± 0.166	1.008 ± 0.018
Subgroup B	0.898 ± 0.075	1.006 ± 0.057	1.045 ± 0.060 ⁴	**1.140 ± 0.176
Kidney (g)	1.775 ± 0.132	**2.082 ± 0.123	**2.359 ± 0.309 ⁹	**3.091 ± 0.371
Subgroup A	1.794 ± 0.071	**2.090 ± 0.073	*2.280 ± 0.394	**2.958 ± 0.274
Subgroup B	1.756 ± 0.182	2.074 ± 0.168	**2.458 ± 0.155 ⁴	**3.224 ± 0.437
Adrenals (g)	0.061 ± 0.006	*0.056 ± 0.004	0.056 ± 0.008 ⁸	*0.079 ± 0.017
Subgroup A	0.059 ± 0.007	0.054 ± 0.004	0.059 ± 0.007 ⁴	*0.080 ± 0.021
Subgroup B	0.063 ± 0.005	0.057 ± 0.003	0.053 ± 0.008 ⁴	0.078 ± 0.015
Ovary (g)	0.084 ± 0.015	0.073 ± 0.009	**0.058 ± 0.017 ⁹	**0.063 ± 0.013
Subgroup A	0.084 ± 0.009	0.072 ± 0.013	**0.048 ± 0.008	**0.057 ± 0.009
Subgroup B	0.085 ± 0.020	0.074 ± 0.005	0.071 ± 0.018 ⁴	0.068 ± 0.015

Values were expressed as mean ± SD. Superscript indicates number of animals, if not 10 in combined Subgroups or 5 in individual Subgroups.

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

Table 11B. Relative Organ Weights with Methyl Testosterone in the Laboratory 3 Study

Methyl Testosterone Dose (mg/kg/day)	Control	Low	Middle	High
	0	10	40	200
Male Body Wt Δ 28d		-0.6%	+1.3%	↓ -7.8%
Male Body Wt Δ Necropsy		-1.1%	+1.4%	↓ -7.4%
Liver	4.00%	3.70%	*4.41%	**4.87%
Subgroup A	3.99%	3.78%	4.15%	4.79%
Subgroup B	4.00%	3.62%	**4.67%	4.95%
Adrenal	0.0131%	0.0120%	0.0145%	*0.0158%
Subgroup A	0.0126%	0.0138%	0.0134%	0.0156%
Subgroup B	0.0136%	**0.0101%	0.0155%	*0.0158%
Brain	0.52%	0.50%	0.51%	0.54%
Subgroup A	0.51%	0.53%	0.53%	0.52%
Subgroup B	0.53%	0.48%	0.49%	0.56%
Kidney	0.68%	0.69%	**0.77%	**0.95%
Subgroup A	0.67%	0.69%	0.77%	**0.90%
Subgroup B	0.70%	0.69%	**0.78%	**1.00%
Thymus	0.19%	0.17%	0.18%	**0.11%
Subgroup A	0.19%	0.17%	0.18%	**0.12%
Subgroup B	0.19%	0.17%	0.17%	**0.10%

Table 11B continued. Relative Organ Weights with Methyl Testosterone in the Laboratory 3 Study

Methyl Testosterone Dose (mg/kg/day)	Control	Low	Middle	High
	0	10	40	200
Testes	0.90%	0.89%	**0.64%	**0.62%
Subgroup A	0.90%	0.94%	*0.68%	**0.58%
Subgroup B	0.91%	0.84%	**0.61%	*0.67%
Ventral prostate	0.102%	0.101%	0.107%	**0.174%
Subgroup A	0.104%	0.105%	0.088%	**0.156%
Subgroup B	0.100%	0.098%	0.125%	**0.195%
Dorsolateral prostate	0.051%	0.053%	0.049%	**0.103%
Subgroup A	0.055%	0.047%	0.048%	0.099%
Subgroup B	0.047%	0.058%	0.051%	**0.107%
Seminal vesicle	0.254% ⁸	0.220% ⁸	*0.161% ⁸	**0.418% ⁸
Subgroup A	0.253% ⁴	0.235% ⁴	0.151% ⁴	*0.423% ⁴
Subgroup B	0.256% ⁴	0.206% ⁴	0.180% ⁴	**0.413% ⁴
Methyl testosterone (mg/kg/day)	Control	Low	Middle	High
	0	10	100	600
Female Body Wt Δ at 28 d		↑ +12.6%	↑↑ +16.1%	↑↑ +11.0%
Female Body Wt Δ at Necropsy		↑↑ +14.2%	↑↑ +17.0%	↑↑ +12.9%
Liver	3.47%	3.59%	**4.39% ⁹	**5.56%
Subgroup A	3.35%	3.65%	**4.53%	**5.64%
Subgroup B	3.58%	3.53%	4.31% ⁴	**5.49%
Heart	0.36%	0.36%	0.35% ⁹	0.38%
Subgroup A	0.37%	0.36%	0.37%	0.38%
Subgroup B	0.35%	0.36%	0.34% ⁴	0.38%
Kidney	0.71%	0.73%	*0.80% ⁹	**1.09%
Subgroup A	0.73%	0.72%	0.83%	**1.10%
Subgroup B	0.69%	0.73%	0.79% ⁴	**1.09%
Adrenals	0.0243%	**0.0196%	**0.0191% ⁸	0.0279%
Subgroup A	0.0240%	*0.0187%	0.0215% ⁴	0.0298%
Subgroup B	0.0247%	0.0201%	**0.0170% ⁴	0.0263%
Brain	0.76%	**0.67%	**0.64% ⁹	**0.65%
Subgroup A	0.76%	**0.67%	*0.69%	*0.69%
Subgroup B	0.75%	*0.67%	**0.61% ⁴	**0.62%
Thymus	0.22%	0.20%	**0.16% ⁹	**0.08%
Subgroup A	0.23%	0.20%	**0.16%	**0.08%
Subgroup B	0.21%	0.19%	*0.17% ⁴	**0.08%
Ovary	0.034%	**0.026%	**0.020% ⁹	**0.022%
Subgroup A	0.034%	**0.025%	**0.017%	**0.021%
Subgroup B	0.033%	0.026%	*0.023% ⁴	*0.023%

Values were expressed as mean ± SD. Superscript indicates number of animals, if not 10 in combined Subgroups or 5 in individual Subgroups.

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

72. At the high dose, statistical differences in males from methyl testosterone administration were typically observed in both combined Subgroups and both individual Subgroups. Absolute increases in the brain and relative increases in the dorsolateral prostate were the only exceptions, and trends in the absolute values were similar in both of these cases. At the mid-dose, only the combined Subgroups were significant for absolute and relative decreases in the seminal vesicles, and one of the individual Subgroups did not achieve statistical significance in the case of absolute and relative kidney weights and relative liver weights. Both the combined Subgroups and both individual Subgroups observed statistically significant testicular decreases at the mid-dose.

73. In females, the combined Subgroups and the individual Subgroups achieved statistical significance when the weight change was strong. At different doses, statistical significance was seen in the combined Subgroups and only one individual Subgroup (absolute adrenal at the low dose, absolute ovarian at the mid and high dose, absolute heart at the low and high doses, absolute kidney at the low dose, relative adrenal at the low and mid-doses, and the relative ovaries at the low doses). Statistical significance was seen only in the combined Subgroups in the absolute liver and adrenal weights at the low dose, absolute heart weight at the mid-dose, and the relative kidney weights at the mid-dose. The absolute trends were present in both individual Subgroups in each case, but did not achieve statistical significance. Thus, the combined Subgroups appear to improve statistical power where the intensity of the effects may be lower.

74. The combined Subgroups would not change the male LOEL as distinct testicular changes were seen by both individual Subgroups at 40 mg/kg/d. The combined Subgroups detected changes and would lower the LOEL based on the seminal vesicles to 40 mg/kg/d, where neither Subgroup achieved significance at that dose. For the female, the ovarian changes were not detected by Subgroups B at the low 10 mg/kg/d dose.

Laboratory 12 – Methyl Testosterone

75. Gross pathology findings in males consisted of small testes, epididymes, and seminal vesicles. Uterine distension due to fluid was noted in a number of females as was reduced thymus size.

76. The absolute weights discussed for this study are shown in Table 12A and the adjusted weights in Table 12B. Absolute brain weights were significantly decreased at the male mid- and high doses and at the female high dose, but adjusted brain weights were not statistically different in these groups. Absolute heart weights were significantly increased at the female high dose, and the adjusted weights were increased in both the mid- and high dose groups. Absolute kidney weights were significantly increased in the male high dose group and in both the female mid- and high dose groups; adjusted weights were increased in all male groups and in the female high dose group. Absolute liver weights were significantly increased in all female groups, and adjusted weights were increased in the mid- and high dose groups. Absolute pituitary weights were significantly decreased in both sexes at the respective mid- and high doses, while adjusted weights were unchanged in males and statistically decreased in all female doses. Absolute spleen weights were statistically decreased at the male mid- and high doses, but the adjusted weights were not statistically different. Absolute thyroid weights were statistically increased in both sexes at the respective high doses, and the adjusted weights were also statistically increased. Absolute thymus weights were statistically decreased in both sexes at the respective mid- and high doses, and the adjusted weights were also statistically decreased at the high dose in the males and at both the mid- and high dose in the females.

77. In the male reproductive tract, absolute and adjusted combined epididymal and testes weights were statistically decreased at the mid- and the high doses.

78. In the female reproductive tract, absolute and adjusted ovary weights were statistically decreased at the mid- and the high doses. The absolute and adjusted uterus plus cervix weights were statistically decreased at the low and the mid-dose, but statistically increased at the high dose.

Table 12A. Absolute Organ Weights with Methyl Testosterone in the Laboratory 12 Study

Methyl Testosterone Dose (mg/kg/day)	Control	Low	Middle	High
	0	10	40	200
Male Body Wt Δ 28d		-3.3%	$\Downarrow\Downarrow$ -10.1%	$\Downarrow\Downarrow$ -16.9%
Male Body Wt Δ Necropsy		-2.6%	$\Downarrow\Downarrow$ -10.2%	$\Downarrow\Downarrow$ -16.6%
Kidney (g)	2.80 \pm 0.33	2.88 \pm 0.37	2.71 \pm 0.16	**3.45 \pm 0.14
Subgroup A	2.87 \pm 0.44	2.69 \pm 0.30	2.77 \pm 0.21	**3.46 \pm 0.13
Subgroup B	2.73 \pm 0.21	*3.08 \pm 0.35	2.65 \pm 0.06	**3.43 \pm 0.16
Heart (g)	1.128 \pm 0.110	1.138 \pm 0.108	1.057 \pm 0.097	1.052 \pm 0.106
Subgroup A	1.120 \pm 0.127	1.082 \pm 0.088	1.052 \pm 0.113	1.117 \pm 0.091
Subgroup B	1.135 \pm 0.105	1.193 \pm 0.103	1.061 \pm 0.090	*0.987 \pm 0.081
Brain (g)	1.99 \pm 0.07	1.96 \pm 0.03	**1.90 \pm 0.07	*1.91 \pm 0.05
Subgroup A	2.03 \pm 0.09	*1.94 \pm 0.03	*1.91 \pm 0.05	*1.93 \pm 0.06
Subgroup B	1.99 \pm 0.07	1.98 \pm 0.01	*1.88 \pm 0.08	*1.90 \pm 0.02
Pituitary (g)	0.009 \pm 0.002	0.007 \pm 0.001	0.007 \pm 0.002	*0.007 \pm 0.001
Subgroup A	0.009 \pm 0.002	0.007 \pm 0.001	0.007 \pm 0.002	*0.007 \pm 0.001
Subgroup B	0.010 \pm 0.002	0.009 \pm 0.001	0.008 \pm 0.001	*0.007 \pm 0.002
Spleen (g)	0.897 \pm 0.119	0.818 \pm 0.068	**0.740 \pm 0.113	**0.699 \pm 0.034
Subgroup A	0.932 \pm 0.214	0.798 \pm 0.097	0.773 \pm 0.129	*0.721 \pm 0.030
Subgroup B	0.869 \pm 0.136	0.837 \pm 0.004	**0.707 \pm 0.098	**0.677 \pm 0.021
Thymus (g)	0.540 \pm 0.073	0.534 \pm 0.083	*0.463 \pm 0.083	**0.368 \pm 0.063
Subgroup A	0.558 \pm 0.048	0.506 \pm 0.054	*0.487 \pm 0.072	**0.418 \pm 0.007
Subgroup B	0.522 \pm 0.094	0.561 \pm 0.103	0.439 \pm 0.095	**0.318 \pm 0.051
Thyroid (g)	0.015 \pm 0.002	0.015 \pm 0.003	0.014 \pm 0.002	*0.017 \pm 0.002
Subgroup A	0.015 \pm 0.002	0.014 \pm 0.002	0.013 \pm 0.002	0.017 \pm 0.002
Subgroup B	0.014 \pm 0.002	0.015 \pm 0.003	0.014 \pm 0.002	0.016 \pm 0.002
Testes (g)	3.24 \pm 0.19	3.10 \pm 0.19	**2.89 \pm 0.17	**1.06 \pm 0.18
Subgroup A	3.23 \pm 0.17	*2.99 \pm 0.16	**2.88 \pm 0.18	**1.04 \pm 0.16
Subgroup B	3.25 \pm 0.23	3.22 \pm 0.15	*2.89 \pm 0.18	**1.09 \pm 0.22
Epididymis (g)	0.827 \pm 0.052	0.780 \pm 0.071	**0.702 \pm 0.039	**0.476 \pm 0.051
Subgroup A	0.814 \pm 0.071	0.748 \pm 0.077	**0.696 \pm 0.048	**0.469 \pm 0.033
Subgroup B	0.840 \pm 0.023	0.811 \pm 0.054	**0.707 \pm 0.032	**0.482 \pm 0.068
R Cauda Epididymis (g)	0.142 \pm 0.019	*0.128 \pm 0.009	**0.119 \pm 0.014	**0.089 \pm 0.008
Subgroup A	0.140 \pm 0.022	0.120 \pm 0.009	*0.117 \pm 0.016	**0.087 \pm 0.008
Subgroup B	0.145 \pm 0.016	0.136 \pm 0.011	*0.122 \pm 0.014	**0.091 \pm 0.009
Methyl testosterone (mg/kg/day)	Control	Low	Middle	High
	0	10	100	600
Female Body Wt Δ at 28 d		$\Uparrow\Uparrow$ +10.9%	$\Uparrow\Uparrow$ +13.8%	+0.2%
Liver (g)	9.7 \pm 1.0	*10.7 \pm 0.6	**12.7 \pm 1.5	**13.7 \pm 1.1
Subgroup A	9.5 \pm 1.2	10.6 \pm 0.7	**11.8 \pm 1.4	**13.5 \pm 1.0
Subgroup B	9.8 \pm 0.9	10.7 \pm 0.5	**13.6 \pm 1.0	**13.9 \pm 1.2
Kidney (g)	1.84 \pm 0.14	1.93 \pm 0.12	**2.18 \pm 0.25	**2.66 \pm 0.20
Subgroup A	1.90 \pm 0.16	1.88 \pm 0.13	2.07 \pm 0.22	**2.69 \pm 0.26
Subgroup B	1.77 \pm 0.08	1.99 \pm 0.22	**2.29 \pm 0.24	**2.63 \pm 0.15
Heart (g)	0.856 \pm 0.051	*0.908 \pm 0.050	0.857 \pm 0.055 ⁹	*0.915 \pm 0.047
Subgroup A	0.837 \pm 0.052	*0.931 \pm 0.018	0.835 \pm 0.073 ⁴	*0.908 \pm 0.040
Subgroup B	0.875 \pm 0.048	0.885 \pm 0.066	0.875 \pm 0.035 ⁴	*0.921 \pm 0.056

Table 12A continued. Absolute Organ Weights with Methyl Testosterone in the Laboratory 12 Study

Methyl Testosterone Dose (mg/kg/day)	Control	Low	Middle	High
	0	10	40	200
Brain (g)	1.83 ± 0.04	1.88 ± 0.07	1.81 ± 0.08	*1.76 ± 0.06
Subgroup A	1.82 ± 0.04	1.86 ± 0.07	1.78 ± 0.10	1.73 ± 0.07
Subgroup B	1.84 ± 0.05	*1.76 ± 0.06	1.84 ± 0.02	1.79 ± 0.04
Pituitary (g)	0.009 ± 0.002	0.008 ± 0.001	**0.007 ± 0.001	**0.007 ± 0.001
Subgroup A	0.009 ± 0.002	0.008 ± 0.001	**0.006 ± 0.002	**0.006 ± 0.002
Subgroup B	0.008 ± 0.002	0.008 ± 0.002	0.007 ± 0.002	0.007 ± 0.001
Thymus (g)	0.487 ± 0.051	0.489 ± 0.055	**0.383 ± 0.063	**0.203 ± 0.075
Subgroup A	0.477 ± 0.060	0.488 ± 0.053	*0.362 ± 0.029	**0.185 ± 0.098
Subgroup B	0.497 ± 0.045	0.489 ± 0.064	*0.401 ± 0.080	**0.221 ± 0.0498
Thyroid (g)	0.013 ± 0.002	0.012 ± 0.004	0.014 ± 0.001	**0.018 ± 0.003
Subgroup A	0.013 ± 0.002	0.013 ± 0.004	0.013 ± 0.000	*0.018 ± 0.003
Subgroup B	0.013 ± 0.003	0.011 ± 0.004	0.014 ± 0.002	**0.019 ± 0.003
Ovary (g)	0.093 ± 0.011	0.092 ± 0.008	**0.056 ± 0.011	**0.050 ± 0.013
Subgroup A	0.096 ± 0.009	0.090 ± 0.007	**0.054 ± 0.013	**0.044 ± 0.008
Subgroup B	0.089 ± 0.013	0.093 ± 0.008	**0.059 ± 0.008	**0.056 ± 0.015
Uterus plus cervix (g)	0.360 ± 0.115	**0.256 ± 0.077	*0.268 ± 0.041	**0.518 ± 0.102
Subgroup A	0.321 ± 0.032	0.254 ± 0.034	0.240 ± 0.027	**0.488 ± 0.125
Subgroup B	0.399 ± 0.158	*0.259 ± 0.110	0.296 ± 0.031	*0.548 ± 0.074

Values were expressed as mean ± SD. Superscript indicates number of animals, if not 10 in combined Subgroups or 5 in individual Subgroups.

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

Table 12B. Relative Organ Weights with Methyl Testosterone in the Laboratory 12 Study^a

Methyl Testosterone Dose (mg/kg/day)	Control	Low	Middle	High
	0	10	40	200
Male Body Wt Δ 28d		-3.3%	↓↓ -10.1%	↓↓ -16.9%
Male Body Wt Δ Necropsy		-2.6%	↓↓ -10.2%	↓↓ -16.6%
Liver	4.65%	4.64%	**4.78%	**5.54%
Subgroup A	4.68%	4.49%	*4.92%	**5.69%
Subgroup B	4.63%	4.78%	4.67%	**5.39%
Brain	0.54%	0.56%	0.61%	0.54%
Subgroup A	0.54%	0.56%	0.61%	0.54%
Subgroup B	0.54%	0.57%	0.62%	0.54%
Kidney	0.75%	*0.80%	**0.80%	**1.11%
Subgroup A	0.74%	0.76%	**0.81%	**1.09%
Subgroup B	0.75%	*0.84%	0.80%	**1.12%
Heart	0.30%	0.31%	0.31%	**0.34%
Subgroup A	0.29%	0.30%	0.31%	**0.35%
Subgroup B	0.31%	0.32%	0.32%	0.32%
Pituitary	0.002%	0.002%	0.002%	0.002%
Subgroup A	0.002%	0.002%	0.002%	0.002%
Subgroup B	0.003%	0.002%	0.002%	0.002%

Table 12B continued. Relative Organ Weights with Methyl Testosterone in the Laboratory 12 Study^a

Methyl Testosterone Dose (mg/kg/day)	Control	Low	Middle	High
	0	10	40	200
Spleen	0.24%	0.23%	0.22%	0.22%
Subgroup A	0.24%	0.22%	0.23%	0.23%
Subgroup B	0.24%	0.23%	0.21%	0.22%
Thymus	0.14%	0.15%	0.14%	*0.12%
Subgroup A	0.14%	0.14%	0.14%	*0.13%
Subgroup B	0.14%	0.15%	0.13%	0.10%
Thyroid	0.004%	0.004%	0.004%	**0.005%
Subgroup A	0.004%	0.004%	0.004%	**0.005%
Subgroup B	0.004%	0.004%	0.004%	0.005%
Testes	0.87%	0.86%	*0.86%	**0.34%
Subgroup A	0.84%	0.84%	0.84%	**0.33%
Subgroup B	0.90%	0.88%	0.87%	**0.36%
Epididymis	0.221%	0.215%	**0.208%	**0.152%
Subgroup A	0.211%	0.210%	0.204%	**0.147%
Subgroup B	0.232%	0.220%	**0.213%	**0.157%
Methyl testosterone (mg/kg/day)	Control	Low	Middle	High
	0	10	100	600
Female Body Wt Δ at 28 d		↑↑ +10.9%	↑↑ +13.8%	+0.2%
Liver	4.10%	4.20%	**4.89%	**5.97%
Subgroup A	4.05%	4.18%	*4.67%	**6.02%
Subgroup B	4.12%	4.18%	**5.10%	**5.91%
Brain	0.77%	**0.71%	**0.70%	0.77%
Subgroup A	0.78%	*0.73%	**0.70%	0.77%
Subgroup B	0.77%	0.69%	0.69%	0.76%
Heart	0.36%	0.36%	*0.33%	**0.40%
Subgroup A	0.36%	0.37%	0.33%	**0.40%
Subgroup B	0.37%	0.35%	0.33%	0.39%
Kidney	0.78%	0.76%	0.84%	**1.16%
Subgroup A	0.81%	0.74%	0.82%	**1.20%
Subgroup B	0.74%	0.78%	0.86%	**1.12%
Adrenal	0.0262%	**0.0216%	**0.0193%	*0.0301%
Subgroup A	0.0264%	**0.0205%	**0.0194%	0.0290%
Subgroup B	0.0256%	0.0227%	*0.0187%	*0.0311%
Pituitary	0.004%	*0.003%	**0.003%	*0.003%
Subgroup A	0.004%	**0.003%	**0.002%	*0.003%
Subgroup B	0.003%	0.003%	0.003%	0.003%
Thymus	0.21%	0.19%	**0.15%	**0.09%
Subgroup A	0.20%	0.19%	*0.14%	**0.08%
Subgroup B	0.21%	0.19%	**0.15%	**0.09%
Thyroid	0.005%	0.005%	0.005%	**0.008%
Subgroup A	0.006%	0.005%	0.005%	*0.008%
Subgroup B	0.005%	*0.004%	0.005%	**0.008%

Table 12B continued. Relative Organ Weights with Methyl Testosterone in the Laboratory 12 Study^a

Methyl testosterone (mg/kg/day)	Control	Low	Middle	High
	0	10	100	600
Ovary	0.039%	0.036%	**0.022%	**0.022%
Subgroup A	0.041%	**0.035%	**0.021%	**0.020%
Subgroup B	0.037%	0.036%	*0.022%	**0.024%
Uterus plus cervix	0.152%	*0.100%	*0.103%	**0.226%
Subgroup A	0.137%	0.100%	0.095%	**0.218%
Subgroup B	0.168%	*0.101%	0.111%	*0.233%

^a Laboratory 12 uses an ANCOVA procedure adjusting organ weights with body weight as a covariate, a slightly different procedure than in most labs. Thus, the statistical significance here is not strictly comparable to the results of other laboratories. These are relative weights in the table, which were calculated by the Secretariat.

Values were expressed as mean \pm SD. Superscript indicates number of animals, if not 10 in combined Subgroups or 5 in individual Subgroups.

*, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively).

79. At the high dose and sometimes the mid-dose, responses in both sexes to methyl testosterone administration were strong and statistical differences were often observed in both combined Subgroups and both individual Subgroups. However, as doses and the intensity of responses decreased, statistical significance occurred in the combined Subgroups and only one of the individual Subgroups. For males, examples include absolute spleen and thymus weights at the mid-dose, adjusted liver weights at the mid-dose, adjusted kidney weights at the low and mid-doses, and adjusted testicular and epididymal weights at the mid-dose. For females, examples include absolute kidney weights at the mid-dose, adjusted brain and heart weights at various doses, and adrenal and pituitary weights. Additionally, amongst the large number of statistical differences observed with this potent androgen, there were instances where only the combined Subgroups were statistically significant and other instances where only one of the individual Subgroups were statistically significant. Overall, the combined Subgroups were often significant at a lower dose where one or both of the individual Subgroups were not. In those instances, the absolute trends were similar between the individual and combined Subgroups, indicating the increased power of the combined Subgroups.

80. In the laboratory 12 study, the combined Subgroups did detect adjusted testicular and uterine changes at a lower doses (40 mg/kg/d and 10 mg/kg/d, respectively) and, for the latter, in a less equivocal fashion, than the individual Subgroups.

Summary – Methyl Testosterone

81. The two methyl testosterone studies were in agreement in several major respects. In both studies, the livers and kidneys of both sexes were increased; the relative adrenal weights in females decreased, particularly at the low and mid-doses; thymus weights were modestly decreased in males and more strongly and at lower doses in females; testicular weights decreased by 36% in the laboratory 3 study and even more strongly by 67% in the laboratory 12 study; and ovarian weights decreased by 25-30% at the mid- and high doses in the laboratory 3 study and 40 and 46%, respectively, in the laboratory 12 study. The trend in the uterine weights were similar, decreasing at the low dose (-39% laboratory 3 and -21% laboratory 12) and increasing at the high dose (+47% laboratory 3 and +52% laboratory 12), but the latter did not achieve statistical significance in the laboratory 3 study. There were some differences in response in the male reproductive tract where the laboratory 3 study saw strong increases in prostate tissues at the high dose (55-80%), while the laboratory 12 study saw moderate losses.

82. Both studies clearly identify significant tissue weight changes in the male and the female reproductive tracts at methyl testosterone doses as low as 10 mg/kg/d that would lead to suspicion of endocrine activity. The changes in absolute and relative tissue weights include the testes and other

accessory reproductive tract tissues in the male and the ovaries and uterus in the female. In Phase-2 of the OECD hershberger validation program, the minimal effective doses for a statistically significant increase in one or more of the five tissues is 2-10 mg/kg/d, indicating that ability of the updated 407 to identify changes due to potent androgens compares favourably to other screening assays. Therefore, with these tissue weight endpoints in the reproductive tracts of both sexes, the updated 407 was successful in identifying the possible activity of methyl testosterone.

Flutamide

Laboratory 2 – Flutamide

83. Gross pathology observations were reduced prostate and seminal vesicles at the mid- and high doses in the males and hepatic enlargement in the females at the high dose.

84. The absolute weights discussed for this study are shown in Table 13A and the relative weights in Table 13B. Absolute and relative liver weights were significantly increased in females at the high dose; absolute brain weights were significantly decreased in females at the high dose; and absolute heart, spleen, and kidney weights were significantly decreased in males at the mid- and high doses. Relative brain and liver weights were significantly increased in males at the high dose and the adrenals at the mid- and high doses.

85. In the male reproductive tract, absolute ventral prostate weights were significantly decreased at all doses and the relative weights at the high dose. Absolute and relative epididymis and seminal vesicle (plus coagulating glands) weights were significantly decreased at the mid- and high doses. The absolute male accessory reproductive organs (m.a.r.o.) weights, consisting of seminal vesicles, coagulating glands, all parts of the prostate, and seminal vesicles, were significantly decreased at all doses, and the relative weight of the m.a.r.o. were significantly decreased at the mid- and high doses.

Table 13A. Absolute Organ Weights with Flutamide in the Laboratory 2 Study

Flutamide Dose (mg/kg/day)	Control	Low	Middle	High
	0	1	10	100
Male Body Wt Δ 28d		-6.7%	-3.4%	↓↓ -14.1%
Male Body Wt Δ Necropsy		-7.1%	-3.813%	↓↓ -12.1%
Kidney (mg)	2297 ± 185.5	2103 ± 212.0	*2084 ± 233.5	**1971 ± 129.0
Subgroup A	2411 ± 88.7	*2083 ± 164.4	2128 ± 253.0	**2005 ± 142.0
Subgroup B	2184 ± 193.6	2122 ± 270.5	2040 ± 232.1	1937 ± 120.1
Heart (mg)	1169 ± 92.4	1064 ± 103.3	1076 ± 129.4	*1026 ± 104.2
Subgroup A	1228 ± 86.3	1085 ± 97.4	1097 ± 157.7	1053 ± 75.7
Subgroup B	1110 ± 55.2	1043 ± 115.7	1054 ± 108.0	999 ± 129.9
Spleen (mg)	729 ± 100.5	699 ± 131.2	634 ± 132.4	*606 ± 54.0
Subgroup A	803 ± 77.9	669 ± 81.6	676 ± 157.8	644 ± 52.8
Subgroup B	656 ± 56.8	728 ± 172.8	592 ± 100.7	569 ± 16.5
Ventral prostate (mg)	417 ± 75.6	**285 ± 38.0	*303 ± 67.0	**92 ± 44.5
Subgroup A	436 ± 63.0	**291 ± 51.8	*313 ± 85.1	**98 ± 42.3
Subgroup B	397 ± 89.0	*279 ± 21.5	*293 ± 51.0	**85 ± 50.6
Seminal vesicles (mg)	1593 ± 260.4	1390 ± 201.2	**1171 ± 242.2	**302 ± 43.4
Subgroup A	1663 ± 364.4	1368 ± 251.6	*1134 ± 242.3	**316 ± 34.0
Subgroup B	1523 ± 86.6	1413 ± 162.8	*1208 ± 264.4	**289 ± 51.3
Epididymis (mg)	648 ± 62.0	574 ± 86.2	**514 ± 62.4	**356 ± 86.1
Subgroup A	651 ± 78.5	588 ± 18.5	*511 ± 65.8	**407 ± 93.2
Subgroup B	645 ± 49.7	559 ± 126.0	*518 ± 66.3	**304 ± 36.4
M.a.r.o.^a (mg)	2010 ± 265.6	**1678 ± 220.3	**1477 ± 263.7	**398 ± 78.8
Subgroup A	2097 ± 362.0	1662 ± 289.4	**1450 ± 301.2	**419 ± 62.5
Subgroup B	1922 ± 92.7	1694 ± 157.6	**1503 ± 253.0	**377 ± 94.8
Female Body Wt Δ at 28 d		-2.4%	-1.0%	-2.9%
Female Body Wt Δ at Necropsy		-2.9%	-1.0%	-1.9%
Liver (mg)	9189 ± 928.9	8668 ± 979.7	9162 ± 941.0	**11459 ± 644.0
Subgroup A	9084 ± 1058.4	8800 ± 941.2	9414 ± 648.7	*11032 ± 552.0
Subgroup B	9295 ± 890.9	8535 ± 1109.1	8910 ± 1188.6	**11886 ± 415.1
Brain (mg)	1769 ± 50.7	1719 ± 89.1	1718 ± 91.3	**1655 ± 81.9
Subgroup A	1752 ± 47.7	1762 ± 72.3	1737 ± 105.6	1642 ± 63.5
Subgroup B	1787 ± 52.5	1676 ± 89.9	1699 ± 81.9	1668 ± 103.2
Pituitary (mg)	13 ± 2.3	12 ± 1.8	11 ± 1.3	* 10 ± 1.6
Subgroup A	12 ± 2.9	^{nc} 13 ± 1.8	^{nc} 11 ± 1.3	^{nc} 11 ± 1.2
Subgroup B	13 ± 1.7	^{nc} 12 ± 1.9	^{nc} 10 ± 1.5	^{nc} 10 ± 1.8

^a Male accessory reproductive organs (Maro): Seminal vesicles, coagulating glands, and dorsolateral prostate
*, **, Significantly different from control (p<0.05 and p<0.01, respectively); nc – statistics were not calculated for this value and Subgroup, where a control or test group size was n ≤ 4; under these conditions the power of the statistics was judged to too low to provide meaningful information.

Table 13B. Relative Organ Weights with Flutamide in the Laboratory 2 Study

Flutamide Dose (mg/kg/day)	Control	Low	Middle	High
	0	1	10	100
Male Body Wt Δ 28d		-6.7%	-3.4%	$\downarrow\downarrow$ -14.1%
Male Body Wt Δ Necropsy		-7.1%	-3.813%	$\downarrow\downarrow$ -12.1%
Liver	4.25%	4.14%	4.31%	**5.37%
Subgroup A	4.35%	4.10%	4.35%	**5.37%
Subgroup B	4.13%	4.19%	4.28%	**5.38%
Kidney	0.68%	0.67%	0.64%	0.66%
Subgroup A	0.68%	0.66%	0.63%	0.65%
Subgroup B	0.67%	0.68%	0.65%	0.67%
Adrenal	0.0192%	0.0216%	*0.0224%	**0.0238%
Subgroup A	0.0199%	0.0223%	0.0207%	0.0237%
Subgroup B	0.0187%	0.0209%	0.0240%	*0.0240%
Brain	0.55%	0.58%	0.56%	*0.61%
Subgroup A	0.54%	0.57%	0.55%	0.59%
Subgroup B	0.56%	0.60%	0.57%	0.62%
Heart	0.34%	0.34%	0.33%	0.34%
Subgroup A	0.35%	0.34%	0.32%	0.34%
Subgroup B	0.34%	0.34%	0.34%	0.35%
Spleen	0.22%	0.22%	0.19%	0.20%
Subgroup A	0.23%	0.21%	0.20%	0.21%
Subgroup B	0.20%	0.23%	0.19%	0.20%
Ventral prostate	0.123%	0.090%	0.093%	**0.031%
Subgroup A	0.124%	0.092%	0.093%	**0.032%
Subgroup B	0.121%	0.090%	0.094%	**0.030%
Seminal vesicles	0.470%	0.441%	**0.359%	**0.101%
Subgroup A	0.472%	0.430%	*0.336%	**0.103%
Subgroup B	0.472%	0.462%	0.391%	**0.101%
Epididymis	0.191%	0.182%	*0.158%	**0.119%
Subgroup A	0.185%	0.185%	0.151%	*0.132%
Subgroup B	0.198%	0.179%	0.166%	**0.106%
M.a.r.o.^a	0.593%	0.540%	**0.455%	**0.134%
Subgroup A	0.596%	0.526%	**0.426%	**0.137%
Subgroup B	0.590%	0.553%	0.485%	**0.132%
Female Body Wt Δ at 28 d		-2.4%	-1.0%	-2.9%
Female Body Wt Δ at Necropsy		-2.9%	-1.0%	-1.9%
Liver	4.42%	4.29%	4.45%	**5.62%
Subgroup A	4.35%	4.36%	4.46%	**5.49%
Subgroup B	4.49%	4.23%	4.46%	**5.77%
Brain	0.85%	0.85%	0.83%	0.81%
Subgroup A	0.84%	0.87%	0.82%	0.82%
Subgroup B	0.86%	0.83%	0.85%	0.81%
Pituitary	0.0063%	0.0059%	0.0053%	0.0049%
Subgroup A	0.0057%	^{nc} 0.0064%	^{nc} 0.0052%	^{nc} 0.0055%
Subgroup B	0.0063%	^{nc} 0.0059%	^{nc} 0.0055%	^{nc} 0.0049%

^a Male accessory reproductive organs (Maro): Seminal vesicles, coagulating glands, and dorsolateral prostate
*, **, Significantly different from control (p<0.05 and p<0.01, respectively); nc – statistics not calculated, control group n < 4 considered too low.

86. In the male reproductive tract, both the combined Subgroups and each individual Subgroup achieved statistical significance together in all cases except one. The male accessory reproductive tract tissues and organs (m.a.r.o) achieved absolute significance at the low dose of 1 mg/kg/d flutamide only in

the combined Subgroups. Similarly, the m.a.r.o. relative weights were significantly decreased, in the combined Subgroups and Subgroup A at the 10 mg/kg/d mid-dose. Relative increases in the male liver were significant in both the combined and individual Subgroups, but the adrenals were significantly increased only in the combined Subgroups at the mid-dose and the combined Subgroups and Subgroup B at the high dose. In the females, absolute and relative increases in the liver were significant in both the combined and individual Subgroups. These data suggest that the combined Subgroups do provide modestly increased statistical power, but were not essential to consistently detect the effects of flutamide versus the individual Subgroups.

Laboratory 11 – Flutamide

87. Gross pathology at necropsy observed liver enlargement in a significant number of males and females. The sex accessory tissues in the males (epididymes, seminal vesicles, and prostate) in the high dose group were small, and, in several individuals, the testes were judged to be soft.

88. The absolute weights discussed for this study are shown in Table 14A and the relative weights in Table 14B. Absolute liver weights were significantly increased in both sexes at the high dose. The absolute adrenal and pituitary weights were significantly increased in the males in the high dose group. In the male reproductive tract, the absolute right and left epididymis were significantly decreased at all doses; the absolute seminal vesicles, prostate, and dorsolateral prostate weights were significantly decreased at the mid- and high dose; and the absolute ventral prostate weight was significantly decreased at the high dose. In the female reproductive tract, the absolute weights of the individual left ovary were significantly decreased at the high dose. On a relative weight basis, additional findings were statistically significant. The thymus was significantly decreased in males at the high dose, the pituitary was significantly decreased in males at the mid-dose, and prostate was significantly decreased at the low dose.

Table 14A. Absolute Organ Weights with Flutamide in the Laboratory 11 Study

Flutamide Dose (mg/kg/day)	Control	Low	Middle	High
	0	1	10	100
Male Body Wt Δ 28d		+4.2%	-0.4%	-5.3%
Male Body Wt Δ Necropsy		4.0%	-0.5%	-5.2%
Liver (g)	14.45 ± 1.68	15.97 ± 1.80	15.47 ± 1.22	**19.88 ± 2.21
Subgroup A	15.19 ± 1.92	15.95 ± 2.17	15.28 ± 1.56	*18.99 ± 1.83
Subgroup B	13.71 ± 1.12	15.98 ± 1.59	15.67 ± 0.91	**20.76 ± 2.39
Right Adrenal (mg)	29.3 ± 3.9	28.6 ± 2.9	32.8 ± 4.4	*35.3 ± 5.7
Subgroup A	29.6 ± 5.5	28.4 ± 2.6	34.0 ± 4.1	36.5 ± 7.9
Subgroup B	29.1 ± 1.9	28.8 ± 3.4	31.5 ± 4.8	34.1 ± 2.6
Left Adrenal (mg)	31.1 ± 3.1	31.6 ± 3.0	34.0 ± 4.9	35.5 ± 4.9
Subgroup A	31.5 ± 3.7	31.5 ± 3.8	35.8 ± 5.0	35.8 ± 6.0
Subgroup B	30.7 ± 2.7	31.7 ± 2.4	32.3 ± 4.7	35.3 ± 4.3
Pituitary (mg)	11.4 ± 1.4	11.7 ± 1.1	12.5 ± 1.7	*13.0 ± 1.2
Subgroup A	11.5 ± 1.6	12.0 ± 0.9	12.8 ± 1.4	12.6 ± 1.3
Subgroup B	11.2 ± 1.4	11.4 ± 1.2	12.2 ± 2.0	13.3 ± 1.2
Seminal Vesicle (g)	1.51 ± 0.20	1.33 ± 0.20	**1.12 ± 0.18	**0.17 ± 0.02
Subgroup A	1.50 ± 0.22	1.34 ± 0.24	1.12 ± 0.21	*0.17 ± 0.03
Subgroup B	1.52 ± 0.21	1.33 ± 0.19	*1.11 ± 0.18	*0.16 ± 0.02
Prostate (g)	0.88 ± 0.17	0.77 ± 0.14	**0.64 ± 0.13	**0.23 ± 0.07
Subgroup A	0.95 ± 0.18	0.73 ± 0.13	*0.67 ± 0.16	**0.20 ± 0.06
Subgroup B	0.81 ± 0.14	0.81 ± 0.14	*0.62 ± 0.10	**0.25 ± 0.07
Ventral Prostate (g)	0.66 ± 0.14	0.60 ± 0.15	0.50 ± 0.12	**0.17 ± 0.05
Subgroup A	0.71 ± 0.17	0.51 ± 0.12	0.52 ± 0.14	**0.15 ± 0.06
Subgroup B	0.61 ± 0.10	0.68 ± 0.12	0.48 ± 0.09	**0.19 ± 0.04
Dorsolateral Prostate (g)	0.39 ± 0.09	0.34 ± 0.07	**0.27 ± 0.06	**0.08 ± 0.01
Subgroup A	0.42 ± 0.11	0.35 ± 0.06	*0.25 ± 0.06	*0.08 ± 0.02
Subgroup B	0.37 ± 0.06	0.33 ± 0.08	0.28 ± 0.05	*0.09 ± 0.01
Epididymis – Right (g)	0.49 ± 0.03	**0.44 ± 0.04	**0.39 ± 0.04	**0.22 ± 0.03
Subgroup A	0.49 ± 0.04	0.44 ± 0.05	*0.40 ± 0.05	**0.21 ± 0.03
Subgroup B	0.49 ± 0.03	**0.44 ± 0.03	**0.38 ± 0.02	**0.23 ± 0.02
Epididymis – Left (g)	0.48 ± 0.04	*0.43 ± 0.04	**0.40 ± 0.04	**0.23 ± 0.03
Subgroup A	0.45 ± 0.03	0.44 ± 0.05	0.41 ± 0.05	**0.22 ± 0.05
Subgroup B	0.51 ± 0.03	**0.43 ± 0.03	**0.38 ± 0.02	**0.24 ± 0.02
Female Body Wt Δ at 28 d		+1.8%	+1.8%	-0.9%
Female Body Wt Δ at Necropsy		+1.3%	+2.0%	-0.4%
Liver (g)	9.95 ± 0.81	10.52 ± 1.28	10.41 ± 1.06	**14.19 ± 1.34
Subgroup A	10.24 ± 0.81	10.42 ± 1.41	10.32 ± 1.43	**14.23 ± 1.40
Subgroup B	9.65 ± 0.76	10.61 ± 1.29	10.50 ± 0.69	**14.16 ± 1.44
Right Ovary (mg)	46.0 ± 7.1	44.6 ± 9.0	50.3 ± 10.5	39.9 ± 8.4
Subgroup A	46.1 ± 5.7	45.4 ± 12.3	49.7 ± 4.6	36.5 ± 9.3
Subgroup B	45.8 ± 9.0	43.7 ± 5.3	50.9 ± 15.0	43.2 ± 6.5
Left Ovary (mg)	46.8 ± 7.5	42.8 ± 6.4	48.0 ± 7.6	*37.9 ± 7.2
Subgroup A	46.1 ± 8.5	42.6 ± 8.0	48.4 ± 6.9	36.8 ± 6.5
Subgroup B	47.6 ± 7.1	43.0 ± 5.2	47.6 ± 9.0	38.9 ± 8.4

Values were expressed as mean ± SD.

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

Table 14B. Relative Organ and Tissue Weights with Flutamide in the Laboratory 11 Study

Flutamide Dose (mg/kg/day)	Control	Low	Middle	High
	0	1	10	100
Male Body Wt Δ 28d		+4.2%	-0.4%	-5.3%
Male Body Wt Δ Necropsy		4.0%	-0.5%	-5.2%
Liver	3.73%	3.96%	*4.02%	**5.42%
Subgroup A	3.83%	3.91%	3.98%	**5.35%
Subgroup B	3.64%	*4.01%	*4.06%	**5.48%
Adrenal	0.0156%	0.0150%	0.0174%	**0.0193%
Subgroup A	0.0154%	0.0147%	0.0182%	*0.0204%
Subgroup B	0.0159%	0.0152%	0.0166%	0.0184%
Pituitary	0.0029%	0.0029%	*0.0032%	**0.0035%
Subgroup A	0.0029%	0.0029%	*0.0033%	**0.0036%
Subgroup B	0.0030%	0.0029%	0.0032%	*0.0035%
Thymus	0.11%	0.13%	0.12%	*0.15%
Subgroup A	0.12%	0.12%	0.11%	0.14%
Subgroup B	0.11%	0.15%	0.13%	*0.16%
Seminal Vesicle	0.39%	0.33%	**0.29%	**0.05%
Subgroup A	0.38%	0.33%	0.29%	*0.05%
Subgroup B	0.40%	0.33%	0.29%	*0.04%
Prostate	0.23%	*0.19%	**0.17%	**0.06%
Subgroup A	0.24%	*0.18%	*0.17%	**0.06%
Subgroup B	0.21%	0.20%	*0.16%	**0.07%
Ventral Prostate	0.17%	0.15%	*0.13%	**0.05%
Subgroup A	0.18%	0.13%	0.14%	**0.04%
Subgroup B	0.16%	0.17%	0.12%	**0.05%
Dorsolateral Prostate	0.10%	0.08%	**0.07%	**0.02%
Subgroup A	0.11%	0.09%	0.07%	*0.02%
Subgroup B	0.10%	0.08%	0.07%	*0.02%
Epididymis	0.25%	*0.22%	**0.21%	**0.12%
Subgroup A	0.24%	0.21%	0.21%	**0.12%
Subgroup B	0.27%	*0.22%	**0.20%	**0.12%
Female Body Wt Δ at 28 d		+1.8%	+1.8%	-0.9%
Female Body Wt Δ at Necropsy		+1.3%	+2.0%	-0.4%
Liver	3.91%	4.08%	4.02%	**5.60%
Subgroup A	4.03%	4.06%	3.99%	**5.54%
Subgroup B	3.80%	4.10%	4.04%	**5.67%
Ovary	0.037%	0.034%	0.038%	0.031%
Subgroup A	0.036%	0.034%	0.038%	0.029%
Subgroup B	0.037%	0.034%	0.038%	0.033%

Values were expressed as mean ± SD.

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

89. In the male reproductive tract, both the combined Subgroups and each individual Subgroup achieved statistical significance together at the high dose with the potent androgen antagonist, flutamide. At the mid-dose, only the combined Subgroups relative weights were statistically significant for seminal vesicles, whole prostate, ventral prostate, and dorsolateral prostate. For the absolute weights, the combined Subgroups and one individual Subgroup achieved significance in the seminal vesicles, whole prostate, and dorsolateral prostate. The combined Subgroups were statistically significant for the absolute pituitary weights at the high dose and the relative liver and pituitary weights at the mid-dose, where one or both of the individual Subgroups did not achieve statistical significance. The absolute trends were similar in these cases. In the females, absolute and relative increases in the liver were significant in both the combined and individual Subgroups at the high dose; absolute weights were significantly increased in the combined and

one individual Subgroup at the mid-dose. Individual ovarian weights were recorded in this lab with the left ovary absolute weight decreased significantly in the combined Subgroups. The relative paired ovary weights were significantly increased only in the combined Subgroups. These data suggest that the combined Subgroups provided increased statistical power in this study.

Summary – Flutamide

90. The two flutamide studies are in relative agreement. There were significant decreases in the accessory tissue of the male reproductive tract. In the laboratory 2 study, these decreases were sometimes seen at Flutamide doses as low as 1 mg/kg/d. In the laboratory 11 study, these decreases were often observed only in the combined Subgroups at a dose of 10 mg/kg/d. Changes in male liver and female liver were evident in both studies. While absolute ovarian weights decreased in both studies, they achieved statistical significance only in left ovary of the laboratory 11 study.

91. Both studies clearly identify significant tissue weight changes in the male reproductive tract at flutamide doses of 1-10 mg/kg/d that would lead to suspicion of endocrine activity. The prostate lobes, seminal vesicles, and epididymis weights were decreased in both studies. Flutamide was effective in the Hershberger assay at doses of 0.1-1 mg/kg/d (Phase 1 report), indicating that the updated 407 compares well to screening assays that are mechanistically specific for androgens and antiandrogens.

92. The updated 407 studies are also consistent with developmental studies that indicate flutamide exposure *in utero* can induce frank malformations in the male reproductive tract (Imperato-McGinley et al 1992; McIntyre et al. 2001; Mylchreest et al. 1999; Neri et al. 1972; Viguiet-Martinez et al 1983). Therefore, with these tissue weight endpoints, the updated 407 was successful in identifying the possible activity of flutamide.

p,p'-DDE

Laboratory 6 –*p,p'*-DDE

93. Gross pathology observations included significantly enlarged livers in both sexes that appeared to increase in a dose related manner. In males, relevant observations included, in some individuals, small prostate, small seminal vesicles, flaccid testes, and thin red liquid in the thoracic cavity.

94. The absolute weights discussed for this study are shown in Table 15A and the relative weights in Table 15B. Absolute liver weights were significantly increased in males at all doses and in females at the respective mid- and high doses. Relative liver weights were significantly increased in males at the high dose. Absolute and relative thyroid weights were significantly increased in females at the high dose. A trend to higher kidney weights in both sexes did not achieve statistical significance on either an absolute or relative basis in either sex.

95. There were no significant absolute or relative weight changes in the reproductive tracts of either sex.

Table 15A. Absolute Organ Weights with *p,p'*-DDE in the Laboratory 6 Study

<i>p,p'</i> -DDE Dose (mg/kg/day)	Control	Low	Middle	High
	0	12.5	50	200/150
Male Body Wt Δ 28d		+2.5%	+6.1%	-1.4%
Male Body Wt Δ Necropsy		+3.6%	+6.2%	-1.7%
Liver (g)	14.55 ± 2.00	*17.35 ± 1.88	**23.58 ± 2.27	**30.12 ± 2.02
Subgroup A	13.34 ± 2.08	**17.84 ± 1.13	**24.26 ± 2.21	**30.14 ± 2.60
Subgroup B	15.76 ± 0.98	16.86 ± 2.46	**22.89 ± 2.36	**30.08 ± 1.67
Thyroid (mg)	26.2 ± 5.5	26.1 ± 5.0	31.6 ± 4.7	27.5 ± 4.5
Subgroup A	24.3 ± 4.4	25.4 ± 2.7	**32.9 ± 3.0	24.5 ± 2.5
Subgroup B	28.0 ± 6.4	26.7 ± 6.9	30.3 ± 6.0	32.0 ± 1.4
<i>p,p'</i> -DDE Dose (mg/kg/day)	0	6.5	25	100
Female Body Wt Δ at 28 d		+5.1%	+1.3%	+5.6%
Female Body Wt Δ at Necropsy		+5.3%	+1.5%	+6.6%
Liver (g)	8.34 ± 0.67	9.94 ± 1.15	**11.63 ± 1.35	**19.91 ± 3.20 ⁹
Subgroup A	8.50 ± 0.50	9.68 ± 0.99	*11.04 ± 1.28	**18.69 ± 2.34
Subgroup B	8.18 ± 0.84	10.20 ± 1.36	*12.21 ± 1.28	**20.83 ± 3.77 ⁴
Thyroid (mg)	22.3 ± 4.0	23.4 ± 2.6	22.7 ± 3.5	**28.7 ± 4.7 ⁹
Subgroup A	23.8 ± 3.1	23.4 ± 1.8	22.0 ± 2.5	*29.2 ± 4.07
Subgroup B	20.9 ± 4.6	23.3 ± 3.4	23.4 ± 4.6	28.4 ± 5.7 ⁴

Values expressed as mean ± SD.

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

Table 15B. Relative Organ Weights with *p,p'*-DDE in the Laboratory 6 Study

<i>p,p'</i> -DDE Dose (mg/kg/day)		Control	Low	Middle	High
		0	12.5	50	200/150
Male Body Wt Δ 28d			+2.5%	+6.1%	-1.4%
Male Body Wt Δ Necropsy			+3.6%	+6.2%	-1.7%
Liver		3.89%	4.48%	**5.94%	**8.20%
Subgroup A		3.76%	*4.53%	**6.14%	**8.30%
Subgroup B		4.01%	4.43%	**5.74%	**8.06%
Kidney	Males	0.75%	0.75%	0.77%	**0.87%
	Subgroup A	0.74%	0.74%	0.75%	**0.88%
	Subgroup B	0.76%	0.75%	0.79%	0.88%
Thyroid	Males	0.0070%	0.0067%	0.0080%	0.0075%
	Subgroup A	0.0068%	0.0065%	*0.0083%	0.0067%
	Subgroup B	0.0071%	0.0070%	0.0076%	0.0086%
Epididymes		0.261%	0.223%	0.235%	0.245%
Subgroup A		0.279%	**0.239%	**0.233%	*0.247%
Subgroup B		0.244%	0.206%	0.237%	0.242%
Ventral Prostate		0.145%	0.151%	0.135%	0.139%
Subgroup A		0.136%	0.168%	0.122%	0.144%
Subgroup B		0.153%	0.134%	0.149%	0.131%
Dorsolateral Prostate		0.046%	0.032%	0.043%	0.045%
Subgroup A		0.047%	0.035%	0.036%	0.042%
Subgroup B		0.045%	0.029%	0.051%	0.049%
Seminal Vesicles		0.353%	0.342%	0.334%	0.382%
Subgroup A		0.345%	0.343%	0.330%	0.396%
Subgroup B		0.360%	0.341%	0.338%	0.361%
<i>p,p'</i> -DDE Dose (mg/kg/day)			6.5	25	100
Female Body Wt Δ at 28 d			+5.1%	+1.3%	+5.6%
Female Body Wt Δ at Necropsy			+5.3%	+1.5%	+6.6%
Liver	Females	3.64%	4.12%	**5.00%	**8.15%
	Subgroup A	3.67%	4.12%	**4.84%	**7.90%
	Subgroup B	3.61%	4.12%	*5.15%	**8.33%
Thyroid	Females	0.0097%	0.0097%	0.0098%	*0.0117%
	Subgroup A	0.0103%	0.0100%	0.0096%	0.0123%
	Subgroup B	0.0092%	0.0094%	0.0099%	0.0114%

Values expressed as mean ± SD.

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

96. Dramatic and significant increases in absolute and relative liver weights occurred in both sexes at the mid- and high doses in the combined Subgroups and both individual Subgroups. At the low dose of 12.5 mg/kg/d DDE, significant increases in males occurred for absolute liver weights in the combined and one individual Subgroup, while for relative weights, a significant increase occurred only in Subgroup A. In females, absolute thyroid weights were significantly increased in the combined Subgroups and Subgroup A at the high dose. The female relative thyroid weights were significantly increased only in the combined Subgroups and neither individual Subgroup at the high dose.

Laboratory 7 – *p,p'*-DDE

97. Gross pathology observations related to treatment were the enlargement and discoloration of the liver in both sexes that increased in a dose related manner.

98. The absolute weights discussed for this study are shown in Table 16A and the relative weights in Table 16B. Absolute liver weights were significantly increased in males at all doses and in females at the respective mid- and high doses. Relative weights of the kidney were significantly increased in males at the high dose. The relative thyroid weight was significantly increased in males at the high dose.

99. There were no significant absolute or relative weight changes in the reproductive tracts of males. In females, the relative weights of the ovaries were significantly increased at the high dose.

Table 16A. Absolute Organ Weights with *p,p'*-DDE in the Laboratory 7 Study

<i>p,p'</i> -DDE Dose (mg/kg/day)		Control	Low	Middle	High
		0	12.5	50	100
Male Body Wt Δ 28 d			-1.6%	-0.8%	-4.7%
Male Body Wt Δ Necropsy			-1.9%	-1.2%	-4.7%
Liver (g)	Males	16.04 ± 1.79	**18.83 ± 1.53	**23.00 ± 1.90	**28.65 ± 2.09
	Subgroup A	16.66 ± 2.23	19.71 ± 1.28	**22.12 ± 2.07	**29.07 ± 2.43
	Subgroup B	15.42 ± 1.11	*17.96 ± 1.32	**23.89 ± 1.36	**28.24 ± 1.87
<i>p,p'</i> -DDE Dose (mg/kg/day)		0	6.5	25	75
Female Body Wt Δ at 28 d			-0.6%	-1.2%	-3.6%
Female Body Wt Δ at Necropsy			-0.2%	-0.7%	-8.0%
Liver (g)	Females	9.38 ± 0.98	10.37 ± 1.10	**12.48 ± 1.61	**16.25 ± 3.14
	Subgroup A	9.32 ± 1.04	10.15 ± 1.53	*12.83 ± 1.95	**17.74 ± 3.33
	Subgroup B	9.44 ± 1.02	10.59 ± 0.52	*12.12 ± 1.30	**14.75 ± 2.36

Values expressed as mean ± SD.

*, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively).

Table 16B. Relative Organ Weights with *p,p'*-DDE in the Laboratory 7 Study

<i>p,p'</i> -DDE Dose (mg/kg/day)		Control	Low	Middle	High
		0	12.5	50	100
Male Body Wt Δ 28 d			-1.6%	-0.8%	-4.7%
Male Body Wt Δ Necropsy			-1.9%	-1.2%	-4.7%
Liver	Males	3.75%	**4.49%	**5.44%	**7.03%
	Subgroup A	3.84%	**4.61%	**5.33%	**7.03%
	Subgroup B	3.64%	**4.35%	**5.55%	**7.03%
Kidney	Males	0.65%	0.67%	0.69%	**0.73%
	Subgroup A	0.67%	0.68%	0.67%	0.72%
	Subgroup B	0.63%	0.65%	*0.72%	**0.75%
Adrenal	Males	0.0125%	0.0124%	0.0123%	0.0139%
	Subgroup A	0.0135%	0.0118%	0.0128%	0.0131%
	Subgroup B	0.0115%	0.0129%	0.0117%	**0.0146%
Thyroid	Males	0.0045%	0.0046%	0.0061%	*0.0063%
	Subgroup A	0.0045%	0.0049%	0.0066%	*0.0062%
	Subgroup B	0.0046%	0.0043%	0.0056%	0.0065%
Epididymes		0.214%	0.222%	0.214%	0.209%
Subgroup A		0.220%	0.222%	0.217%	0.204%
Subgroup B		0.209%	0.222%	0.212%	0.213%
Ventral Prostate		0.158%	0.152%	0.175%	0.154%
Subgroup A		0.155%	0.172%	0.178%	0.157%
Subgroup B		0.160%	0.133%	0.171%	0.151%
Dorsolateral Prostate		0.129%	0.120%	0.118%	0.110%
Subgroup A		0.129%	0.124%	0.110%	0.106%
Subgroup B		0.130%	0.115%	0.126%	0.114%

Table 16B continued. Relative Organ Weights with *p,p'*-DDE in the Laboratory 7 Study

<i>p,p'</i> -DDE Dose (mg/kg/day)		Control	Low	Middle	High
		0	12.5	50	100
Seminal Vesicles		0.319%	0.321%	0.327%	0.325%
Subgroup A		0.331%	0.317%	0.312%	0.318%
Subgroup B		0.306%	0.325%	0.342%	0.331%
<i>p,p'</i> -DDE Dose (mg/kg/day)		0	6.5	25	75
Female Body Wt Δ at 28 d			-0.6%	-1.2%	-3.6%
Female Body Wt Δ at Necropsy			-0.2%	-0.7%	-8.0%
Liver	Females	3.66%	4.05%	**4.89%	**6.98%
	Subgroup A	3.68%	4.01%	*4.98%	**6.90%
	Subgroup B	3.64%	4.10%	*4.81%	**7.07%
Kidneys	Females	0.66%	0.68%	0.70%	0.79%
	Subgroup A	0.68%	0.70%	*0.70%	0.69%
	Subgroup B	0.63%	0.66%	0.70%	*0.84%
Thyroid	Females	0.0073%	0.0065%	0.0068%	0.0087%
	Subgroup A	0.0072%	0.0068%	0.0073%	0.0090%
	Subgroup B	0.0073%	0.0061%	0.0063%	0.0085%
Ovaries		0.030%	0.031%	0.034%	**0.041%
	Subgroup A	0.030%	0.033%	0.032%	**0.042%
	Subgroup B	0.030%	0.030%	0.036%	*0.039%

Values expressed as mean ± SD. *, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively).

100. Dramatic and significant increases in absolute and relative liver weights occurred in both sexes at the mid- and high doses in the combined Subgroups and both individual Subgroups. In males, the liver increases extended to the low dose of 12.5 mg/kg/d. Relative liver weights increased significantly in the combined Subgroups and both individual Subgroups, and the absolute liver weights increased significantly in the combined Subgroups and Subgroup B. Relative male kidney and thyroid weights were significantly increased at the high dose in the combined Subgroups, but in only one of the individual Subgroups. Relative kidney weights were increased at the mid-dose in Subgroup A and the high dose in Subgroup B, but at no dose in the combined Subgroups. The relative ovarian weights were significantly increased at the high doses in the combined Subgroups and both individual Subgroups.

Summary – *p,p'*-DDE

101. The dramatic effects of DDE on the liver in both sexes were repeated in both studies. An increase in the male relative kidney weights were seen in both studies. The female relative thyroid weights increased in both studies. The relative weights of the male thyroid in the laboratory 7 study were significantly increased, but not in the laboratory 6 study. There were no consistent effects on the male reproductive tract in either study, other than in the epididymes of one Subgroup in the laboratory 6 study, which could be interpreted as a spurious result due to high control values.

102. The antiandrogenic activity of DDE has been reported in several studies (Gray et al. 1999; Kelce et al. 1995; You et al. 1998), but the effects have typically been limited to exquisitely sensitive parameters have in utero exposure such as anogenital distance and nipple retention at doses of approximately 100 mg/kg/d. Continued exposure through puberty appears necessary to delay preputial separation and to result in modest decreases in some tissues in the male reproductive tract (Gray et al. 1999; Kelce et al. 1995). In Phase 2 of the Hershberger validation, with 2 of 3 laboratories reporting, the minimal effective doses of *p,p'*-DDE on one or more of the five reproductive tract tissues has been 50-160 mg/kg/d.

103. In addition, screening assays which incorporate endpoints capable of detecting several endocrine modes of action, do not detect *p,p'*-DDE as an antiandrogen, but rather thyroid toxicity is detected including significant increases in both liver and thyroid weights at 50 mg/kg/d similar to the current 407 studies (O'Connor et al. 1999, 2002). In the absence of a long-term reproductive study to indicate whether *p,p'*-DDE acts via antiandrogenic, antithyroid, or both mechanisms, the predictive ability of the 407 for weak antiandrogens may be unresolved.

Propylthiouracil

Laboratory 1 – Propylthiouracil

104. The significant gross pathology findings were enlarged thyroids in both the male and female mid- and high dose groups. Other observations were in single individuals with no obvious dose relationship.

105. The absolute weights discussed for this study are shown in Table 17A and the relative weights in Table 17B. Absolute liver, kidney, heart, spleen and adrenal weights were significantly decreased in both sexes at the high dose. Absolute thymus weights were significantly decreased and brain weights significantly increased in males at the high dose. However, body weight decreases at the high dose were 17-18% in both sexes. Relative liver, kidney, heart, spleen and adrenal weights were significantly decreased in the females at the high dose; relative kidney weights were significantly decreased in males at the high dose, relative adrenal weights were significantly decreased in males at the mid- and high dose, relative brain weights were significantly increased in both sexes at the mid- and high dose, relative pituitary weights were significantly increased in males at the high dose, and relative pituitary weights were significantly increased in females at the mid- and high doses.

106. In the reproductive tract of males, the absolute seminal vesicle weights were significantly increased at the high dose, and the relative weights of the testes, epididymides, seminal vesicles and prostate were significantly increased at the high dose. In females, the absolute uterine weights were significantly decreased at all doses.

107. The absolute thyroid weights were significantly increased in both sexes at the mid- and high doses (1 and 10 mg/kg/d, respectively).

Table 17A. Absolute Organ Weights with Propylthiouracil in the Laboratory 1 Study

Propylthiouracil Dose (mg/kg/day)		Control	Low	Middle	High
		0	0.1	1	10
Male Body Wt Δ 28d			+4.6%	-1.0%	$\Downarrow\Downarrow$ -17.8%
Male Body Wt Δ Necropsy			+4.2%	-1.1%	$\Downarrow\Downarrow$ -17.7%
Liver (g)	Male	9.94 \pm 1.13	10.80 \pm 1.42	10.07 \pm 1.28	**7.86 \pm 0.68
	Subgroup A	9.68 \pm 0.78	10.78 \pm 1.66	10.03 \pm 1.26	**7.72 \pm 0.56
	Subgroup B	10.21 \pm 1.45	10.82 \pm 1.34	10.10 \pm 1.45	*8.00 \pm 0.83
Kidneys (g)	Male	1.944 \pm 0.146	2.016 \pm 0.174	1.910 \pm 0.125	**1.43 \pm 0.095
	Subgroup A	1.940 \pm 0.136	2.038 \pm 0.237	1.852 \pm 0.088	**1.396 \pm 0.122
	Subgroup B	1.948 \pm 0.173	1.994 \pm 0.105	1.968 \pm 0.138	**1.444 \pm 0.062
Heart (g)	Male	0.905 \pm 0.083	0.947 \pm 0.054	0.865 \pm 0.068	**0.664 \pm 0.042
	Subgroup A	0.918 \pm 0.034	0.942 \pm 0.064	0.854 \pm 0.050	**0.652 \pm 0.052
	Subgroup B	0.892 \pm 0.118	0.952 \pm 0.048	0.876 \pm 0.088	**0.676 \pm 0.029
Adrenals (mg)	Male	61.8 \pm 10.62	63.6 \pm 7.72	63.5 \pm 11.12	**45.3 \pm 6.48
	Subgroup A	63.0 \pm 10.63	61.4 \pm 5.41	69.4 \pm 13.33	*45.6 \pm 8.74
	Subgroup B	60.6 \pm 11.72	65.8 \pm 9.63	57.6 \pm 3.65	*45.0 \pm 4.24
Spleen (g)	Male	0.548 \pm 0.120	0.551 \pm 0.039	0.502 \pm 0.093	**0.376 \pm 0.034
	Subgroup A	0.532 \pm 0.101	0.552 \pm 0.036	0.504 \pm 0.071	*0.394 \pm 0.040
	Subgroup B	0.564 \pm 0.146	0.550 \pm 0.046	0.500 \pm 0.121	**0.358 \pm 0.015
Thymus (mg)	Male	528.4 \pm 108.0	509.6 \pm 99.7	476.5 \pm 108.9	**334.0 \pm 53.0
	Subgroup A	477.0 \pm 69.16	507.0 \pm 111.3	504.8 \pm 78.4	*325.0 \pm 64.97
	Subgroup B	579.8 \pm 121.96	512.2 \pm 99.77	448.2 \pm 136.2	*343.0 \pm 43.62
Brain (g)	Male	1.896 \pm 0.061	1.916 \pm 0.070	1.895 \pm 0.070	**1.812 \pm 0.054
	Subgroup A	1.882 \pm 0.037	1.926 \pm 0.051	1.860 \pm 0.034	1.808 \pm 0.073
	Subgroup B	1.910 \pm 0.080	1.906 \pm 0.090	1.930 \pm 0.083	1.816 \pm 0.034
Thyroid (mg)	Male	16.9 \pm 3.64	16.3 \pm 3.13	**48.4 \pm 9.54	**100.4 \pm 19.41
	Subgroup A	17.4 \pm 3.58	14.4 \pm 2.70	**46.4 \pm 12.05	**102.8 \pm 22.84
	Subgroup B	16.4 \pm 4.04	18.2 \pm 2.39	**50.4 \pm 7.02	**98.0 \pm 17.65
Seminal vesicles (g)		0.619 \pm 0.131	0.651 \pm 0.162	0.543 \pm 0.125	*0.785 \pm 0.154
	Subgroup A	0.682 \pm 0.086	0.56 \pm 0.176	0.548 \pm 0.158	0.7425 \pm 0.178
	Subgroup B	0.556 \pm 0.146	*0.742 \pm 0.085	0.538 \pm 0.100	*0.828 \pm 0.132

Table 17A continued. Absolute Organ Weights with Propylthiouracil in the Laboratory 1 Study

Propylthiouracil Dose (mg/kg/day)		Control	Low	Middle	High
		0	0.1	1	10
Female Body Wt Δ at 28 d			-0.9%	-4.3%	\downarrow -12.8%
Female Body Wt Δ at Necropsy			+6.7%	-0.2%	$\downarrow\downarrow$ -17.6%
Liver (g)	Female	7.30 \pm 0.607	7.30 \pm 0.555	6.72 \pm 0.655	**5.80 \pm 0.607
	Subgroup A	7.16 \pm 0.724	7.39 \pm 0.261	6.62 \pm 0.558	*5.73 \pm 0.809
	Subgroup B	7.45 \pm 0.501	7.21 \pm 0.7781	6.82 \pm 0.793	**5.88 \pm 0.398
Kidneys (g)	Female	1.416 \pm 0.092	1.489 \pm 0.113	1.421 \pm 0.103	**1.13 \pm 0.076
	Subgroup A	1.382 \pm 0.092	1.518 \pm 0.125	1.372 \pm 0.08	**1.164 \pm 0.086
	Subgroup B	1.450 \pm 0.087	1.460 \pm 0.106	1.470 \pm 0.108	**1.100 \pm 0.054
Heart (g)	Female	0.708 \pm 0.062	0.724 \pm 0.068	0.679 \pm 0.080	**0.564 \pm 0.054
	Subgroup A	0.690 \pm 0.076	0.724 \pm 0.069	0.684 \pm 0.091	*0.568 \pm 0.051
	Subgroup B	0.726 \pm 0.044	0.724 \pm 0.076	0.674 \pm 0.078	**0.560 \pm 0.063
Adrenals (mg)	Female	78.4 \pm 11.97	79.0 \pm 9.25	73.9 \pm 7.96	**49.5 \pm 7.47
	Subgroup A	76.2 \pm 13.03	74.4 \pm 5.51	70.4 \pm 9.15	*50.2 \pm 7.33
	Subgroup B	80.6 \pm 11.87	83.6 \pm 10.46	77.4 \pm 5.32	*48.8 \pm 8.41
Spleen (g)	Female	0.523 \pm 0.075	*0.460 \pm 0.045	0.473 \pm 0.087	**0.308 \pm 0.042
	Subgroup A	0.546 \pm 0.068	*0.460 \pm 0.036	*0.428 \pm 0.068	**0.312 \pm 0.022
	Subgroup B	0.500 \pm 0.082	0.460 \pm 0.057	0.518 \pm 0.085	*0.304 \pm 0.059
Thyroid (mg)	Female	15.3 \pm 2.54	16.5 \pm 5.50	**36.4 \pm 8.46	**101.7 \pm 24.22
	Subgroup A	14.6 \pm 1.95	**18.2 \pm 1.30	**33.8 \pm 7.98	**104.0 \pm 27.39
	Subgroup B	16.0 \pm 3.08	14.8 \pm 7.69	**39.0 \pm 8.97	**99.4 \pm 23.59
Uterus plus cervix (g)		0.445 \pm 0.077	**0.359 \pm 0.054	*0.397 \pm 0.040	**0.359 \pm 0.037
Subgroup A		0.414 \pm 0.103	0.362 \pm 0.054	0.376 \pm 0.019	0.362 \pm 0.041
Subgroup B		0.476 \pm 0.021	**0.356 \pm 0.060	0.418 \pm 0.045	**0.356 \pm 0.038

Values were expressed as mean \pm SD.

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

Table 17B. Relative Organ Weights with Propylthiouracil in the Laboratory 1 Study

Propylthiouracil Dose (mg/kg/day)		Control	Low	Middle	High
		0	0.1	1	10
Male Body Wt Δ 28d			+4.6%	-1.0%	$\downarrow\downarrow$ -17.8%
Male Body Wt Δ Necropsy			+4.2%	-1.1%	$\downarrow\downarrow$ -17.7%
Liver	Male	3.66%	3.81%	3.74%	3.51%
	Subgroup A	3.60%	3.76%	3.74%	3.49%
	Subgroup B	3.71%	3.87%	3.74%	3.54%
Kidneys	Male	0.71%	0.71%	0.71%	**0.63%
	Subgroup A	0.72%	0.71%	0.69%	*0.63%
	Subgroup B	0.71%	0.71%	0.73%	*0.64%

Table 17B continued. Relative Organ Weights with Propylthiouracil in the Laboratory 1 Study

Propylthiouracil Dose (mg/kg/day)		Control	Low	Middle	High
		0	0.1	1	10
Heart	Male	0.33%	0.33%	*0.32%	**0.30%
	Subgroup A	0.34%	0.33%	**0.32%	**0.29%
	Subgroup B	0.32%	0.34%	0.32%	0.30%
Adrenals	Male	0.0227%	0.0224%	0.0236%	0.0203%
	Subgroup A	0.0234%	0.0214%	0.0259%	0.0206%
	Subgroup B	0.0220%	0.0235%	0.0213%	0.0199%
Spleen	Male	0.20%	0.19%	0.19%	0.17%
	Subgroup A	0.20%	0.19%	0.19%	0.18%
	Subgroup B	0.20%	0.20%	0.19%	0.16%
Thymus	Male	0.19%	0.18%	0.18%	**0.15%
	Subgroup A	0.18%	0.18%	0.19%	0.15%
	Subgroup B	0.21%	0.18%	0.17%	0.15%
Brain	Male	0.70%	0.68%	0.70%	**0.81%
	Subgroup A	0.70%	0.67%	0.69%	**0.82%
	Subgroup B	0.69%	0.68%	0.71%	*0.80%
Pituitary	Male	0.0030%	0.0030%	0.0031%	**0.0041%
	Subgroup A	0.0030%	0.0029%	0.0032%	*0.0042%
	Subgroup B	0.0030%	0.0031%	0.0030%	**0.0039%
Thyroid	Male	0.006%	0.006%	**0.018%	**0.045%
	Subgroup A	0.006%	0.005%	**0.017%	**0.046%
	Subgroup B	0.006%	0.007%	**0.019%	**0.043%
Testes		1.08%	1.06%	1.08%	**1.32%
Subgroup A		1.11%	1.02%	1.11%	**1.33%
Subgroup B		1.04%	1.10%	1.05%	**1.32%
Seminal vesicles		0.228%	0.230%	0.202%	**0.351%
Subgroup A		0.254%	0.195%	0.204%	0.335%
Subgroup B		0.202%	0.265%	0.199%	*0.366%
Ventral Prostate		0.108%	0.105%	0.103%	*0.140%
Subgroup A		0.103%	0.097%	0.099%	0.145%
Subgroup B		0.112%	0.114%	0.106%	0.134%
Subgroup Body Wt Δ at 28 d			-0.9%	-4.3%	↓ -12.8%
Subgroup Body Wt Δ at Necropsy			+6.7%	-0.2%	↓↓ -17.6%
Liver	Female	3.68%	3.74%	3.54%	**3.37%
	Subgroup A	3.66%	3.76%	3.47%	3.36%
	Subgroup B	3.69%	3.71%	3.61%	3.39%
Kidneys	Female	0.71%	0.76%	0.75%	**0.66%
	Subgroup A	0.71%	0.77%	0.72%	0.68%
	Subgroup B	0.72%	0.75%	0.78%	**0.63%
Heart	Female	0.36%	0.37%	0.36%	**0.33%
	Subgroup A	0.35%	0.37%	0.36%	0.33%
	Subgroup B	0.36%	0.37%	0.36%	*0.32%
Brain	Female	0.90%	0.91%	*0.94%	**1.03%
	Subgroup A	0.91%	0.91%	0.94%	*1.00%
	Subgroup B	0.89%	0.90%	0.94%	*1.05%
Adrenals	Female	0.0395%	0.0404%	0.0389%	**0.0288%
	Subgroup A	0.0390%	0.0379%	0.0369%	0.0294%
	Subgroup B	0.0399%	0.0430%	0.0410%	*0.0282%

Table 17B continued. Relative Organ Weights with Propylthiouracil in the Laboratory 1 Study

Propylthiouracil Dose (mg/kg/day)	Control	Low	Middle	High
	0	0.1	1	10
Spleen	0.26%	0.24%	0.25%	**0.18%
Subgroup A	0.28%	0.23%	0.22%	**0.18%
Subgroup B	0.25%	0.24%	0.27%	*0.18%
Thymus	0.22%	0.26%	0.21%	0.20%
Subgroup A	0.20%	0.28%	0.19%	0.21%
Subgroup B	0.25%	0.25%	0.22%	*0.19%
Pituitary	0.0048%	0.0054%	*0.0057%	**0.0067%
Subgroup A	0.0046%	0.0059%	0.0054%	*0.0066%
Subgroup B	0.0050%	0.0048%	*0.0061%	**0.0068%
Thyroid	0.008%	0.008%	**0.019%	**0.059%
Subgroup A	0.007%	*0.009%	**0.018%	**0.061%
Subgroup B	0.008%	0.008%	**0.021%	**0.057%
Uterus plus cervix	0.224%	**0.184%	0.209%	0.209%
Subgroup A	0.212%	0.184%	0.197%	0.212%
Subgroup B	0.236%	**0.183%	0.221%	0.205%

Values were expressed as mean \pm SD.

*, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively).

108. The declines in body weights approached 18% in both sexes at the high dose of PTU and resulted in numerous statistically significant changes in absolute organ weights. Statistical significance usually occurred in the combined Subgroups and both individual Subgroups. Simultaneously, there were statistically significant increases in thyroid weights at the mid- and high doses in both sexes in the combined Subgroups and both individual Subgroups. Statistically significant relative weight increases in conserved organs such as the brain, testes, and pituitary were observed in the combined Subgroups and both individual Subgroups at the high dose of PTU. In the case of the relative heart, thymus, seminal vesicle, and ventral prostate weights in males and the relative liver, kidney, heart, and adrenal weights in females, significant changes in the combined Subgroups were observed in only one or in neither of the individual Subgroups. This would support the increased statistical power of the combined Subgroups, but this would not have contributed to any substantive changes in the LOELs or the detection of the thyroid effects in this PTU study.

Laboratory 10 – Propylthiouracil

109. No gross pathology findings were recorded in the final report.

110. The absolute weights discussed for this study are shown in Table 18A and the relative weights in Table 18B. The liver, left and right kidney, lung, and spleen weights were significantly decreased in males at the high dose. The heart and thymus weights were significantly decreased in both sexes at the high dose. Thyroid weights were not recorded in the final report received by the Secretariat.

Table 18A. Absolute Organ Weights with Propylthiouracil in the Laboratory 10 Study

Propylthiouracil Dose (mg/kg/day)	Control	Low	Middle	High
	0	0.1	1	10
Male Body Wt Δ at Necropsy		-0.9%	+0.7%	↓ -16.8%
Liver (g)	11.34 ± 1.26	10.72 ± 2.00	11.59 ± 1.01	*8.90 ± 3.38
L Kidney (g)	1.20 ± 0.14	1.15 ± 0.20	1.22 ± 0.07	*0.92 ± 0.35
R Kidney (g)	1.16 ± 0.07	1.13 ± 0.20	1.21 ± 0.08	*0.92 ± 0.35
Spleen (g)	0.83 ± 0.05	0.76 ± 0.13	0.79 ± 0.10	*0.59 ± 0.24
Heart (g)	1.13 ± 0.07	1.11 ± 0.09	1.13 ± 0.17	*0.90 ± 0.32
Lung (g)	2.09 ± 0.16	*1.76 ± 0.13	2.25 ± 0.28	*1.80 ± 0.64
Thymus (g)	0.82 ± 0.24	*0.46 ± 0.13	0.74 ± 0.09	*0.49 ± 0.26
Female Body Wt Δ at Necropsy		+1.0%	+0.8%	↓ -8.7%
Heart (g)	0.83 ± 0.06	0.78 ± 0.10	0.75 ± 0.10	*0.62 ± 0.10
Thymus (g)	0.41 ± 0.08	0.46 ± 0.14	0.37 ± 0.08	*0.27 ± 0.10

Values were expressed as mean ± SD.

*, Significantly different from control (p<0.05) – no indication that p<0.01 was analyzed

Table 18B. Relative Organ Weights with Propylthiouracil in the Laboratory 10 Study

Propylthiouracil Dose (mg/kg/day)	Control	Low	Middle	High
	0	0.1	1	10
Male Body Wt Δ at Necropsy		-0.9%	+0.7%	↓ -16.8%
Liver	3.37%	3.21%	3.42%	*3.17%
Kidney	0.70%	0.68%	0.72%	*0.66%
Spleen	0.25%	0.23%	0.23%	*0.21%
Heart	0.34%	0.33%	0.33%	0.32%
Lung	0.62%	*0.53%	0.66%	0.64%
Thymus	0.24%	*0.14%	0.22%	*0.17%
Female Body Wt Δ at Necropsy		+1.0%	+0.8%	↓ -8.7%
Heart	0.38%	0.35%	0.34%	0.31%
Thymus	0.19%	0.21%	0.17%	*0.13%

Values were expressed as mean ± SD.

*, Significantly different from control (p<0.05) – no indication that p<0.01 was analyzed

Summary – Propylthiouracil

111. The profile of changes between the two studies has limited similarities, particularly, given that gross pathology and thyroid weights were not recorded in the final report of the laboratory 10 study. The pattern of relative organ weight increases in conserved organs such as the brain, testes, and pituitary in the laboratory 1 study were not found in the laboratory 10 study, despite similar changes in body weights. Due to the body weight changes, only changes in relative weights will be considered further in making the comparison. For males, only decreases in relative thymus and kidney weights were consistent between the two studies. For females, only the decrease in relative thymus weights was consistent.

112. The laboratory 1 study clearly identifies significant changes in the thyroid at PTU doses of 1 and 10 mg/kg/d. There was clear enlargement of the thyroid visually evident in gross pathology and dramatic weight increases in the thyroid that would lead to suspicion of endocrine activity. Therefore, with these endpoints, the updated 407 was successful in identifying the possible activity of PTU.

L-Thyroxine**Laboratory 9 – L-Thyroxine**

114. Gross pathology findings in the high dose groups of both sexes were thyroid discoloration, spleen enlargement, and bilateral kidney enlargement.

115. The absolute weights discussed for this study are shown in Table 19A and the relative weights in Table 19B. The absolute and relative weights of heart, spleen, kidney, and adrenals were significantly increased in both sexes at the high dose. The absolute and relative liver weights were significantly increased and the relative thyroid weight was significantly decreased in the female high dose group. The absolute adrenal weights were significantly increased in the female mid-dose group.

116. The putative target organ is the thyroid. The female absolute thyroid weights were not significantly different at any dose; the male thyroids were significantly increased in the low dose group, but this was judged to be a spurious result. The female relative thyroid weights were significantly decreased at the $p < 0.05$ level at the high dose, but the male relative weights were unchanged.

Table 19A. Absolute Organ Weights with l-Thyroxine in the Laboratory 9 Study

L-thyroxine Dose (mg/kg/day)	Control	Low	Middle	High
	0	0.01	0.1	1
Male Body Wt Δ 28d		+0.8%	+3.6%	-2.4%
Male Body Wt Δ Necropsy		+0.6%	+2.5%	-2.2%
Kidneys (g)	2.75 \pm 0.166	2.74 \pm 0.171	2.79 \pm 0.207	**3.38 \pm 0.225
Subgroup A	2.77 \pm 0.175	2.69 \pm 0.169	2.83 \pm 0.241	**3.37 \pm 0.288
Subgroup B	2.73 \pm 0.173	2.79 \pm 0.176	2.74 \pm 0.182	**3.39 \pm 0.176
Adrenals (mg)	56.01 \pm 7.47	58.81 \pm 9.23	58.20 \pm 11.48	**69.47 \pm 5.77
Subgroup A	55.88 \pm 6.67	53.94 \pm 6.34	53.30 \pm 11.38	69.02 \pm 7.18
Subgroup B	56.14 \pm 8.99	63.68 \pm 9.60	63.10 \pm 10.35	69.92 \pm 4.79
Heart (g)	1.22 \pm 0.097	1.25 \pm 0.093	1.28 \pm 0.133	**1.72 \pm 0.106
Subgroup A	1.21 \pm 0.138	1.22 \pm 0.110	1.35 \pm 0.149	**1.77 \pm 0.055
Subgroup B	1.22 \pm 0.043	1.28 \pm 0.070	1.20 \pm 0.057	**1.68 \pm 0.130
Spleen (g)	0.67 \pm 0.076	0.73 \pm 0.091	0.77 \pm 0.114	**1.09 \pm 0.153
Subgroup A	0.65 \pm 0.061	0.71 \pm 0.095	0.78 \pm 0.145	**1.10 \pm 0.141
Subgroup B	0.69 \pm 0.091	0.74 \pm 0.096	0.76 \pm 0.089	**1.08 \pm 0.181
Thyroid (mg)	24.65 \pm 4.34	*29.75 \pm 6.04	23.33 \pm 4.12	23.15 \pm 3.04
Subgroup A	26.16 \pm 4.24	31.50 \pm 7.70	23.56 \pm 3.27	22.26 \pm 3.83
Subgroup B	23.14 \pm 4.32	28.00 \pm 3.90	23.10 \pm 5.23	24.04 \pm 2.05
L-thyroxine Dose (mg/kg/day)	Control	Low	Middle	High
	0	0.01	0.1	1
Female Body Wt Δ at 28 d		-0.9%	+1.0%	+1.4%
Female Body Wt Δ at Necropsy		-0.5%	+1.3%	+1.6%
Liver (g)	9.57 \pm 1.08	9.36 \pm 1.28	10.09 \pm 1.24	*11.16 \pm 1.17
Subgroup A	9.51 \pm 0.47	8.78 \pm 1.58	9.94 \pm 1.40	11.25 \pm 1.67
Subgroup B	9.63 \pm 1.55	9.94 \pm 0.59	10.23 \pm 1.22	11.07 \pm 0.48
Kidneys (g)	1.96 \pm 0.203	1.84 \pm 0.161	1.98 \pm 0.191	**2.36 \pm 0.222
Subgroup A	2.00 \pm 0.156	1.77 \pm 0.131	1.97 \pm 0.172	*2.31 \pm 0.162
Subgroup B	1.92 \pm 0.253	1.91 \pm 0.174	1.98 \pm 0.229	*2.40 \pm 0.282

Table 19A (continued). Absolute Organ Weights with l-Thyroxine in the Laboratory 9 Study

L-thyroxine Dose (mg/kg/day)	Control	Low	Middle	High
	0	0.01	0.1	1
Heart (g)	0.99 ± 0.127	0.93 ± 0.076	0.98 ± 0.069	**1.22 ± 0.127
Subgroup A	0.96 ± 0.047	0.91 ± 0.083	0.97 ± 0.054	**1.27 ± 0.144
Subgroup B	1.02 ± 0.180	0.95 ± 0.073	1.00 ± 0.085	1.17 ± 0.096
Adrenals (mg)	71.95 ± 7.78	68.59 ± 8.14	*82.21 ± 9.01	**94.00 ± 10.28
Subgroup A	70.40 ± 8.08	68.12 ± 8.24	80.38 ± 12.43	**95.32 ± 8.48
Subgroup B	73.50 ± 8.05	69.06 ± 8.99	84.04 ± 4.44	*92.68 ± 12.71
Spleen (g)	0.59 ± 0.080	0.59 ± 0.066	0.64 ± 0.085	**0.81 ± 0.077
Subgroup A	0.63 ± 0.065	0.57 ± 0.072	0.64 ± 0.061	**0.82 ± 0.106
Subgroup B	0.55 ± 0.081	0.61 ± 0.061	0.64 ± 0.112	**0.81 ± 0.044
Thyroid (mg)	22.08 ± 2.73	20.72 ± 4.09	20.91 ± 2.22	18.45 ± 2.40
Subgroup A	21.46 ± 3.10	21.00 ± 4.65	22.38 ± 2.08	18.36 ± 1.11
Subgroup B	22.70 ± 2.50	20.44 ± 3.97	19.44 ± 1.158	18.54 ± 3.42

Values were expressed as mean ± SD. *, **, Significantly different from control (p<0.05 and p<0.01, respectively). n = 10 Combined Subgroups; n = 5 individual Subgroups.

Table 19B. Relative Organ Weights with l-Thyroxine in the Laboratory 9 Study

L-thyroxine Dose (mg/kg/day)	Control	Low	Middle	High
	0	0.01	0.1	1
Male Body Wt Δ 28d		+0.8%	+3.6%	-2.4%
Male Body Wt Δ Necropsy		+0.6%	+2.5%	-2.2%
Kidneys	0.69%	0.68%	0.68%	**0.87%
Subgroup A	0.69%	0.67%	0.67%	**0.86%
Subgroup B	0.70%	0.69%	0.68%	**0.88%
Adrenals	0.0141%	0.0147%	0.0141%	**0.0179%
Subgroup A	0.0138%	0.0135%	0.0126%	*0.0176%
Subgroup B	0.0143%	0.0158%	0.0157%	0.0182%
Heart	0.31%	0.31%	0.31%	**0.44%
Subgroup A	0.30%	0.31%	0.32%	**0.45%
Subgroup B	0.31%	0.32%	0.30%	**0.44%
Spleen	0.17%	0.18%	0.19%	**0.28%
Subgroup A	0.16%	0.18%	0.18%	**0.28%
Subgroup B	0.18%	0.18%	0.19%	**0.28%
Thyroid	0.006%	*0.007%	0.006%	0.006%
Subgroup A	0.007%	0.008%	0.006%	0.006%
Subgroup B	0.006%	0.007%	0.006%	0.006%
L-thyroxine Dose (mg/kg/day)		Low	Middle	High
		0.01	0.1	1
Female Body Wt Δ at 28 d		-0.9%	+1.0%	+1.4%
Female Body Wt Δ at Necropsy		-0.5%	+1.3%	+1.6%
Liver	3.42%	3.36%	3.56%	**3.92%
Subgroup A	3.31%	3.30%	3.53%	*3.94%
Subgroup B	3.51%	3.22%	3.53%	3.94%
Kidneys	0.70%	0.66%	0.70%	**0.83%
Subgroup A	0.70%	0.67%	0.70%	**0.81%
Subgroup B	0.72%	0.63%	0.69%	**0.83%

Table 19B continued. Relative Organ Weights with L-Thyroxine in the Laboratory 9 Study

L-thyroxine Dose (mg/kg/day)		Low	Middle	High
		0.01	0.1	1
Heart		0.35%	0.35%	**0.43%
Subgroup A		0.33%	0.34%	**0.44%
Subgroup B		0.36%	0.34%	0.43%
Adrenals		0.0257%	0.0290%	**0.0331%
Subgroup A		0.0245%	0.0285%	**0.0334%
Subgroup B		0.0264%	0.0288%	0.0332%
Spleen		0.21%	0.23%	**0.28%
Subgroup A		0.22%	0.23%	**0.29%
Subgroup B		0.22%	0.22%	**0.29%
Thyroid		0.008%	0.007%	*0.006%
Subgroup A		0.008%	0.008%	0.006%
Subgroup B		0.008%	0.007%	*0.007%

Values were expressed as mean \pm SD. *, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively). n = 10 Combined Subgroups; n = 5 individual Subgroups.

117. Statistically significant changes were observed in the combined Subgroups and both individual Subgroups in the male absolute kidney, heart and spleen weights; the female absolute kidney, adrenal, and spleen weights; the male relative kidney, heart, and spleen weights; and the female relative kidney and spleen weights at the high dose. The combined Subgroups were changed significantly when one or both individual Subgroups did not achieve significance in several cases. These included male absolute kidney weights at the high dose, the absolute female liver and heart weights at the high dose, the absolute female adrenal weights at the mid-dose, the relative male adrenal weights at the high dose, and the relative female liver, heart, adrenal, and thyroid weights at the high dose.

Laboratory 13 – L-Thyroxine

118. Gross pathology findings were limited to increased spleen size in females.

119. The absolute weights discussed for this study are shown in Table 20A and the relative weights in Table 20B. Absolute and relative adrenal weights were significantly increased in males at the high dose and in females at the mid- and high dose. Absolute and relative heart weights were significantly increased in both sexes at the high dose and in females at the mid-dose. Absolute and relative kidney heart weights were significantly increased in both sexes at the high dose and in males at the mid-dose. Absolute liver weights were significantly increased in females at the high dose, and relative liver weights in both sexes at the high dose and females at the mid-dose. Absolute and relative spleen weights were significantly increased in both sexes at the high dose, and absolute weights in the males at the mid-dose.

120. No significant changes were observed in thyroid weights or the reproductive tract organs and tissues of either sex. However, clear histopathological evidence indicating an atrophic effect on the thyroid was observed in both studies (Annex 4).

Table 20A. Absolute Organ Weights with l-Thyroxine in the Laboratory 13 Study

L-thyroxine Dose (mg/kg/day)	Control	Low	Middle	High
	0	0.01	0.1	1
Male Body Wt Δ 28d		-0.4%	+3.5%	+1.7%
Male Body Wt Δ Necropsy		-0.3%	+3.0%	-0.03%
Kidneys (g)	2.259 \pm 0.203	2.280 \pm 0.164	*2.550 \pm 0.177	**3.046 \pm 0.327
Heart (g)	1.085 \pm 0.111	1.063 \pm 0.090	1.162 \pm 0.105	**1.414 \pm 0.192
Adrenals (g)	0.067 \pm 0.007	0.065 \pm 0.013	0.070 \pm 0.008	**0.095 \pm 0.011
Spleen (g)	0.692 \pm 0.075	0.687 \pm 0.093	*0.830 \pm 0.136	**1.048 \pm 0.152
Thyroid (mg)	27.3 \pm 4.8	23.1 \pm 4.5	23.4 \pm 5.6	25.8 \pm 5.2
Female Body Wt Δ at 28 d		+0.3%	+2.9%	+6.8%
Female Body Wt Δ at Necropsy		-0.7%	+3.4%	+4.2%
Liver (g)	8.34 \pm 0.86	8.46 \pm 0.63	9.21 \pm 0.83	**10.32 \pm 1.07
Kidneys (g)	1.696 \pm 0.144	1.788 \pm 0.163	1.877 \pm 0.181	**2.276 \pm 0.306
Heart (g)	0.812 \pm 0.127	0.849 \pm 0.089	*0.933 \pm 0.103	**1.087 \pm 0.115
Adrenals (g)	0.084 \pm 0.014	0.095 \pm 0.012	*0.099 \pm 0.012	**0.117 \pm 0.15
Spleen (g)	0.640 \pm 0.080	0.622 \pm 0.078	0.702 \pm 0.122	**0.957 \pm 0.228
Thyroid (mg)	23.6 \pm 4.4	26.2 \pm 4.4	25.7 \pm 3.3	27.5 \pm 3.9

Values were expressed as mean \pm SD. *, **, Significantly different from control (p<0.05 and p<0.01, respectively). n = 10 Combined Subgroups; n = 5 individual Subgroups.

Table 20B. Relative Organ Weights with l-Thyroxine in the Laboratory 13 Study

L-thyroxine Dose (mg/kg/day)	Control	Low	Middle	High
	0	0.01	0.1	1
Male Body Wt Δ 28d		-0.4%	+3.5%	+1.7%
Male Body Wt Δ Necropsy		-0.3%	+3.0%	-0.03%
Liver	4.41%	4.27%	4.52%	**4.79%
Kidneys	0.74%	0.75%	0.81%	**1.00%
Heart	0.36%	0.35%	0.37%	**0.47%
Adrenals	0.0220%	0.0214%	0.0223%	**0.0313%
Spleen	0.23%	0.23%	0.27%	**0.34%
Thyroid	0.0090%	0.0076%	0.0075%	0.0085%
Female Body Wt Δ at 28 d		+0.3%	+2.9%	+6.8%
Female Body Wt Δ at Necropsy		-0.7%	+3.4%	+4.2%
Liver	4.19%	4.28%	*4.47%	**4.97%
Kidneys	0.85%	0.90%	0.91%	**1.10%
Heart	0.41%	0.43%	*0.45%	**0.52%
Adrenals	0.0422%	0.0480%	0.0481%	**0.0564%
Spleen	0.32%	0.31%	0.34%	**0.46%
Thyroid	0.0118%	0.0132%	0.0125%	0.0133%

Values were expressed as mean \pm SD. *, **, Significantly different from control (p<0.05 and p<0.01, respectively). n = 10 Combined Subgroups; n = 5 individual Subgroups.

Summary – l-Thyroxine

122. The two l-thyroxine studies are in relative agreement in terms of the organ and tissue weights. The absolute and the relative changes seen in the major organs of both sexes were similar in pattern, dose and degree in both studies. This includes changes in liver, kidney, heart, adrenal, and spleen weights.

123. The evidence from the organ and tissue weights for possible endocrine effect of l-thyroxine was limited and equivocal. The only evidence was the decrease in female relative thyroids in the laboratory 9 study, but this was not replicated in the laboratory 13 study. It should be noted that both studies did

observe histopathological changes at the high dose consistent with thyroxine's negative feedback on TSH levels and the subsequent thyroid gland stimulation an atrophy of the thyroid apparent in the flattening of the follicular epithelium.

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ANNEX 6

Histopathology Data Generated for the Updated TG 407 Validation Studies

1. The purposes of this Annex are to record for the significant histopathological observations in the updated TG 407 validation studies:

- whether the histopathological observations were judged to be treatment related;
- the basic statistical findings for each sex from each Subgroups and the combined Subgroups, when these data were available from the final reports; and
- the histopathological terms and grades for the significant observations, when these data were available from the final reports.

2. Additional purposes of this Annex are to compare the reproducibility and reliability of the experimental findings for histopathology:

- the added value and power of combined the Subgroups (i.e., a group size of five versus ten animals per sex); and
- between the two laboratories studying each chemical.

3. This Annex is organized in the following manner:

- each of the ten chemicals tested in the updated TG 407 validation program has its own individual section. The chemicals are roughly grouped as:
 - Estrogens: ethinyl oestradiol, genistein, and nonylphenol.
 - Antiestrogens: tamoxifen
 - Aromatase inhibitors: CGS 18320B
 - Androgens: methyl testosterone
 - Antiandrogens: flutamide and *p,p'*-DDE
 - Thyroid toxicants and hormones: propylthiouracil and l-thyroxine
- in each section, the histopathological observations of both laboratories testing the chemical are presented with the laboratories in alphabetical order;
- the data are presented both in text and tabular form as extracted from the original reports;
- the dose groups are arranged from left to right in the following order: the vehicle control, the low dose, the mid-dose, and the high dose of each given test substance; and
- the achievement of statistical significance, is denoted with an asterisk, usually one asterisk for $p < 0.05$ and two asterisks for $p < 0.01$, as most laboratories reported the statistics in this manner.

4. Overall integration and interpretation of the histopathological data with other findings are noted only when the original laboratory referred to their own data.

Ethinyl Oestradiol

Laboratory 2 – Ethinyl Oestradiol

5. A brief, overall summary of the histopathological findings obtained in study A and study B is shown in Table 1. The results of both studies are almost identical. Details of the incidences at which individual findings were observed are contained in the histopathology reports shown in Tables 2-5. The most sensitive alterations in male rats were changes in the mammary gland (increased ratio of small basophilic over large acidophilic cells) observed at either >0.01 or >0.05 mg/kg body weight per day in studies A or B. Reduced vacuolation of the zona fasciculata cells of the adrenals were observed in studies A and B at 0.2 mg/kg/d. Atrophic changes in testicular Leydig cells, prostate, seminal vesicles and coagulation glands were all observed at 0.2 mg/kg/d. At 0.05 mg/kg/d, seminal vesicles were affected in study B and coagulation glands in study A.

6. Estrogenic effects of ethinylestradiol on the uterus and vagina of female rats were seen in both study A and B at ≥ 0.01 mg/kg/d. Main effects on the uterus were an increase in epithelial height and an infiltration of the endometrium by granulocytes. The morphology of the vaginal epithelium suggested a reduction in the number of diestric females. The number of early stage ovarian follicles appeared to be increased in some females at 0.2 mg/kg/d in studies A and B.

7. A histopathological no observed effect level (NOEL) was only obtained in males of study B. Males of study A and females of studies A and B presented treatment-related alterations in all dose groups.

Table 1. Histopathological findings – Laboratory 2 EE

Organ System	Histopathological Finding	Subgroup A	Subgroup B
		[mg EE/kg b.w. per day]	
MALES			
testes	Leydig cell atrophy	0.2	0.2
	degeneration of germinal epithelium	0.2	0.2
epididymides	minimal spermatic debris	--	from 0.05
prostate	atrophy	0.2	0.2
coagulation gland	atrophy	0.05	0.2
seminal vesicles	atrophy	0.2	from 0.05
mammary gland	increased ratio of small basophilic: over large acidophilic cells	from 0.01	from 0.05
adrenal glands	reduced vacuolation of zona fasciculata cells	0.2	0.2
NOEL	Not observed in A	< 0.01	0.01
FEMALES			
ovary	increased number of follicles	0.2	0.2
	increased number of apoptotic corpora lutea cells	--	from 0.05
uterus	increased height of luminal and glandular epithelium	from 0.01	from 0.01
	granulocytic infiltration of endometrium	from 0.01	from 0.01
	thickened uterine wall	0.2	--
	dilation of the uterus	from 0.01	0.2
vagina	squamous metaplasia, of uterine glands	from 0.01	--
	reduction in diestric females	from 0.01	from 0.01
	keratinization	from 0.01	from 0.01
	mucification	from 0.05	from 0.01
adrenal glands	thickening of epithelium	from 0.01	from 0.05
	cytoplasmic eosinophilia	--	0.2
NOEL	Not observed in either Subgroup	< 0.01	< 0.01

Histopathological Findings -Subgroup A – Laboratory 2 EE study

8. The data for Subgroup A of the laboratory 2 EE report have been extracted in Tables 2 (males) and 3 (females) with the text immediately below.

9. Liver: Two females of the high dose group had cytoplasmic change of centrilobular liver cells.

10. Testis/Epididymides: Diffuse Leydig cell atrophy (grade 1 or 2, minimal or slight) was seen in the testes of 3 out of 5 males, which had received 0.2 mg/kg EE. One additional male of the high dose group revealed moderate Leydig cell atrophy, concomitantly the germinal epithelium of this male was immature, and the epididymides were atrophic with reduced sperm content (grade 4). One male of the high-dose group had a minimal (grade 1) degeneration of the germinal epithelium of both testes characterized by minimally increased numbers of apoptotic bodies.

11. Prostate (Ventral and Dorsolateral Part, Coagulating Gland): Atrophy of the dorsolateral (2/4) and ventral (3/4) prostate and the coagulating gland (5/5) was found in males of the high dose group. One male at 0.05 mg/kg EE had minimal atrophic changes of the coagulating gland. These findings were characterized by narrowed acinar lumina and increased connective tissue. The severity of atrophy varied between grade 1 and 4 (minimal and marked).

12. Seminal vesicle: The histopathological correlate for the diminished size of the seminal vesicles seen at necropsy was also slight atrophy (grade 2) in two males and marked atrophy (grade 4) in one male of the high dose group.

13. Ovary: The number of early stage follicles appeared to be increased in two females of the high dose group.

14. Uterus: The epithelial height of luminal and glandular cells of the uterus demonstrated a dose dependent increase of severity in females at 0.01 mg/kg and above. Granulocytic endometrial stromal infiltrates were observed in females of all groups including controls. However, the severity of granulocytic infiltration of the endometrial stroma was minimally increased in all dose groups. Uterus dilation was found in two females at 0.2 mg/kg and one female each at 0.05 and 0.01 mg/kg EE. The uterus wall was thickened in two females at 0.2 mg/kg. One female of each dose group showed incipient squamous metaplasia (grade 1) of uterine glands.

15. Vagina/Estrous Cycle: The vaginal epithelium of all control females was considered to be in the stage of diestrous. In the high dose group, no female in diestrous was seen. In the other treatment groups lower numbers of females were regarded to be in diestrous, two at 0.05 and four at 0.01 mg/kg EE. Keratinization of the vagina was found in females of all treatment groups, which coincided in some of females dosed at 0.2 or 0.05 mg/kg with distinct mucification in other epithelial segments. The vaginal epithelium was thickened in all females at 0.2 mg/kg, in three females at 0.05 mg/kg, and in one female at 0.01 mg/kg EE.

16. Mammary Gland: In the mammary gland tissue, males of all treatment groups had an increased ratio of basophilic to acidophilic tubular/ductular cells when compared to control males. In controls, male mammary tissue is predominantly characterized by large acidophilic foamy cells arranged in larger lobular groups. In the EE-treated groups, the proportion of small basophilic tubular/ductular cells typical for female mammary tissue was increased dose dependently. No differences concerning mammary gland morphology was found between untreated and treated females.

17. Adrenal Glands: In males, vacuolation of zona fasciculata cells was reduced in the high dose group.

18. No treatment related findings were seen in the other organs and tissues investigated (brain, thyroid gland, pituitary gland, kidneys, pancreas, thymus, oviducts). All findings are listed in the incidence tables, the table of individual microscopic findings and the individual animal data sheets.

Table 2. Histopathological Findings for Males: Subgroup A

Dose of EE in µg/kg/d	0	10	50	200
TESTES No. Examined	5	5	5	5
- Degeneration germinal epithelium	---	---	---	1
Grade 1	---	---	---	1
- Atrophy/immature	---	---	---	1
Grade 4	---	---	---	1
- Leydig cell atrophy	---	---	---	4
Grade 1	---	---	---	2
Grade 2	---	---	---	1
Grade 3	---	---	---	1
EPIDIDYMIDES No. Examined	5	5	5	5
- Mononuclear cell infiltration	---	---	1	---
Grade 1	---	---	1	---
- Sperm granuloma	1	---	---	1
Grade 3	1	---	---	1
- Atrophy/reduced sperm content	---	---	---	1
Grade 4	---	---	---	1
DORSOLATERAL PROST. No. Examined	5	5	5	4
- Atrophy	---	---	---	2
Grade 1	---	---	---	1
Grade 3	---	---	---	1
VENTRAL PROSTATE No. Examined	5	5	5	4
- Atrophy	---	---	---	3
Grade 1	---	---	---	2
Grade 2	---	---	---	1
SEMINAL VESICLES No. Examined	5	5	5	5
- Atrophy	---	---	---	3
Grade 2	---	---	---	2
Grade 4	---	---	---	1
COAGULATING GLANDS No. Examined	5	5	4	5
- Atrophy	---	---	1	5
Grade 1	---	---	1	1
Grade 2	---	---	---	2
Grade 3	---	---	---	1
Grade 4	---	---	---	1
ADRENAL GLANDS No. Examined	5	5	5	4
- Single cell necrosis	---	---	---	1
Grade 1	---	---	---	1
- Vacuolation Zona fasciculata	5	3	3	1
Grade 1	5	3	3	1
- Accessory adrenocortical tissue	1	---	---	---
MAMMARY GLAND No. Examined	5	4	5	5
- Increased ratio basophilic acidophilic cells	1	2	2	5
Grade 1	1	1	1	2
Grade 2	---	1	1	3

Grade 1 = Minimal / very few / very small; Grade 2 = Slight / few / small; Grade 3 = Moderate / moderate number / moderate size; Grade 4 = Marked / many / large

Table 3. Histopathological Findings for Females: Subgroup A

Dose of EE in µg/kg/d	0	10	50	200
No. Animals per Dose Group	5	5	5	5
LIVER No. Examined	5	5	5	5
- Cytoplasmic change	---	---	---	2
Grade 1	---	---	---	2
- Kupffer cell foci	4	3	3	2
Grade 1	3	3	3	2
Grade 2	1	---	---	---
- Fatty change	3	5	5	4
Grade 1	2	4	3	3
Grade 2	1	1	1	1
Grade 3	---	---	1	---
OVARIES No. Examined	5	5	5	5
- Increased number of follicles	---	---	---	2
Grade 2	---	---	---	2
UTERUS No. Examined	5	5	5	5
- Epithelial Height	5	5	5	5
Grade 2	5	4	4	1
Grade 3	---	1	1	1
Grade 4	---	---	---	3
- Granulocytes	5	5	5	5
Grade 1	3	---	1	1
Grade 2	2	5	4	4
- Degeneration epithelium	3	4	5	3
Grade 1	---	1	3	1
Grade 2	3	3	2	2
- Thickened Wall	---	---	---	2
Grade 2	---	---	---	2
- Dilation	---	1	1	2
Grade 2	---	1	1	1
Grade 3	---	---	---	1
- Squamous metaplasia uterine glands	---	1	1	1
Grade 1	---	1	1	1
VAGINA No. Examined	5	5	5	5
- Thickened epithelium	---	1	3	5
Grade 2	---	1	2	3
Grade 3	---	---	1	2
- Keratinization	---	1	2	3
Grade 1	---	---	1	1
Grade 2	---	---	---	1
Grade 3	---	1	1	1
- Mucification	---	---	1	2
Grade 2	---	---	1	1
Grade 4	---	---	---	1
- Diestrous	5	4	2	---
ADRENAL GLANDS No. Examined	5	5	5	5
- Vacuolation Zona fasciculata	---	1	---	---
Grade 1	---	1	---	---
- Mononuclear cell infiltration	---	---	1	---
Grade 1	---	---	1	---

Grade 1 = Minimal / very few / very small; Grade 2 = Slight / few / small; Grade 3 = Moderate / moderate number / moderate size; Grade 4 = Marked / many / large

Histopathological Findings -Subgroup B – Laboratory 2 EE Study

19. The data for Subgroup B of the laboratory 2 EE report have been extracted in Tables 4 (males) and 5 (females) with the text immediately below.
20. Liver: The hepatocyte glycogen content was increased in 5 females of the high dose group and in one female of the intermediate dose group.
21. Testes: Diffuse Leydig cell atrophy (grade 1, minimal) was seen in the testes of 4 out of 5 males, which had received 0.2 mg/kg EE. One male of the mid dose group revealed moderate Leydig cell atrophy, the germinal epithelium of this male was immature (grade 4, bilateral). One male of the high dose group had a minimal (grade 1) degeneration of the germinal epithelium of both testes characterized by flattening, vacuolation and/or exfoliation of premature spermatids in single seminiferous tubules.
22. Epididymides: Spermatic debris in minimal amount (grade 1) was found in the epididymidal tubules of two males each, which were given doses of 0.2 and 0.05 mg/kg EE.
23. Prostate (Ventral and Dorsolateral Part, Coagulating Gland): Atrophy of the dorsolateral (3/5) and ventral (3/5) prostate and the coagulating gland (5/5) was found in males of the high dose group. This finding was characterized by narrowed acinar lumina and increased connective tissue. The severity of atrophy was scored grade 2 or 3 (slight or moderate).
24. Seminal Vesicle: The histopathological correlate for the diminished size of the seminal vesicles seen at necropsy was also atrophy of moderate degree in all high dose males and of minimal severity in 4 out of 5 males of the intermediate dose group.
25. Ovary: The number of early stage follicles appeared to be increased in two females of the high dose group. Apoptotic luteal cells (“apoptosis c. lutea”) were found in higher frequency in two females at 0.2 mg/kg EE and in one female at 0.05 mg/kg EE.
26. Uterus: The epithelial height of luminal and glandular cells of the uterus was increased dose dependently in females at 0.01 mg/kg and above. Degeneration of luminal and glandular epithelial cells was more pronounced (grade 1 or 2) in all treated groups when compared to controls (grade 1). Granulocytic endometrial stromal infiltrates were observed in females of all groups including controls. However, the severity of granulocytic infiltration of the endometrial stroma was minimally increased in all dose groups. The uterus of one high dose female was moderately dilated.
27. Vagina/Estrous Cycle: The vaginal epithelium of all control females was considered to be in the stage of diestrous. In the treatment groups lower numbers of females were regarded to be in diestrous, two at 0.2 mg/kg, three each at 0.05 and 0.01 mg/kg EE. Keratinization of the vagina was found in females of all treatment groups which coincided in some of these females with distinct mucification in other epithelial segments. The vaginal epithelium was thickened in all females at 0.2 mg/kg and one female at 0.05 mg/kg EE.
28. Mammary Gland: In the mammary gland tissue, males given 0.05 mg/kg EE and above had an increased ratio of basophilic to acidophilic tubular/alveolar cells when compared to control males. In control males, the mammary tissue is predominantly characterized by large acidophilic foamy cells arranged in large lobular groups. In the-EE treated groups, the proportion of small (cuboidal) basophilic tubular/alveolar cells resembling female mammary tissue was increased dose dependently (severity). No differences were found between untreated and treated females.

29. Adrenal Glands: In males, vacuolation of cortical Zona fasciculata cells was reduced after 0.2 mg/kg EE. Two females of the same group revealed a more eosinophilic cytoplasm of zona fasciculata cells.

30. No treatment related findings were seen in the other organs and tissues investigated (brain, pituitary gland, thyroid gland, kidneys, pancreas, thymus, oviducts). All findings are listed in the incidence tables, the table of individual microscopic findings and the individual animal data sheets.

Table 4. Histopathological Findings for Males: Subgroup B

Dose of EE in µg/kg/d	0	10	50	200
No. Animals per Dose Group	5	5	5	5
TESTES No. Examined	5	5	5	5
- Degeneration germinal epithelium	---	1	---	1
Grade 1	---	---	---	1
Grade 3	---	1	---	---
- Atrophy/immature	---	---	1	---
Grade 4	---	---	1	---
- Leydig cell atrophy	---	---	1	4
Grade 1	---	---	---	4
Grade 3	---	---	1	---
EPIDIDYMIDES No. Examined	5	5	5	5
- Debris	---	---	2	2
Grade 1	---	---	2	2
- Atrophy/Reduced sperm content	---	---	1	---
Grade 4	---	---	1	---
- Sperm granuloma	---	2	---	---
Grade 3	---	2	---	---
- Oligospermia	---	1	---	---
Grade 3	---	1	---	---
DORSOLATERAL PROST. No. Examined	5	5	4	5
- Atrophy	---	---	---	3
Grade 2	---	---	---	3
VENTRAL PROSTATE No. Examined	5	5	4	5
- Atrophy	---	---	---	3
Grade 2	---	---	---	1
Grade 3	---	---	---	2
SEMINAL VESICLES No. Examined	4	5	5	5
Atrophy	---	---	4	5
Grade 1	---	---	4	---
Grade 3	---	---	---	5
COAGULATING GLANDS No. Examined	5	5	4	5
Atrophy	---	---	---	5
Grade 3	---	---	---	5
ADRENAL GLANDS No. Examined	5	5	5	5
- Single cell necrosis	---	---	---	1
Grade 1	---	---	---	1
- Vacuolization Zona fasciculata	3	5	3	---
Grade 1	2	4	3	---
Grade 2	1	1	---	---
- Accessory adrenocortical tissue	---	---	---	1

Grade 1 = Minimal / very few / very small; Grade 2 = Slight / few / small; Grade 3 = Moderate / moderate number / moderate size; Grade 4 = Marked / many / large

Table 4 continued. Histopathological Findings for Males: Subgroup B

MAMMARY GLAND/REGION No. Examined	5	5	5	4
- Increased ratio basophilic acidophilic cells	---	---	3	3
Grade 1	---	---	2	---
Grade 2	---	---	1	3

Grade 1 = Minimal / very few / very small; Grade 2 = Slight / few / small; Grade 3 = Moderate / moderate number / moderate size; Grade 4 = Marked / many / large

Table 5. Histopathological Findings for Females: Subgroup B

Dose of EE in µg/kg/d	0	10	50	200
No. Animals per Dose Group	5	5	5	5
LIVER No. Examined	5	5	5	5
- Increased mitotic rate	2	---	1	---
Grade 1	2	---	1	---
- Kupffer cell foci	4	2	4	1
Grade 1	3	2	3	1
Grade 2	1	---	1	---
- Glycogen increased	---	---	1	5
Grade 1	---	---	1	---
Grade 2	---	---	---	5
LIVER (ORO) No. Examined	5	5	5	5
- Fatty change	3	4	2	2
Grade 1	2	2	2	2
Grade 2	1	2	---	---
OVARIES No. Examined	5	5	5	5
- Apoptosis Corpea lutea	---	---	1	2
Grade 1	---	---	---	1
Grade 2	---	---	1	1
- Increased number of follicles	---	---	---	2
Grade 2	---	---	---	2
- Cyst	1	---	---	---
Grade 2	1	---	---	---
- Mineralization	---	---	1	---
Grade 1	---	---	1	---
UTERUS No. Examined	5	5	5	5
- Epithelial Height	5	5	5	5
Grade 1	2	---	---	---
Grade 2	3	4	3	4
Grade 3	---	1	2	---
Grade 4	---	---	---	1
- Granulocyte	5	5	5	5
Grade 1	5	3	3	4
Grade 2	---	2	2	1
- Degeneration epithelium	3	2	2	3
Grade 1	3	---	---	1
Grade 2	---	2	2	2
- Dilatation	---	---	---	1
Grade 3	---	---	---	1

Grade 1 = Minimal / very few / very small; Grade 2 = Slight / few / small; Grade 3 = Moderate / moderate number / moderate size; Grade 4 = Marked / many / large

Table 5 continued. Histopathological Findings for Females: Subgroup B

Dose of EE in µg/kg/d	0	10	50	200
No. Animals per Dose Group	5	5	5	5
VAGINA No. Examined	5	5	5	5
- Thickened epithelium	---	---	1	5
Grade 2	---	---	1	5
- Keratinisation	---	2	2	3
Grade 1	---	1	---	---
Grade 2	---	---	2	3
Grade 3	---	1	---	---
- Diestrous	5	3	3	2
- Mucification	---	1	---	2
Grade 2	---	1	---	1
Grade 3	---	---	---	1
ADRENAL GLANDS No. Examined	5	5	5	5
- Eosinophilic cytoplasm	---	---	---	2
Grade 1	---	---	---	2
- Vacuolation Zona fasciculata	---	---	1	---
Grade 1	---	---	1	---

Grade 1 = Minimal / very few / very small; Grade 2 = Slight / few / small; Grade 3 = Moderate / moderate number / moderate size; Grade 4 = Marked / many / large

Laboratory 5 – Ethinyl Oestradiol

31. The discussion summary of the results of the histopathological changes in the sex and accessory sex organs from the laboratory 5 EE study report are shown in Table 6.

32. Atrophic changes of the prostate, seminal vesicle and mammary gland, spermacytic degeneration in the testis and splenic hemosiderosis were observed in male rats in the 50 and 200 µg/kg groups, and adrenal cortical hyperplasia was detected in males in the 200 µg/kg group. Atrophic changes of the ovary, squamous metaplasia of the epithelium, hypertrophy of the epithelial and glandular cells in the uterus, mucification in the vagina and splenic hemosiderosis were detected in females in the 200 µg/kg group.

33. The data for the histopathological changes in the sex and accessory sex organs that are discussed have been extracted into three Tables that follow: combined Subgroups (Table 7), Subgroup A (Table 8), and Subgroup B (Table 9).

Table 6. Histopathological changes of the sex and sex accessory glands in rats given ethinyl estradiol

Ethinyl Estradiol ($\mu\text{g}/\text{kg}/\text{day}$). Organs Findings	Male				Female			
	Control	10	50	200	Control	10	50	200
Testis Degeneration of spermatocytes	0/10 ^{a)}	0/10	1/10	2/10				
Prostate (ventral lobe) Atrophy	0/10	0/10	1/10	4/10				
Prostate (dorsolateral lobe) Atrophy	0/10	0/10	1/10	3/10				
Seminal vesicle Atrophy	0/10	0/10	1/10	5/10				
Ovary Atrophy					0/10	0/10	0/10	2/10
Uterus Squamous metaplasia surface epithel.					0/10	0/10	0/10	1/10
Tall columnar glandular epithel cells					0/10	0/10	0/10	1/10
Tall columnar surface epithel cells					0/10	0/10	0/10	6/10
Vagina Mucification					0/10	0/10	0/10	7/10
Mammary gland Atrophy	0/10	0/10	1/10	4/10	0/10	- -	- -	0/10

^{a)} Number of animals affected / Number of animals examined; - - Not examined

Table 7. Summary of histopathological findings from the Laboratory 5 EE study. (Combined Subgroups, n=10)

Ethinyl oestradiol (µg/kg/day). Organ or tissue Findings	Grade	Male				Female			
		Vehicle control	10	50	200	Vehicle control	10	50	200
		ta 10 ^a	ta 10	ta 10	ta 10	ta 10	ta 10	ta 10	ta 10
Liver									
No abnormalities detected.		10/10 ^b	--	--	10/10	9/10	--	--	10/10
Microgranuloma	+	0/10	--	--	0/10	1/10	--	--	0/10
Testis									
No abnormalities detected.		10/10	10/10	9/10	8/10				
Degeneration of spermatocytes	+	0/10	0/10	1/10	2/10				
Epididymis									
No abnormalities detected.		8/10	--	--	10/10				
Spermatic granuloma	+	2/10	--	--	0/10				
Prostate (ventral lobe)									
No abnormalities detected.		7/10	8/10	7/10	4/10				
Atrophy	+	0/10	0/10	1/10	4/10				
Round cell infiltration	+	2/10	1/10	1/10	2/10				
	++	1/10	1/10	1/10	1/10				
Prostate (dorsolateral lobe)									
No abnormalities detected.		10/10	10/10	9/10	7/10				
Atrophy	+	0/10	0/10	1/10	3/10				
Seminal vesicle									
No abnormalities detected.		10/10	10/10	9/10	5/10				
Atrophy	+	0/10	0/10	1/10	5/10				
Ovary									
No abnormalities detected.						10/10	10/10	10/10	8/10
Atrophy	+					0/10	0/10	0/10	2/10

ta, terminal autopsy. ^a Number of animals autopsied. ^b Number of animals affected / Number of animals examined.

--, Not examined.

±, very slight; +, slight; ++, moderate; +++ severe.

Table 7 continued. Summary of histopathological findings from the Laboratory 5 study. (Combined Subgroups, n=10)

Ethinyl oestradiol (µg/kg/day). Organ or tissue Findings	Grade	Male				Female			
		Vehicle control	10	50	200	Vehicle control	10	50	200
		ta 10 ^a	ta 10	ta 10	ta 10	ta 10	ta 10	ta 10	ta 10
Uterus									
No abnormalities detected						10/10 ^b	10/10	10/10	4/10
Squamous metaplasia of surface epithelium	±					0/10	0/10	0/10	1/10
Tall columnar glandular epithelial cells	+					0/10	0/10	0/10	1/10
Tall columnar surface epithelial cells	+					0/10	0/10	0/10	4/10
	++					0/10	0/10	0/10	2/10
Vagina									
No abnormalities detected						10/10	10/10	10/10	3/10
Mucification	±					0/10	0/10	0/10	1/10
	+					0/10	0/10	0/10	1/10
	++					0/10	0/10	0/10	3/10
	+++					0/10	0/10	0/10	2/10
Adrenal									
No abnormalities detected		10/10	10/10	10/10	7/10	10/10	--	--	10/10
Hypertrophy of cortex	+	0/10	0/10	0/10	3/10	0/10	--	--	0/10
Mammary gland									
No abnormalities detected		10/10	10/10	9/10	6/10	10/10	--	--	10/10
Atrophy	+	0/10	0/10	1/10	4/10	0/10	--	--	0/10

ta, terminal autopsy. ^a Number of animals autopsied. ^b Number of animals affected / Number of animals examined.

--, Not examined.

±, very slight; +, slight; ++, moderate; +++ severe.

Table 8. Summary of histopathological findings from the Laboratory 5 study. (Subgroup A, n=5)

Ethinyl oestradiol (µg/kg/day). Organ or tissue Findings	Grade	Male				Female			
		Vehicle control	10	50	200	Vehicle control	10	50	200
		ta 5 ^a	ta 5	ta 5	ta 5	ta 5	ta 5	ta 5	ta 5
Testis									
No abnormalities detected		5/5 ^b	5/5	5/5	4/5				
Degeneration of spermatocytes	+	0/5	0/5	0/5	1/5				
Epididymis									
No abnormalities detected.		3/5	--	--	5/5				
Spermatoc granuloma	+	2/5	--	--	0/5				
Prostate (ventral lobe),									
No abnormalities detected		3/5	4/5	4/5	3/5				
Atrophy	+	0/5	0/5	0/5	2/5				
Round cell infiltration	+	1/5	1/5	0/5	0/5				
	++	1/5	0/5	1/5	0/5				
Prostate (dorsolateral lobe)									
No abnormalities detected		5/5	5/5	5/5	3/5				
Atrophy	+	0/5	0/5	0/5	2/5				
Seminal vesicle									
No abnormalities detected		5/5	5/5	5/5	3/5				
Atrophy	+	0/5	0/5	0/5	2/5				
Ovary									
No abnormalities detected.						5/5	5/5	5/5	4/5
Atrophy	+					0/5	0/5	0/5	1/5

ta, terminal autopsy. ^a Number of animals autopsied. ^b Number of animals affected / Number of animals examined.

--, Not examined.

±, very slight; +, slight; ++, moderate;+++ severe.

Table 8 continued. Summary of histopathological findings from the Laboratory 5 study. (Subgroup A, n=5)

Ethinyl oestradiol (µg/kg/day). Organ or tissue Findings	Grade	Male				Female			
		Vehicle control	10	50	200	Vehicle control	10	50	200
		ta 5 ^a	ta 5	ta 5	ta 5	ta 5	ta 5	ta 5	ta 5
Uterus									
No abnormalities detected						5/5 ^b	5/5	5/5	1/5
Squamous metaplasia of surface epithelium	±					0/5	0/5	0/5	1/5
Tall columnar glandular epithelial cells	+					0/5	0/5	0/5	1/5
Tall columnar surface epithelial cells	+					0/5	0/5	0/5	2/5
	++					0/5	0/5	0/5	2/5
Vagina									
No abnormalities detected						5/5	5/5	5/5	2/5
Mucification	++					0/5	0/5	0/5	2/5
	+++					0/5	0/5	0/5	1/5
Adrenal									
No abnormalities detected.		5/5	5/5	5/5	3/5	5/5	--	--	5/5
Hypertrophy of cortex	+	0/5	0/5	0/5	2/5	0/5	--	--	0/5
Mammary gland									
No abnormalities detected		5/5	5/5	5/5	3/5	5/5	--	--	5/5
Atrophy	+	0/5	0/5	0/5	2/5	0/5	--	--	0/5

ta, terminal autopsy. ^a Number of animals autopsied. ^b Number of animals affected / Number of animals examined.

--, Not examined.

±, very slight; +, slight; ++, moderate;+++ severe.

Table 9. Summary of histopathological examinations from the Laboratory 5 study. (Subgroup B, n=5). Ethinyl estradiol (µg/kg/day)

Ethinyl oestradiol (µg/kg/day). Organ or tissue Findings	Grade	Male				Female			
		Vehicle control	10	50	200	Vehicle control	10	50	200
		ta 5 ^a	ta 5	ta 5	ta 5	ta 5	ta 5	ta 5	ta 5
Testis									
No abnormalities detected.		5/5 ^b	5/5	4/5	4/5				
Degeneration of spermatocytes	+	0/5	0/5	1/5	1/5				
Epididymis									
No abnormalities detected		5/5	--	--	5/5				
Prostate (ventral lobe)									
No abnormalities detected.		4/5	4/5	3/5	1/5				
Atrophy	+	0/5	0/5	1/5	2/5				
Round cell infiltration	+	1/5	0/5	1/5	2/5				
	++	0/5	1/5	0/5	1/5				
Prostate (dorsolateral lobe)									
No abnormalities detected		5/5	5/5	4/5	4/5				
Atrophy	+	0/5	0/5	1/5	1/5				
Seminal vesicle									
No abnormalities detected		5/5	5/5	4/5	2/5				
Atrophy	+	0/5	0/5	1/5	3/5				
Ovary									
No abnormalities detected						5/5	5/5	5/5	4/5
Atrophy	+					0/5	0/5	0/5	1/5

ta, terminal autopsy. ^a Number of animals autopsied. ^b Number of animals affected / Number of animals examined.

--, Not examined.

±, very slight; +, slight; ++, moderate;+++ severe.

Table 9 continued. Summary of histopathological examinations from the Laboratory 5 study. (Subgroup B, n=5).

Ethinyl estradiol ($\mu\text{g}/\text{kg}/\text{day}$)

Ethinyl oestradiol ($\mu\text{g}/\text{kg}/\text{day}$). Organ or tissue Findings	Grade	Male				Female			
		Vehicle control	10	50	200	Vehicle control	10	50	200
		ta 5	ta 5	ta 5	ta 5	ta 5	ta 5	ta. 5	ta 5
Uterus						5/5 ^b	5/5	5/5	3/5
No abnormalities detected									
Tall columnar surface epithel cells	+					0/5	0/5	0/5	2/5
Vagina						5/5	5/5	5/5	1/5
No abnormalities detected.									
Mucification	±					0/5	0/5	0/5	1/5
	+					0/5	0/5	0/5	1/5
	++					0/5	0/5	0/5	1/5
	+++					0/5	0/5	0/5	1/5
Adrenal									
No abnormalities detected		5/5	5/5	5/5	4/5	5/5	--	--	5/5
Hypertrophy of cortex	+	0/5	0/5	0/5	1/5	0/5	--	--	0/5
Mammary gland									
No abnormalities detected.		5/5	5/5	4/5	3/5	5/5	--	--	5/5
Atrophy	+	0/5	0/5	1/5	2/5	0/5	--	--	0/5

ta, terminal autopsy. ^a Number of animals autopsied. ^b Number of animals affected / Number of animals examined.

--, Not examined.

±, very slight; +, slight; ++, moderate; +++ severe.

Genistein

Laboratory 4 - Genistein

34. The histopathological observations described below were judged to be incidental based upon the low rate at which they were observed, the lack of dose response, their pathological nature and the lack of corresponding evidence from other measurements.

- heart: myocarditis (1/10 males in the control group).
- kidney: pelvic dilatation of one side and aplasia of another side (one female in the 400 mg/kg group in which aplasia was found at necropsy), tubular basophilia (1/10 males in the 1000 mg/kg group) and hyaline urinary cast (1/10 males in the control group).
- oviduct: cystic dilatation (one female in the 400 mg/kg group in which cyst was found at necropsy).
- pancreas: focal atrophy (1/10 females in the control group).
- prostate: interstitial cell infiltration (5/10 in the control group and 6/10 in the 1000 mg/kg group).
- ileum (including Peyer's patch): diverticulum (one female in the 1000 mg/kg group in which diverticulum was found at necropsy).
- skin (cervical): ulcer (one female in the 400 mg/kg group in which crust was found at necropsy).
- uterus: unilateral aplasia (one female in the 400 mg/kg group in which agenesis was found at necropsy).

35. The histopathological observations described below were judged to be treatment-related lesions.

- liver: Slight or mild increase in glycogen deposit in the peri-portal hepatocytes in the control, 120, 400 and 1000 mg/kg groups was observed in 1/10, 3/10, 5/10 and 8/10 females, respectively, and the incidence in each dose group increased in a dose dependent manner. On the other hand, in males, although the above change was found in 8/10 in the 1000 mg/kg group, the incidence in the 1000 mg/kg group was comparable to that of the control group (6/10). Slight focal necrosis (2/10 males in the control group and 1/10 females in the 120 mg/kg group) was incidental lesion.
- vagina: Slight or mild vacuolation of the epithelium (vacuole or cleft formation containing cell debris) was observed in 2/10 in the 400 (one female each in group A and group B) and 2/10 in the 1000 mg/kg group (one female each in group A and group B). The epithelium of the above animals became columnar containing mucous.

36. The specific data for the treatment-related observations is provided in Tables 10 (Combined Subgroups), 11 (Subgroup A), and 12 (Subgroup B).

Table 10. Histopathological findings – Genistein – Laboratory 4 Study - Combined Subgroups

Organs Findings	Genistein (mg/kg/day): Number:	Males				Females			
		0 10	120 10	400 10	1000 10	0 10	120 10	400 10	1000 10
Liver									
Number examined		10	0	0	10	10	10	10	10
Increased glycogen deposit, hepatocyte, periportal		6	--	--	8	1	3	5	8
slight		3	--	--	5	1	2	4	5
mild		3	--	--	3	0	1	1	3
Necrosis, focal		2	--	--	0	0	1	0	0
slight		2	--	--	0	0	1	0	0
Uterus									
Number examined						10	0	1	10
Aplasia (unilateral.) present						0	--	1	0
						0	--	1	0
Vagina									
Number examined						10	10	10	10
Vacuolation, epithelium						0	0	2	2
slight						0	0	0	1
mild						0	0	2	1

Table 11: Histopathological findings – Genistein – Laboratory 4 Study - Subgroup: A

Organs Findings	Genistein (mg/kg/day): Number:	Males				Females			
		0 5	120 5	400 5	1000 5	0 5	120 5	400 5	1000 5
Liver									
Number examined	5	0	0	5	5	5	5	5	
Increased glycogen deposit, hepatocyte, periportal	4	0	0	4	0	2	2	4	
slight	1	0	0	1	0	1	2	2	
mild	3	0	0	3	0	1	0	2	
Necrosis, focal	1	0	0	0	0	0	0	0	
slight	1	0	0	0	0	0	0	0	
Uterus									
Number examined					5	0	1	5	
Aplasia (unilateral.) present					0	0	1	0	
					0	0	1	0	
Vagina									
Number examined					5	5	5	5	
Vacuolation, epithelium					0	0	1	1	
slight					0	0	0	0	
mild					0	0	1	1	

Table 12: Histopathological findings – Genistein – Laboratory 4 Study - Subgroup B

Organs Findings	Genistein (mg/kg/day): Number:	Males				Females			
		0 5	120 5	400 5	1000 5	0 5	120 5	400 5	1000 5
Liver									
Number examined		5	0	0	5	5	5	5	5
Increased glycogen deposit, hepatocyte, periportal		2	0	0	4	1	1	3	4
slight		2	0	0	4	1	1	2	3
mild		0	0	0	0	0	0	1	1
Necrosis, focal		1	0	0	0	0	1	0	0
slight		1	0	0	0	0	1	0	0
Uterus									
Number examined						5	0	0	5
Aplasia (unilateral.) present						0	0	0	0
						0	0	0	0
Vagina									
Number examined						5	5	5	5
Vacuolation, epithelium						0	0	1	1
slight						0	0	0	1
mild						0	0	1	0

Laboratory 12 - Genistein

37. Microscopic changes were seen in the male kidneys and in the uterus and vagina, in both the 400 and 1000 mg/kg/day groups. The data for the major findings are provided in Table 13 (males by Subgroup) and 14 (females by Subgroup).

38. There was a dose-related increase in incidence and severity of intra-tubular microlithiasis in the male kidney in the 400 and 1000 mg/kg/day groups in comparison with the control group. There was also a minimal increase in severity, but not in incidence of tubular basophilia in males in the 1000 mg/kg/day group compared with the controls.

39. Smear data (Appendix 11 of the laboratory 12 Genistein study) indicated that while the control females were sent for examination *post mortem* in dioestrus, the majority of Genistein treated females remained in oestrus or met-oestrus (as determined by both vaginal smears and histological examination) in the 400 and 1000 mg/kg/day groups. The histological changes characteristic of oestrus and/or met-oestrus (endometrial epithelial hypertrophy, increased apoptosis/vacuolation of endometrial epithelium, increased stromal neutrophils and vaginal keratinisation) were seen in these groups. There was a dosage relationship in that the overall incidence and/or severity of some of the changes were slightly greater at 1000 mg/kg/day. The incidence in females in the 120 mg/kg/day group was similar to that observed in the control group. The changes were of a physiological nature, i.e. were within normal limits for oestrous cycle variation. The only treatment-related 'pathological' change was an increase in incidence of minimal squamous metaplasia of endometrial glands in the uterus confined to females in the 1000 mg/kg/day group.

40. The only differences between Subgroups A and B were a slight increase in the incidence of mononuclear cell infiltration of the female liver and no increase in the incidence of vaginal keratinisation in the 1000 mg/kg/day group compared with the control group in Subgroup B.

Table 13 – Histopathological Findings in Males in Laboratory 12 Genistein Study

Genistein	0 mg/kg/d		120 mg/kg/d		400 mg/kg/d		1000 mg/kg/d	
	A	B	A	B	A	B	A	B
Subgroups								
ON STUDY/ COMPLETED	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
KIDNEY								
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	2	2	2	0	1	1	0	1
Unilateral hydronephrosis (Total)	0	0	1	0	0	3	2	0
minimal	0	0	0	0	0	2	0	0
slight	0	0	1	0	0	1	0	0
moderate	0	0	0	0	0	0	2	0
Eosinophilic casts minimal	0	0	0	0	0	1	0	0
Intratubular microlithiasis (Total)	0	0	0	0	2	1	4	3
minimal	0	0	0	0	2	1	3	3
slight	0	0	0	0	0	0	1	0
Tubular basophilia (Total)	3	3	1	5	2	1	4	2
minimal	3	3	1	5	2	1	2	2
slight	0	0	0	0	0	0	2	0
Tubular dilatation minimal	0	0	1	0	0	0	0	0
Transitional cell hyperplasia minimal	0	0	1	0	0	0	0	0
Cystic tubule/s - minimal	0	0	0	0	0	0	0	1

Table 14 – Histopathological Findings in Females in Laboratory 12 Genistein Study

Genistein Subgroups	0 mg/kg/d		120 mg/kg/d		400 mg/kg/d		1000 mg/kg/d	
	A	B	A	B	A	B	A	B
ON STUDY/ COMPLETED	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
UTERUS								
Examined	5	5	5	5	5	5	5	5
Luminal dilatation	0	0	0	0	1	0	0	0
Dioestrus	4	4	3	5	1	3	0	2
Metoestrus	0	1	1	0	1	1	2	3
Oestrus	1	0	1	0	3	1	3	0
Endomet. epith. hypertrophy (Total)	1	0	1	0	3	2	4	2
minimal	0	0	0	0	0	1	0	1
slight	1	0	0	0	1	0	2	1
moderate	0	0	1	0	2	1	2	0
Increased apoptosis/ vacuolation, endometrial epithelium (Total)	1	1	2	0	4	3	5	2
minimal	0	1	1	0	2	2	2	0
slight	1	0	0	0	0	1	2	2
moderate	0	0	1	0	2	0	1	0
Increased stromal neutrophils (Total)	2	1	2	1	4	4	5	5
minimal	1	1	1	1	2	3	0	0
slight	1	0	1	0	1	1	0	2
moderate	0	0	0	0	1	0	2	3
marked	0	0	0	0	0	0	3	0
Squamous metaplasia, endometrial glands (Total)	1	1	3	0	1	2	4	2
minimal	1	1	3	0	1	2	4	2
VAGINA								
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	4	5	4	5	2	4	2	5
Epithelial keratinisation	1	0	1	0	3	1	3	0

Nonylphenol**Laboratory 1 - Nonylphenol**

41. There were no morphological or histopathological correlates for the significant increase changes in absolute and relative weights. An attempt to correlate the increase of the weights of males with $\alpha 2\mu$ microglobulin failed. Only the following findings (n = number of findings) in liver and kidneys were apparent substance-related alterations with NP (Table 15):

Table 15. Overall Summary of Laboratory 1 Nonylphenol histopathological observations

Sex		Male (n)											
Nonylphenol (mg/kg/d)		0			20			80			200/150		
Organ \ Subset(s)		A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
Liver													
Peliosis (grade 1)		0	2	2	2	0	2	2	2	4	0	2	2
Kidneys													
Calcification		0	0	0	0	0	0	0	0	0	0	0	0
Nephropathy		1	0	1	0	0	0	0	0	0	1	0	1
Dilation, tubular		0	0	0	0	0	0	0	0	0	0	1	1
Basophilic tubules		0	0	0	0	0	0	0	0	0	0	1	1
Sex		Female (n)											
Nonylphenol (mg/kg/d)		0			20			80			200/150		
Organ \ Subset(s)		A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
Liver													
Peliosis (grade 1)		1	0	1	2	1	3	4	2	6	2	3	5
Kidneys													
Calcification		0	0	0	1	3	4	0	0	0	3	3	6
Nephropathy		0	1	1	0	0	0	0	0	0	1	2	3
Dilation, tubular		0	0	0	0	0	0	0	0	0	2	4	6
Basophilic tubules		0	0	0	0	0	0	0	0	0	0	3	3

42. The changes in kidneys of females seemed to be dose related. The findings in the liver (a minimal peliosis) were of such a low severity, that a treatment related effect was questionable. All other microscopic findings recorded were either single observations, or they occurred in control animals only, or they were recorded at comparable incidence and graded severity in control and high dose males and/or females. The detailed findings are reported for Subgroup A (Table 16), Subgroup B (Table 17), and the combined Subgroups (Table 18).

Table 16. Histopathological findings in Laboratory 1 Nonylphenol Study - Subgroup A

Sacrifice group Sex Nonylphenol (mg/kg/day)	Grade	Subgroup A							
		M				F			
		0	20	80	200/150	0	20	80	200/150
Animals in selected Group		5	5	5	5	5	5	5	5
Liver		5	5	5	5	5	5	5	5
- Peliosis	1	--	2	2	--	1	2	4	2,
		--	2	2	--	1	2	4	2
- Vacuolizat., periport	1	1	--	1	2	1	--	1	--
		1	--	1	2	1	--	1	--
- Hematopoiesis, extrm.	1	1	1	1	1	1	--	2	--
	1	1	--	1-	--	1	--	2	--
	2	--	1	--	1	--	--	--	--
Kidneys		5	5	5	5	5	5	5	5
- Calcification, multifocal	1	--	--	--	--	--	1	--	3
	2	--	--	--	--	--	--	--	1
	3	--	--	--	--	--	1	--	1
		--	--	--	--	--	--	--	1
- Hyperemia	P	--	--	--	--	--	--	--	1
		--	--	--	--	--	--	--	1
- Dilation, pelvic	1	--	--	1	1	--	--	--	1
	P	--	--	1	--	--	--	--	1
		--	--	--	1	--	--	--	--
- Chronic nephropathy	1	1	--	--	1	--	--	--	1
	2	--	--	--	--	--	--	--	1
	3	--	--	--	1	--	--	--	--
		1	--	--	--	--	--	--	--
- Tubul. dilat, multifocal	4	--	--	--	--	--	--	--	2
		--	--	--	--	--	--	--	2
Testes		5	--	--	5				
Epididymides		5	--	--	5				
Seminal vesicle +cq		5	--	--	5				
Prostate (dl + v)		5	--	--	5				
Ovaries						5	--	--	5
Oviducts						5	--	--	5
Uterus						5	1	--	5
- Infiltration, granular focal	2					--	--	--	1
						--	--	--	1
- Cyst (s) squamous	3					--	1	--	--
						--	1	--	--
- Dilatation	2					--	--	--	1
						--	--	--	1

Table 17. Histopathological findings in Laboratory 1 Nonylphenol Study - Subgroup B

Sacrifice group Sex Nonylphenol (mg/kg/day)	Grade	Subgroup B							
		M				F			
		0	20	80	200/150	0	20	80	200/150
Animals in selected Group		5	5	5	5	5	5	5	5
Glandular stomach					1				
Liver		5	5	5	5	5	5	5	5
- Peliosis	1	2	--	2	2	--	1	2	3
	2	2	--	2	2	--	1	2	3
- Vacuolizat., periport	1	1	--	1	1	1	1	1	2
	2	--	--	1	1	1	1	1	2
	3	1	--	--	--	--	--	--	--
- Hematopoiesis, extrm.	1	1	2	1	2	1	3	1	1
	2	--	2	1	2	--	3	1	1
	3	1	--	--	--	1	--	--	--
Kidneys		5	5	5	5	5	5	5	5
- Calcification, mf	1	--	--	--	--	--	3	--	3
	2	--	--	--	--	--	3	--	2
	3	--	--	--	--	--	--	--	1
- Dilation, pelvic	1	--	1	--	--	--	--	--	--
	2	--	1	--	--	--	--	--	--
- Chronic nephropathy	1	--	--	--	--	1	--	--	2
	2	--	--	--	--	1	--	--	--
	3	--	--	--	--	--	--	--	1
- Basophil. Tubules, mf	1	--	--	--	1	--	--	--	3
	2	--	--	--	1	--	--	--	1
	3	--	--	--	--	--	--	--	2
- Tubul. dilat, mf	1	--	--	--	1	--	--	--	4
	2	--	--	--	1	--	--	--	--
	3	--	--	--	--	--	--	--	3
	4	--	--	--	--	--	--	--	1
Testes		5	--	--	5				
Epididymides		5	1	--	5				
- Granuloma, spermatog.	P	1	1	--	--				
		1	1	--	--				
Seminal vesicle +cq		5	--	--	5				
Prostate (dl + v)		5	--	--	5				
Ovaries						5	--	--	5
Oviducts						5	--	--	5
Uterus						5	--	--	5
- Dilatation	4					1	--	--	--
						1	--	--	--
Vagina						5	--	--	5
Mammary gland						5	--	--	5

Table 18. Histopathological findings in Laboratory 1 Nonylphenol Study – Combined Subsets

Sacrifice group Sex Nonylphenol (mg/kg/day)	Grade	Combined Subgroups							
		M				F			
		0	20	80	200/150	0	20	80	200/150
Kidneys		10	10	10	10	10	10	10	10
- Calcification, mf		--	--	--	--	--	4	--	6
	1	--	--	--	--	--	3	--	3
	2	--	--	--	--	--	1	--	2
	3	--	--	--	--	--	--	--	1
- Hyperemia		--	--	--	--	--	--	--	1
	P	--	--	--	--	--	--	--	1
- Dilation, pelvic		--	1	1	1	--	--	--	1
	1	--	1	1	--	--	--	--	1
	P	--	--	--	1	--	--	--	--
- Chronic nephropathy		1	--	--	1	1	--	--	3
	1	--	--	--	--	1	--	--	1
	2	--	--	--	1	--	--	--	1
	3	1	--	--	--	--	--	--	1
- Basophil. Tubules, mf		--	--	--	1	--	--	--	3
	1	--	--	--	1	--	--	--	1
	2	--	--	--	--	--	--	--	2
- Tubul. dilat, mf		--	--	--	1	--	--	--	6
	1	--	--	--	1	--	--	--	--
	2	--	--	--	--	--	--	--	3
	3	--	--	--	--	--	--	--	1
	4	--	--	--	--	--	--	--	2
Testes		10	--	--	10				
Epididymides		10	1	1	10				
- Granuloma & spermatog.		1	1	1	--				
	P	1	1	1	--				

Laboratory 6 Study - Nonylphenol

44. No microscopic changes related to administration of the test substance were observed in any of the male or female rats given 20 or 80 mg/kg/day or female rats given 300/250 mg/kg/day. All observed changes were found only in the high dose male group. The significant findings are summarized in Table 19.

45. Treatment-related microscopic changes were observed in the kidneys of male rats given 300/250 mg/kg/day and female rats given 300/250 mg/kg/day of the test substance and in the liver of male rats of the 300/250 mg/kg/day dosage group.

46. The treatment-related change in the kidney was classified as tubular nephrosis. Histomorphologically, this lesion consisted of a combination of dilatation of cortical and medullary tubules and papillary ducts. These dilated tubules often had a flattened and atrophic epithelial lining. In a few instances, there were focal areas of smaller cortical tubules which were basophilic. Occasionally, focal areas of interstitial inflammation and neutrophilic exudate occurred in affected tubules and ducts. The degree of the nephrosis ranged from minimal to mild focal lesions to a moderate to marked diffuse change.

47. The treatment-related effect in the liver was observed in 5/10 high dose male rats and consisted of a very minimal prominence (hypertrophy) of centrilobular hepatocytes. This change was not observed in female rats of the high dosage group or male and female rats of the lower dosage group.

48. All other microscopic changes that were observed in the various organs and tissues were considered to have occurred spontaneously and to be unrelated to treatment with the test substance. These changes generally occurred at single, low, similar frequencies among the groups and the type, incidence or severity was not affected by test substance administration.

49. All other microscopic changes that were observed were considered to have occurred spontaneously and to be unrelated to treatment with the test substance. In the reproductive organs, these included atrophy of the prostate of one mid-dose male rat; single incidences each of focal unilateral testicular atrophy (one high dosage group male rat) and diffuse bilateral testicular atrophy (one low dosage group male rat); and the seminal vesicles were small with atrophy in one male from both the control and the high dose groups.

Table 19. Laboratory 6 Nonylphenol - incidence and severity of histopathologic observations

Nonylphenol (mg/kg/day)	Males				Females			
	0	20	80	300/ 250	0	20	80	300/ 250
Number of Animals/Group:	10	10	10	10	10	10	10	10
KIDNEYS:								
NO. Examined	10	10	10	10	10	10	10	10
NO. NORMAL	7	10	4	0	7	6	8	0
-agenesia, unilateral	0	0	0	0	0	1	0	0
-basophilia/degeneration, cort. tubules, focal/multifocal	[0]	[0]	[0]	[1]	[1]	[0]	[0]	[0]
minimal	0	0	0	1	1	0	0	0
-cyst(s), medulla	1	0	1	0	0	0	0	0
-dilatation, medullary tubules, focal minimal	[1] 1	[0] 0	[2] 2	[0] 0	[0] 0	[0] 0	[0] 0	[0] 0
-dilatation, pelvis minimal	[0] 0	[0] 0	[1] 0	[1] 1	[0] 0	[0] 0	[1] 1	[1] 0
mild	0	0	0	0	0	0	0	1
moderate	0	0	1	0	0	0	0	0
-hyaline droplets, cort. tubular epithel. minimal	[0] 0	[0] 0	[2] 1	[1] 0	[0] 0	[0] 0	[0] 0	[0] 0
mild	0	0	1	1	0	0	0	0
-infiltration, mononuclear-cell, focal minimal	[1] 1	[0] 0	[1] 1	[0] 0	[1] 1	[1] 1	[0] 0	[2] 2
-mineralization, multifocal minimal	[0] 0	[0] 0	[0] 0	[0] 0	[1] 1	[1] 1	[1] 1	[1] 1
-nephrosis, tubular minimal	[0] 0	[0] 0	[0] 0	[9] 3	[0] 0	[1] 1	[0] 0	[9] 5
mild	0	6	0	3	0	0	0	3
moderate	0	0	0	2	0	0	0	1
marked	0	0	0	1	0	0	0	0
- pyelitis, chronic mild	[0] 0	[0] 0	[1] 1	[0] 0	[0] 0	[0] 0	[0] 0	[0] 0
LIVER:								
NO. Examined	10	10	10	10	10	10	10	10
NO. NORMAL	8	9	9	4	7	9	9	8
-hematopoiesis, extramedullary, focal minimal	[0] 0	[0] 0	[0] 0	[0] 0	[0] 0	[1] 1	[0] 0	[0] 0
-hypertrophy, hepatocel., centrilobular minimal	[0] 0	[0] 0	[0] 0	[5] 5	[0] 0	[0] 0	[0] 0	[0] 0
-inflam., chronic, focal/multifocal minimal	[2] 2	[1] 1	[1] 1	[3] 3	[2] 2	[1] 1	[0] 0	[2] 2
-necrosis, focal minimal	[0] 0	[0] 0	[0] 0	[0] 0	[0] 0	[0] 0	[1] 1	[0] 0
-vacuol., hepatocellular, multifocal minimal	[0] 0	[0] 0	[0] 0	[0] 0	[1] 1	[0] 0	[0] 0	[0] 0
-vacuol., hepatocellular, periportal minimal	[0] 0	[0] 0	[0] 0	[1] 1	[0] 0	[0] 0	[0] 0	[0] 0

Table legend is below final portion of Table 19.

Table 19 (continued). Laboratory 6 Nonylphenol - incidence and severity of histopathologic observations

Nonylphenol (mg/kg/day)	Males				Females			
	0	20	80	300/ 250	0	20	80	300/ 250
Number of Animals/Group:	10	10	10	10	10	10	10	10
SEMINAL VESICLES:								
NO. Examined	10	0	0	10				
NO. NORMAL	9	0	0	9				
-small/atrophy minimal	[1] 1	[0] 0	[0] 0	[1] 1				
TESTES:								
NO. Examined	10	1	0	10				
NO. NORMAL	10	0	0	9				
-atrophy, bilateral, diffuse marked	[0] 0	[1] 1	[0] 0	[0] 0				
-atrophy, unilateral, focal mild	[0] 0	[0] 0	[0] 0	[1] 1				
UTERUS:								
NO. Examined					10	0	0	10
NO. NORMAL					9	0	0	7
-distention, lumen minimal					[1] 1	[0] 0	[0] 0	[3] 1
mild					0	0	0	1
moderate					0	0	0	1
VAGINA:								
NO. Examined					10	0	0	9
NO. NORMAL					10	0	0	9

0 = No change (not remarkable, within normal histologic limits or indicated change not present).

P = Indicated change or lesion present

[number] = Total observations for finding

1 = Minimal degree or amount of indicated change or lesion.

2 = Mild degree or amount of indicated change or lesion.

3 = Moderate degree or amount of indicated change or lesion.

4 = Marked degree or amount of indicated change or lesion.

Tamoxifen**Laboratory 3 - Tamoxifen**

50. Treatment-related changes were seen in the prostate gland, seminal vesicle, and mammary gland in males and in the ovary, uterus and vagina in females.

51. The prostate gland and/or seminal vesicle showed a decreased secretion in all males at 200 $\mu\text{g}/\text{kg}/\text{day}$ and in some males at 30 $\mu\text{g}/\text{kg}/\text{day}$, in both subgroups and in the Combined Subgroups (Table 20).

Table 20. Incidence of treatment-related changes in the prostate gland and seminal vesicle

Sex	Males			
Tamoxifen Dose ($\mu\text{g}/\text{kg}/\text{day}$)	0	5	30	200
Number of animals examined	10	10	10	10
Prostate gland: decreased secretion				
Subgroup A	0	0	1	5
Subgroup B	0	0	4	5
combined Subgroups	0	0	5	10
Seminal vesicle: decreased secretion				
Subgroup A	0	0	2	4
Subgroup B	0	0	2	5
combined Subgroups	0	0	4	9

52. There was an acinar atrophy of the mammary gland in most males at 200 and 30 $\mu\text{g}/\text{kg}/\text{day}$, in both Subgroups and in the combined Subgroups.

Table 21. Incidence of treatment-related changes in the mammary gland

Sex	Males			
Tamoxifen Dose ($\mu\text{g}/\text{kg}/\text{day}$)	0	5	30	200
Number of animals examined	9	10	10	8
Acinar atrophy				
Subgroup A	0	0	4	2
Subgroup B	0	0	5	4
combined Subgroups	0	0	9	6

53. The females at 200 and 30 $\mu\text{g}/\text{kg}/\text{day}$ showed a diffuse interstitial gland hypertrophy in the ovary, a diffuse epithelial and glandular hyperplasia/hypertrophy in the uterus and a diffuse mucoid metaplasia in the vagina. In addition at 200 $\mu\text{g}/\text{kg}/\text{day}$, a diffuse epithelial hyperplasia/hypertrophy was noted in the vagina. These changes were detected in both Subgroups and in the combined Subgroups.

Table 22. Incidence of treatment-related changes in the ovary, uterus and vagina

Sex	Females			
Tamoxifen Dose ($\mu\text{g}/\text{kg}/\text{day}$)	0	5	30	200
Number of animals examined	10	10	10	10
Ovary: hypertrophy, interstitial gland, diffuse				
Subgroup A	0	0	2	5
Subgroup B	0	0	1	4
combined Subgroups	0	0	3	9
Uterus: hyperplasia/hypertrophy, epithelial and glandular, diffuse				
Subgroup A	0	0	5	4
Subgroup B	0	0	3	5
combined Subgroups	0	0	8	9
Vagina: mucoïd metaplasia, diffuse				
Subgroup A	0	0	4	5
Subgroup B	0	0	2	5
combined Subgroups	0	0	6	10
Vagina: epithelial hyperplasia/hypertrophy, diffuse				
Subgroup A	0	0	0	4
Subgroup B	0	0	0	4
combined Subgroups	0	0	0	8

54. The other changes seen were few and considered to be incidental in origin and within the range of expected changes for animals of this age kept under laboratory conditions.

Table 23. Histopathological findings from the laboratory 3 tamoxifen study.

Animal sex and Group: Tamoxifen µg/kg/d		Males (Subgroup A)				Males (Subgroup B)				Males (combined)			
		0	5	30	200	0	5	30	200	0	5	30	200
TESTIS(ES)	Number examined:	5	5	5	5	5	5	5	5	10	10	10	10
	Unremarkable:	5	4	5	3	5	4	3	3	10	8	8	6
	Vacuolation, germinal epithelium, multifocal	0	1	0	1	0	0	2	1	0	1	2	2
	Eosinophilia, germinal epithelium, multifocal	0	0	0	2	0	1	0	1	0	1	0	3
	Giant Cell, luminal, focal	0	0	0	1	0	0	0	0	0	0	0	1
PROSTATE	Number examined:	5	5	5	5	5	5	5	5	10	10	10	10
	Unremarkable:	5	4	3	0	4	2	1	0	9	6	4	0
	Decreased secretion	0	0	1	5	0	0	4	5	0	0	5	10
	Infiltrate, mononuclear cell, multifocal	0	1	1	2	1	3	0	2	1	4	1	4
SEMINAL VESICLES	Number examined:	5	5	5	5	5	5	5	5	10	10	10	10
	Unremarkable:	5	5	3	1	5	5	3	0	10	10	6	1
	Decreased secretion	0	0	2	4	0	0	2	5	0	0	4	9
MAMMARY GLAND(S)	Number examined:	4	5	5	3	5	5	5	5	9	10	10	8
	Unremarkable:	4	5	1	0	5	5	0	1	9	10	1	1
	Acinar atrophy	0	0	4	2	0	0	5	4	0	0	9	6
	Acinar secretion, focal	0	0	0	1	0	0	0	0	0	0	0	1
Animal sex and Group		Females (Subgroup A)				Females (Subgroup B)				Females (combined)			
Tamoxifen µg/kg/d		0	5	30	200	0	5	30	200	0	5	30	200
OVARY(IES)	Number examined:	5	5	5	5	5	5	5	5	10	10	10	10
	Unremarkable:	4	5	3	0	5	5	4	1	9	10	7	1
	Hypertrophy, interstitial gland, diffuse	0	0	2	5	0	0	1	4	0	0	3	9
	Follicular atresia, multifocal	0	0	0	1	0	0	0	0	0	0	0	1
	Cystic follicle(s), unilateral	1	0	0	1	0	0	0	0	1	0	0	1
UTERUS	Number examined:	5	5	5	5	5	5	5	5	10	10	10	10
	Unremarkable:	5	4	0	1	5	4	2	0	10	8	2	1
	Hyperplasia/hypertrophy, epithelial and glandular, diffuse	0	0	5	4	0	0	3	5	0	0	8	9
	Dilatation of horns	0	1	0	0	0	1	0	0	0	2	0	0
VAGINA	Number examined:	5	5	5	5	5	5	5	5	10	10	10	10
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
	Mucoid metaplasia, diffuse	0	0	4	5	0	0	2	5	0	0	6	10
	Epithelial hyperplasia/hypertrophy, diffuse	0	0	0	4	0	0	0	4	0	0	0	8
	Proestrus morphology	0	1	0	0	0	1	0	0	0	2	0	0
	Diestrus morphology	5	4	5	5	5	4	5	5	8	10	0	10

Laboratory 10 - Tamoxifen

52. Although detailed histopathological data were not originally reported, upon the request of the Secretariat, laboratory 10 provided an overall summary of the histopathological findings with tamoxifen, and provided detailed summaries and grades for the primary target tissues, the ovaries and uterus. For this Tamoxifen study, there were no abnormal findings reported by this laboratory in the following tissues: salivary gland, esophagus, thyroid gland, parathyroid gland, heart, kidney, thymus, adrenal gland, pancreas, hypophysis, stomach, intestine, peripheral nerve, femur, brain, spleen, testis, and epididymis.

Tamoxifen(μ g/kg/day). Animals per Group.	male				female			
	0	5	30	200	0	5	30	200
	6	6	6	6	6	6	6	6
Liver								
lymphocyteinfiltration		1					1	1
Lung								
Interstitial pneumonia		1	1	1			2	
peribronchiolar Lymphoid hyperplasia			2	1				
Trachea								
tracheitis	2	1		1			1	
Prostate								
Neutrophil infiltration		1		1				

Tamoxifen(μ g/kg/day)	0	5	30	200
Ovary - Follicular cystic change				
normal	6	6	5	
grade1			1	
grade2				2
grade3				4
Uterus - Endometrial gland - atrophy, depletion.				
normal	6	5	5	
grade1		1	1	
grade2				3
grade3				3
Uterus - Endometrial epithelium - squamous metaplasia				
normal	6	5	6	
grade1		1		
grade2				2
grade3				4

grade 1, mild ; grade 2, moderate ; grade 3, severe.

CGS 18320B**Laboratory 8 Study – CGS 18320B**

55. In males, the incidence of diffuse vacuolation of adrenocortical cell was significantly increased at the 30.0 mg/kg/day dose in Subgroup A, Subgroup B, and the combined Subgroups. The incidence of atrophy of the mammary gland was significantly increased at the 0.30, 3.0 and/or 30.0 mg/kg/day doses in Subgroup A, Subgroup B, and/or combined Subgroups. There were no significant changes in the epididymis, seminal vesicle/coagulating gland, prostate (dorso-lateral part and ventral part), and testis, although atrophy of the epididymis, seminal vesicle/coagulating gland, and prostate (dorsolateral part and ventral part) was observed in one male at Subgroup B.

56. In females, the incidences of increased extramedullary hematopoiesis of the spleen, and diffuse vacuolation of adrenocortical cell were significantly increased at the 3.0 and 30.0 mg/kg/day doses in Subgroup A, Subgroup B, and the combined Subgroups. The incidences of hydropic degeneration of basophilic cell of the pituitary, follicular cyst and atrophy of interstitial cell of the ovary, atrophy of the uterus (uterine horn and cervix) and vagina, and hypertrophy of the mammary gland were significantly increased at the 0.3, 3.0, and/or 30.0 mg/kg/day doses in Subgroup A, Subgroup B, and the combined Subgroups. Although increased small follicles of thyroid was found in 4 of 5 animals at the 30.0 mg/kg/day dose in group A, and 6 of 10 animals at the 30.0 mg/kg/day dose in the combined Subgroups, these incidences was not significantly different as compared with the controls (1 of 5 animals in Subgroup A, and 2 of 10 animals in the combined Subgroups).

57. Statistically significant changes in microscopic lesions observed in treated groups are shown in Table 24.

Table 24. Incidence of microscopic lesions

Site & Lesions		Group/CGS 18320B (mg/kg/day)											
		Subgroup A				Subgroup B				Combined Subgroups			
		0	0.3	3.0	30.0	0	0.3	3.0	30.0	0	0.3	3.0	30.0
Spleen: Increased extra medullary hemato- poiesis	F	0/5	1/5	5/5 ↑↑	5/5 ↑↑	0/5	0/5	5/5 ↑↑	5/5 ↑↑	0/10	1/10	10/10 ↑↑	10/10 ↑↑
Pituitary: Hydropic degen- eration, basophilic cells	F	0/5	3/5	4/5 ↑	5/5 ↑↑	0/5	5/5 ↑↑	5/5 ↑↑	5/5 ↑↑	0/10	8/10 ↑↑	9/10 ↑↑	10/10 ↑↑
Adrenal: Vacuolation, cortical cell, diffuse	M	0/5	0/5	1/5	4/5 ↑	0/5	0/5	0/5	3/4 ↑	0/10	0/10	1/10	7/9 ↑↑
	F	0/5	0/5	5/5 ↑↑	5/5 ↑↑	0/5	0/5	4/5 ↑	4/5 ↑	0/10	0/10	9/10 ↑↑	9/10 ↑↑
Thyroid: Increased small follicle	M	1/5	0/5	2/5	2/5	1/5	0/5	1/5	1/4	2/10	0/10	3/10	3/9
	F	1/5	1/5	1/5	4/5	1/5	1/5	0/5	2/5	2/10	2/10	1/10	6/10
Ovary: Cyst, follicular	F	0/5	5/5 ↑↑	5/5 ↑↑	5/5 ↑↑	0/5	5/5 ↑↑	5/5 ↑↑	5/5 ↑↑	0/10	10/10 ↑↑	10/10 ↑↑	10/10 ↑↑
Atrophy, interstitial cell	F	0/5	5/5 ↑↑	5/5 ↑↑	5/5 ↑↑	0/5	5/5 ↑↑	5/5 ↑↑	5/5 ↑↑	0/10	10/10 ↑↑	10/10 ↑↑	10/10 ↑↑
Uterine horn/ cervix: Atrophy	F	0/5	2/5	5/5 ↑↑	5/5 ↑↑	0/5	5/5 ↑↑	5/5 ↑↑	5/5 ↑↑	0/10	7/10 ↑↑	10/10 ↑↑	10/10 ↑↑
Vagina: Atrophy	F	0/5	2/5	5/5 ↑↑	5/5 ↑↑	0/5	5/5 ↑↑	5/5 ↑↑	5/5 ↑↑	0/10	7/10 ↑↑	10/10 ↑↑	10/10 ↑↑
Mammary gland: Atrophy	M	0/5	2/5	5/5 ↑↑	4/5 ↑	0/5	3/5	2/5	4/4 ↑↑	0/10	5/10 ↑	7/10 ↑↑	8/9 ↑↑
Hypertrophy	F	0/5	5/5 ↑↑	5/5 ↑↑	5/5 ↑↑	0/5	4/5 ↑	4/5 ↑	5/5 ↑↑	0/10	9/10 ↑↑	9/10 ↑↑	10/10 ↑↑

↑↓, ↑↑↓↓: p≤0.05 or 0.01.

Abbreviations: Male (M), Female (F).

58. The details of the histopathological findings are reported for males in Tables 25A-C and for females in Tables 26 A-C.

Table 25A. Histopathological findings for male rats (Subgroup A) in the Laboratory 8 CGS 18320B study

Site & Lesion	CGS 18320B (mg/kg/day)		0	0.3	3.0	30.0
	No. of animals examined		5	5	5	5
Prostate (ventral part)		[N=]	5	5	5	5
Cellular infiltration, mononuclear cell			0	1	1	0
Adrenal:		[N=]	5	5	5	5
Vacuolation, cortical cell, diffuse			0	0	1	4*
Mammary gland:		[N=]	5	5	5	5
Atrophy			0	2	5**	4*

Table 25B. Histopathological findings for male rats (Subgroup B) in the Laboratory 8 CGS 18320B study

Site & Lesion	CGS 18320B (mg/kg/day)		0	0.3	3.0	30.0
	No. of animals examined		5	5	5	4
Seminal vesicle:		[N=]	5	5	5	4
Atrophy			0	0	0	1
Coagulating gland:		[N=]	5	5	5	4
Decreased of secreted material			0	1	0	0
Atrophy			0	0	0	1
Prostate (dorso-lateral part):		[N=]	5	5	5	4
Atrophy			0	0	0	1
Prostate (ventral part):		[N=]	5	5	5	4
Atrophy			0	0	0	1
Adrenal:		[N=]	5	5	5	4
Vacuolation, cortical cell, diffuse			0	0	0	3*
Mammary gland		[N=]	5	5	5	4
Atrophy			0	3	2	4**

Table 25C. Histopathological findings for male rats (Combined Subgroups) in the Laboratory 8 CGS 18320B study

Site & Lesion	CGS 18320B mg/kg/day)		0	0.3	3.0	30.0
	No. of animals examined		10	10	10	9
Seminal vesicle:		[N=]	10	10	10	9
Atrophy			0	0	0	1
Coagulating gland:		[N=]	10	10	10	9
Decreased of secreted material			0	1	0	0
Atrophy			0	0	0	1
Prostate (dorso-lateral part):		[N=]	10	10	10	9
Atrophy			0	0	0	1
Prostate (ventral part):		[N=]	10	10	10	9
Atrophy			0	0	0	1
Cellular infiltration, mononuclear cell			0	1	1	0
Adrenal:		[N=]	10	10	10	9
Vacuolation, cortical cell, diffuse			0	0	1	7**
Mammary gland		[N=]	10	10	10	9
Atrophy		-	0	5*	7**	8**

[N=]: Number of animals examined microscopically at the site.

* and **: Significantly different from the control at 5 and 1% levels of probability, respectively (Fisher's exact probability test).

Table 26A. Histopathological findings for female rats (Subgroup A) in the Laboratory 8 CGS 18320B study

Site & Lesion	CGS 18320B (mg/kg/day) No. of animals examined		0 5	0.3 5	3.0 5	30.0 5
Spleen : Hematopoiesis, extramedullary, increased	[N=]	5 0	5 1	5 5**	5 5**	5 5**
Pituitary : Hydropic degeneration, basophilic cell	[N=]	5 0	5 3	5 4*	5 5**	5 5**
Thyroid : Increased small-sized follicles Ultimobranchial remnant	[N=]	5 1 0	5 1 1	5 1 1	5 4 0	5 4 0
Adrenal : Vacuolation, cortical cell, diffuse	[N=]	5 0	5 0	5 5**	5 5**	5 5**
Ovary : Cyst, follicular Atrophy, interstitial cell	[N=]	5 0 0	5 5** 5**	5 5** 5**	5 5** 5**	5 5** 5**
Uterine horn Atrophy	[N=]	5 0	5 2	5 5**	5 5**	5 5**
Uterine cervix Atrophy	[N=]	5 0	5 2	5 5**	5 5**	5 5**
Vagina : Atrophy	[N=]	5 0	5 2	5 5**	5 5**	5 5**
Mammary gland Hypertrophy	[N=]	5 0	5 5**	5 5**	5 5**	5 5**

Table 26B. Histopathological findings for female rats (Subgroup B) in the Laboratory 8 CGS 18320B study

Site Lesion	CGS 18320B (mg/kg/day) No. of animals examined		0 5	0.3 5	3.0 5	30.0 5
Spleen: Hematopoiesis, extramedullary, increased		5 0	5 0	5 5**	5 5**	5 5**
Pituitary: Hydropic degeneration, basophilic cell	[N=]	5 0	5 5**	5 5**	5 5**	5 5**
Thyroid: Increased small-sized follicles Ultimobranchial remnant	[N=]	5 1 1	5 1 0	5 0 1	5 2 1	5 2 1
Adrenal: Vacuolation, cortical cell, diffuse	[N=]	5 0	5 0	5 4*	5 4*	5 4*
Ovary: Cyst, follicular Atrophy, interstitial cell	[N=]	5 0 0	5 5** 5**	5 5** 5**	5 5** 5**	5 5** 5**
Uterine horn: Atrophy	[N=]	5 0	5 5**	5 5**	5 5**	5 5**
Uterine cervix: Atrophy	[N=]	5 0	5 5**	5 5**	5 5**	5 5**
Vagina: Atrophy	[N=]	5 0	5 5**	5 5**	5 5**	5 5**
Mammary gland: Hypertrophy	[N=]	5 0	5 4*	5 4*	5 5**	5 5**

[N]: Number of animals examined microscopically at the site.

* and **: Significantly different from the control at 5 and 1% levels of probability, respectively (Fisher's exact probability test).

Table 26C. Histopathological findings for female rats (Combined Subgroups) in the Laboratory 8 CGS 18320B study

Site & Lesion	CGS 183320B (mg/kg/day) No. of animals examined		0 10	0.3 10	3.0 10	30.0 10
Spleen: Hematopoiesis, extramedullary., increased	[N=]		10 0	10 1	10 10**	10 10**
Pituitary: Hydropic degeneration, basophilic cell	[N=]		10 0	10 8**	10 9**	10 10**
Thyroid: Increased small-sized follicles Ultimobranchial remnant	[N=]		10 2 1	10 2 1	10 1 2	10 6 1
Adrenal: Vacuolation, cortical cell, diffuse	[N=]		10 0	10 0	10 9**	10 9**
Ovary: Cyst, follicular Atrophy, interstitial cell	[N=]		10 0 0	10 10** 10**	10 10** 10**	10 10** 10**
Uterine horn: Atrophy	[N=]		10 0	10 7**	10 10**	10 10**
Uterine cervix: Atrophy	[N=]		10 0	10 7**	10 10**	10 10**
Vagina: Atrophy	[N=]		10 0	10 7**	10 10**	10 10**
Mammary gland: Hypertrophy	[N=]		10 0	10 9**	10 9**	10 10**

[N]: Number of animals examined microscopically at the site.

* and **: Significantly different from the control at 5 and 1% levels of probability, respectively (Fisher's exact probability test).

Laboratory 13 – CGS 18320B

59. The results, illustrations and tabulated incidences of selected histopathologic findings are presented below in Table 20. The dose groups for CGS 18320B in both sexes were groups 1 (Vehicle control), 2 (0.3 mg/kg/day), 3 (3 mg/kg/day) and 4 (30 mg/kg/day).

60. Treatment-related microscopic findings: The following findings are considered related to the treatment, because of their distribution among the groups in a dose-related manner.

61. Adrenal glands: Bilateral diffuse cortical fatty change occurred with increased incidence or grading in male and female groups 2 (0.3 mg/kg/day), 3 (3 mg/kg/day), and 4 (30 mg/kg/day). The adrenal cortex was increased in thickness, especially the zona fasciculata, and contained cortical cells with vacuolated cytoplasm. This finding represented change known as "lipid hyperplasia", which can be experimentally induced by adrenostatic drugs¹. This change was associated with adrenal enlargement observed at the necropsy. The terms such as "hyperplasia" or "hypertrophy" were not used for reporting, because the exact analysis of the findings would require support by markers of cell proliferation and morphometry.

62. Kidneys: Renal tubular atrophy had an increased incidence in male group 4 and increased grading in female group 4. Two males and one female of group 4 had renal tubular dilatation. Cortical mineralization occurred in one male and six females of group 4. Corticomedullary mineralization, normally

¹ Landes Ch, Krinke G, Zak F (1996) Lipid hyperplasia, adrenal cortex, rat. In: Jones TC, Capen CC, Mohr U, (eds) Monographs on pathology of laboratory animals. Endocrine system, Second Edition. Springer, Berlin Heidelberg New York Tokyo, pp 48-486.

occurring especially in female rats, was unaffected by the treatment, as it had about the same distribution among the female groups.

63. Liver: Centrilobular hepatocellular hypertrophy occurred with an increased incidence in male and female group 4.

64. Mammary gland (females): Lobulo-alveolar development was more pronounced in treated than in control females. This finding was most prominent in the group 2 and occurred with grading decreasing in relationship to the increasing dose level.

65. Ovaries: The ovaries of all treated females had large antral follicles (Graafian follicles). This finding, reported as "increased follicles" had grading increased in a dose - related manner.

66. Pituitary gland: Hypertrophy of cells in the pars distalis occurred in none of the control females, two females of group 2, five females of group 3 and 4 females of group 4. The hypertrophic cells had pale "secretory vacuoles" in the cytoplasm, which are known to occur in thyrotrops.

67. Thyroid gland: Follicular cell hypertrophy occurred in none of the control females, two females each of groups 2 and 3 and 3 females of group 4.

68. Uterus: Atrophy of the uterus occurred in 8 females of group 2 and all ten females each of groups 3 and 4. The grading was increased in a dose - related manner. The change was characterized by small size of the organ and high density of cell nuclei with little cytoplasm especially in the smooth muscle tissue.

69. Incidental microscopic findings: Additionally, a variety of other changes was found in this study. They commonly occur in laboratory rats of this strain and age, and, neither their incidences nor their distribution or morphologic appearance gave any indication of a treatment-related association.

70. The details of the major histopathological findings in the laboratory 12 study are shown for males in Table 27 and for females in Table 28.

Table 27. Histopathological findings in males with CGS 18320B in the Laboratory 13 study

DOSE GROUP CGS18320B mg/kg/d:	0	0.3	3	30
NO.ANIMALS:	10	10	10	10
ADRENAL GLANDS:	10	10	10	10
- CHANGE FATTY CORT.				
Grade 1:	3	5	5	1
Grade 2:	-	-	4	2
Grade 3:	-	-	-	3
Grade 4:	-	-	-	4
Total Affected:	3	5	9	10
Mean Severity:	1.0	1.0	1.4	3.0
KIDNEYS:	10	10	10	10
- ATROPHY TUBULAR				
Grade 1:	-	-	2	3
Grade 2:	-	-	-	1
Grade 3:	-	1	-	1
Grade 4:	-	-	-	1
Total Affected:	-	1	2	6
Mean Severity:	-	3.0	1.0	2.0
- DILATATION TUBULAR				
Grade 2:	-	-	-	1
Grade 3:	-	-	-	1
Total Affected:	-	-	-	2
Mean Severity:	-	-	-	2.5
- MINERALIZATION CORT.				
Grade 1:	-	-	-	1
Total Affected:	-	-	-	1
Mean Severity:	-	-	-	1.0
LIVER:	10	10	10	10
- HYPERTROPH.CENTRILOB				
Grade 1:	1	-	-	6
Total Affected:	1	-	-	6
Mean Severity:	1.0	-	-	1.0

Table 28. Histopathological findings in females with CGS 18320B in the Laboratory 13 study

DOSE GROUP CGS18320B mg/kg/d:	0	0.3	3	30
NO.ANIMALS:	10	10	10	10
ADRENAL GLANDS:	10	10	10	10
- CHANGE FATTY CORT.				
Grade 1:	5	8	-	4
Grade 2:	-	1	6	3
Grade 3:	-	-	4	2
Grade 4:	-	-	-	1
Total Affected:	5	9	10	10
Mean Severity:	1.0	1.1	2.4	2.0
KIDNEYS:	10	10	10	10
- ATROPHY TUBULAR				
Grade 1:	1	2	3	-
Grade 2:	5	7	6	5
Grade 3:	4	1	1	5
Total Affected:	10	10	10	10
Mean Severity:	2.3	1.9	1.8	2.5
- DILATATION TUBULAR				
Grade 2:	-	-	-	1
Total Affected:	-	-	-	1
Mean Severity:	-	-	-	2.0
- MINERALIZATION CORT.				
Grade 1:	1	-	-	4
Grade 2:	-	-	-	2
Total Affected:	1	-	-	6
Mean Severity:	1.0	-	-	1.3
LIVER:	10	10	10	10
- HYPERTROPH.CENTRILOB				
Grade 1:	-	2	-	8
Total Affected:	-	2	-	8
Mean Severity:	-	1.0	-	1.0
PITUITARY GLAND:	10	10	10	10
- HYPERTROPHY P. DIST.				
Grade 1:	-	2	3	3
Grade 2:	-	-	2	1
Total Affected:	-	2	5	4
Mean Severity:	-	1.0	1.4	1.3
THYROID GLAND:	10	10	10	10
- HYPERTROPHY FOLLIC.				
Grade 1:	-	2	2	3
Total Affected:	-	2	2	3
Mean Severity:	-	1.0	1.0	1.0
MAMMARY GLAND:	10	10	10	10
- ALVEOLAR DEVELOPMENT				
Grade 1:	8	-	-	-
Grade 2:	2	-	1	5
Grade 3:	-	7	8	4
Grade 4:	-	3	1	1
Total Affected:	10	10	10	10
Mean Severity:	1.2	3.3	3.0	2.6

Table 28 continued. Histopathological findings in females with CGS 18320B in the Laboratory 13 study

DOSE GROUP CGS18320B mg/kg/d:	0	0.3	3	30
NO.ANIMALS:	10	10	10	10
OVARIES:	10	10	10	10
- INCREAS. FOLLICLES				
Grade 2:	2	4	-	1
Grade 3:	-	4	7	3
Grade 4:	-	2	3	6
Total Affected:	2	10	10	10
Mean Severity:	2.0	2.8	3.3	3.5
UTERUS:	10	10	10	10
- ATROPHY				
Grade 3:	-	8	9	5
Grade 4:	-	-	1	5
Total Affected:	-	8	10	10
Mean Severity:	-	3.0	3.1	3.5

Methyl Testosterone**Laboratory 3 - 17 α -Methyl Testosterone**

71. *Male genital organs* Treatment-related histopathologic changes were observed in the testis and epididymis. The predominant testicular finding was a diffuse bilateral, low number of Leydig cells in all males treated at 200 and the majority of males treated at 40 mg/kg/day, in the Combined Subgroups and both Subgroups. Furthermore, in individual males of both Subgroups at 200 or 40 mg/kg/day, a bilateral diffuse degeneration and/or multifocal vacuolation of the germinal epithelium was observed (Table 29).

Table 29. Incidence of treatment-related changes in the testis

Sex	Males			
Methyl Testosterone (mg/kg/day)	0	10	40	200
Low number of Leydig cells, bilateral, diffuse				
Subgroup A	0/5	0/5	4/5	5/5
Subgroup B	0/5	0/5	4/5	5/5
Combined Subgroups	0/10	0/10	8/10	10/10
Germinal epithelium, degeneration, bilateral, diffuse				
Subgroup A	0/5	0/5	0/5	1/5
Subgroup B	0/5	0/5	1/5	1/5
Combined Subgroups	0/10	0/10	1/10	2/10
Germinal epithelium, intraepithelial vacuoles, multifocal				
Subgroup A	0/5	0/5	1/5	1/5
Subgroup B	0/5	0/5	1/5	0/5
Combined Subgroups	0/10	0/10	2/10	1/10

72. Corresponding to the lower epididymal weights, individual males of both Subgroups treated at 200 or 40 mg/kg/day showed a diffuse oligospermia and/or diffuse intratubular spermatic debris in the epididymis (Table 30).

Table 30. Incidence of treatment-related changes in the epididymis

Sex	Males			
Methyl Testosterone (mg/kg/day)	0	10	40	200
Oligospermia, diffuse				
Subgroup A	0/5	0/5	1/5	2/5
Subgroup B	0/5	0/5	1/5	1/5
Combined Subgroups	0/10	0/10	2/10	3/10
Intratubular spermatic debris, diffuse				
Subgroup A	0/5	0/5	1/5	2/5
Subgroup B	0/5	0/5	1/5	0/5
Combined Subgroups	0/10	0/10	2/10	2/10

73. *Female genital organs* Changes of the female genital organs were principally characterized by a gonadal atrophy with an activation and/or hyperplasia of uterus and vagina. These changes were restricted to dose groups 600 and 100 mg/kg/day. All females in both Subgroups at 600 mg/kg/day and a proportion of females in Subgroups 1 and 2 at 100 mg/kg/day showed a diffuse atrophy of ovarian interstitial cells and/or a focal or multifocal follicular atresia (Table 31).

Table 31. Incidence of treatment-related changes in the ovary

Sex	Females			
Methyl Testosterone (mg/kg/day)	0	10	100	600
Atrophy, interstitial cells, diffuse				
Subgroup A	0/5	0/5	4/5	5/5
Subgroup B	0/5	0/5	2/5	5/5
Combined Subgroups	0/10	0/10	6/10	10/10
Follicular atresia, focal/multifocal				
Subgroup A	0/5	1/5	3/5	5/5
Subgroup B	0/5	0/5	1/5	5/5
Combined Subgroups	0/10	1/10	4/10	10/10

74. Histopathologic findings of the uterus were multiple at 600 and 100 mg/kg/day. The predominant change was a diffuse epithelial hyperplasia in all females in both subgroups at 600 mg/kg/day and in a proportion of females in the Combined Subgroups and both subgroups at 100 mg/kg/day. This change was accompanied in some animals, but inconsistently in both subgroups, by epithelial vacuolation, epithelial squamous metaplasia and/or intraluminal/intraglandular secretory material (Table 32).

Table 32. Incidence of treatment-related changes in the uterus

Sex	Females			
Methyl Testosterone (mg/kg/day)	0	10	100	600
Epithelial hyperplasia, diffuse				
Subgroup A	0/5	0/5	3/5	5/5
Subgroup B	0/5	1/5	2/5	5/5
Combined Subgroups	0/10	1/10	5/10	10/10
Epithelial vacuolation, diffuse				
Subgroup A	0/5	0/5	0/5	3/5
Subgroup B	0/5	0/5	0/5	1/5
Combined Subgroups	0/10	0/10	0/10	4/10
Epithelial squamous metaplasia, focal/ multifocal				
Subgroup A	0/5	0/5	2/5	0/5
Subgroup B	0/5	0/5	0/5	0/5
Combined Subgroups	0/10	0/10	2/10	0/10
Secretory material, intraluminal and/or intraglandular				
Subgroup A	0/5	0/5	0/5	2/5
Subgroup B	0/5	0/5	0/5	0/5
Combined Subgroups	0/10	0/10	0/10	2/10

75. Furthermore, in the Combined Subgroups and both subgroups treated at 600 and 100 mg/kg/day, a tendency towards a higher incidence and severity of luminal or glandular uterine dilatation was noted.

76. In accordance with the uterine changes observed, vaginal histology was characterized by a diffuse epithelial hypertrophy/hyperplasia and/or a diffuse epithelial mucoid metaplasia in females in both subgroups treated at 600 or 100 mg/kg/day (Table 33).

Table 33. Incidence of treatment-related changes in the vagina

Sex	Females			
Dose (mg/kg/day)	0	10	100	600
Epithelial hypertrophy/hyperplasia, diffuse				
Subgroup A	0/5	0/5	3/5	5/5
Subgroup B	0/5	0/5	2/5	5/5
Combined Subgroups	0/10	0/10	5/10	10/10
Epithelial mucoid metaplasia, diffuse				
Subgroup A	0/5	0/5	5/5	5/5
Subgroup B	0/5	0/5	5/5	5/5
Combined Subgroups	0/10	0/10	10/10	10/10

77. *Mammary gland* A multifocal or diffuse acinar secretion of the mammary gland occurred in all males in both subgroups treated at 200 mg/kg/day and in all females in both subgroups treated at 600 or 100 mg/kg/day (Table 34).

Table 34. Incidence of treatment-related changes in the mammary gland

Sex	Males				Females			
Dose (mg/kg/day)	0	10	40	200	0	10	100	600
Acinar secretion, multifocal/ diffuse								
Subgroup A	1/5	1/5	0/4	5/5	0/5	0/4	3/3	5/5
Subgroup B	1/5	1/5	2/5	5/5	0/5	0/5	5/5	4/4
Combined Subgroups	2/10	2/10	2/9	10/10	0/10	0/9	8/8	9/9

78. *Non-genital organs* Treatment-related liver changes were seen in the high and mid dose groups of both sexes. There was an increased diffuse cytoplasmic eosinophilia in the centrilobular area (with decreased glycogen storage in these areas) in the Combined Subgroups and both subgroups in males and females, with the exception of Subgroup A, males, treated at 40 mg/kg/day. Furthermore, in the combined groups at high and mid dose, there was a tendency towards a higher degree of diffuse intracellular glycogen storage (Table 35).

Table 35. Incidence of treatment-related changes in the liver

Sex	Males				Females			
Dose (mg/kg/day)	0	10	40	200	0	10	100	600
Increased cytoplasmic eosinophilia with decreased intracellular glycogen storage, centrilobular, diffuse								
Subgroup A	0/5	0/5	0/5	5/5	0/5	0/5	2/5	3/5
Subgroup B	0/5	0/5	3/5	4/5	0/5	0/5	4/5	3/5
Combined group	0/10	0/10	3/10	9/10	0/10	0/10	6/10	6/10

79. In the thyroid gland, a slight diffuse follicular cell hypertrophy was noted in 3/10 males at 200 mg/kg/day as well as in 4/10 females at 600 mg/kg/day and 2/10 females at 100 mg/kg/day, the low incidence in the subgroups making this change difficult to interpret at this level.

Table 36. Incidence of treatment-related changes in the thyroid gland

Sex	Males				Females			
Dose (mg/kg/day)	0	10	40	200	0	10	100	600
Follicular cell hypertrophy, diffuse								
Subgroup A	0/5	0/5	0/5	1/5	0/5	0/5	2/5	1/5
Subgroup B	0/5	0/5	0/5	2/5	0/5	0/5	0/5	3/5
Combined Subgroups	0/10	0/10	0/10	3/10	0/10	0/10	2/10	4/10

80. Treatment-related kidney changes, corresponding to the higher kidney weights, were observed in both sexes. In a proportion of males at 200 mg/kg/day (combined Subgroups and Subgroup A) and of

females at 600 mg/kg/day (Combined Subgroups and both subgroups), a slight or mild diffuse cortical tubular dilatation was noticed. A higher incidence and severity of cortical basophilic tubules in both sexes treated at the high dose (Combined Subgroups and both individual Subgroups) as well as in males at 40 mg/kg/day (Combined Subgroups and both individual subgroups) was judged as accompanying change, indicating tubular regeneration. Furthermore, in a proportion of males of the Combined Subgroups and both individual Subgroups at 200 mg/kg/day, there was a slight or mild accumulation of hyaline droplets in the corticotubular epithelium (Table 37).

Table 37. Incidence of treatment- related changes in the kidney

Sex	Males				Females			
	0	10	40	200	0	10	100	600
Tubular dilatation, cortical, diffuse								
Subgroup A	0/5	0/5	0/5	3/5	0/5	0/5	0/5	5/5
Subgroup B	0/5	0/5	1/5	1/5	0/5	0/5	0/5	3/5
Combined Subgroups	0/10	0/10	1/10	4/10	0/10	0/10	0/10	8/10
Basophilic tubules, cortical, focal/multifocal								
Subgroup A	3/5	2/5	5/5	4/5	2/5	2/5	2/5	5/5
Subgroup B	1/5	2/5	4/5	5/5	0/5	2/5	2/5	3/5
Combined Subgroups	4/10	4/10	9/10	9/10	2/10	4/10	4/10	8/10
Increased hyaline droplets, corticotubular epithelium, multifocal/ diffuse								
Subgroup A	0/5	0/5	1/5	2/5	0/5	0/5	0/5	0/5
Subgroup B	0/5	0/5	1/5	3/5	0/5	0/5	0/5	0/5
Combined Subgroups	0/10	0/10	2/10	5/10	0/10	0/10	0/10	0/10

81. A proportion of males at 200 and 40 mg/kg/day and females at 600 and 100 mg/kg/day showed an increased diffuse vacuolation of the adrenal zona fasciculata in the combined Subgroups and both individual Subgroups. In females, this change was accompanied by a multifocal or diffuse deposition of intracellular eosinophilic droplets in the zona fasciculata, in the Combined Subgroups and both subgroups at 600 and 100 mg/kg/day (Table 38).

Table 38. Incidence of treatment-related changes in the adrenal gland

Sex	Males				Females			
	0	10	40	200	0	10	100	600
Increased vacuolation, zona fasciculata, diffuse								
Subgroup A	0/5	0/5	2/5	2/5	0/5	0/5	2/5	3/5
Subgroup B	0/5	0/5	5/5	3/5	0/5	0/5	1/5	2/5
Combined Subgroups	0/10	0/10	7/10	5/10	0/10	0/10	3/10	5/10
Intracellular eosinophilic droplets, zona fasciculata, multifocal/ diffuse								
Subgroup A	0/5	0/5	0/5	0/5	0/5	0/5	4/5	4/5
Subgroup B	0/5	0/5	0/5	0/5	0/5	0/5	3/5	5/5
Combined Subgroups	0/10	0/10	0/10	0/10	0/10	0/10	7/10	9/10

82. Corresponding to the lower thymus weights noted, the thymus of a proportion of males at 200 mg/kg/day and of all females at 600 mg/kg/day showed a slightly to moderately diminished size, predominantly of the cortex.

83. All other histological changes were judged to be incidental and not treatment-related.

84. The overall histopathological data are summarized for males in Table 39 and for females in Table 40.

Table 39. Histopathological findings for males in the laboratory 3 Methyl Testosterone study

Methyl Testosterone (mg/kg/day)	Animal sex: Group dose levels Number in groups	MALES (subgroup A)				MALES (subgroup B)				MALES (combined)			
		Ctls	10	40	200	Ctls.	10	40	200	Ctls	10	40	200
		5	5	5	5	5	5	5	5	10	10	10	10
LIVER	Number examined:	5	5	5	5	5	3	5	5	10	10	10	10
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
	Intracellular glycogen storage, diffuse	5	4	3	5	5	4	5	4	10	8	8	9
	increased cytoplasmic eosinophilia with decreased intracellular glycogen storage, centrilobular, diffuse	0	0	0	5	0	0	3	4	0	0	3	9
	Necrotic focus(i), focal/multifocal	3	2	3	2	5	2	1	4	8	4	4	6
	Interstitial infiltration, mononuclear cell, -focal/multifocal	2	2	2	0	0	2	0	1	2	4	2	1
	Capsular fibrosis, focal/multifocal	0	0	0	0	0	0	0	0	0	0	0	0
	Misshapen lobe	0	0	0	0	0	0	0	0	0	0	0	0
KIDNEY(S)	Number examined:	5	5	5	5	5	5	5	5	10	10	10	10
	Unremarkable:	2	3	0	1	2	3	0	0	4	6	0	1
	Tubular dilatation, cortical, diffuse	0	0	0	3	0	0	1	1	0	0	1	4
	increased hyaline droplets, corticotubular epithelium, multifocal/diffuse	0	0	1	2	0	0	1	3	0	0	2	5
	Basophilic tubules, cortical, focal/multifocal	3	2	5	4	1	2	4	5	4	4	9	9
	Interstitial infiltration, mononuclear cell, cortical, focal/multifocal	1	0	0	0	2	1	2	0	3	1	2	0
	Pelvic dilatation, unilateral	0	0	1	1	1	0	1	2	1	0	2	3
	Pelvic dilatation, bilateral	0	0	0	1	0	0	0	0	0	0	0	1
	Urothelial hyperplasia, pelvis, focal/multifocal	0	0	1	1	0	0	0	0	0	0	1	1
	Mineralization, corticomedullary junction, focal/multifocal	0	0	1	2	0	0	2	3	0	0	3	5
TESTIS(ES)	Number examined:	5	5	5	5	5	5	5	5	10	10	10	10
	Unremarkable:	5	5	0	0	5	3	1	0	10	8	1	0
	Low numbers of Leydig cells, bilateral, diffuse	0	0	4	5	0	0	4	5	0	0	8	10
	Germinal epithelium, degeneration, bilateral, diffuse	0	0	0	1	0	0	1	1	0	0	1	2
	Germinal epith., intraepithelial vacuoles, multifocal	0	0	1	1	0	0	1	0	0	0	2	1
	Atrophic tubules, focal/multifocal	0	0	0	0	0	2	0	0	0	2	0	0
EPIDIDYMIS(DES)	Number examined:	5	5	5	5	5	5	5	5	10	10	10	10
	Unremarkable:	4	2	4	1	2	1	2	0	6	3	6	1
	Oligospermia, diffuse	0	0	1	2	0	0	1	1	0	0	2	3
	Intratubular spermatic debris, diffuse	0	0	1	2	0	0	1	0	0	0	2	2
	Interstitial infiltration, mononuclear cell, focal/multifocal	1	3	0	3	3	4	2	5	4	7	2	8

Table 39 continued. Histopathological findings for males in the laboratory 3 Methyl Testosterone study

Methyl Testosterone (mg/kg/day)	Animal sex: Group dose levels Number in groups	MALES (subgroup A)				MALES (subgroup B)				MALES (combined)			
		Ctls	10	40	200	Ctls.	10	40	200	Ctls	10	40	200
PROSTATE	Number examined: Unremarkable:	5 0	5 3	5 1	5 3	5 1	5 2	5 0	5 3	10 1	10 5	10 1	10 6
	Secretory inactivity, diffuse	0	0	1	0	0	0	1	0	0	0	2	0
	Interstitial infiltration, mononuclear cell, -focal/multifocal	5	2	3	2	4	3	5	2	9	5	8	4
SEMINAL VESICLES	Number examined: Unremarkable:	5 5	5 5	5 5	5 5	5 5	5 5	5 5	5 5	10 10	10 10	10 10	10 10
MAMMARY GLAND (S)	Number examined: Unremarkable:	5 4	5 4	4 4	5 0	5 4	5 4	5 3	5 0	10 8	10 8	9 7	10 0
	Acinar secretion, multifocal/diffuse	1	1	0	5	1	1	2	5	2	2	2	10
ADRENAL GLAND (S)	Number examined: Unremarkable:	5 5	5 5	5 3	5 3	5 5	5 5	5 0	5 2	10 10	10 10	10 3	10 5
	Increased vacuolation, zona fasciculata, diffuse	0	0	2	2	0	0	5	3	0	0	7	5
	Intracellular eosinophilic droplets, zona fasciculata, -multifocal/diffuse	0	0	0	0	0	0	0	0	0	0	0	0
	Congestion and/or hemorrhage, z. fasciculata, multifocal/diffuse	0	0	0	0	0	0	0	0	0	0	0	0
	Extracortical nodule(s)	0	0	0	0	0	0	0	0	0	0	0	0
THYROID GLAND(S)	Number examined: Unremarkable:	5 5	5 4	5 3	5 4	5 1	5 4	5 1	5 2	10 6	10 8	10 4	10 6
	Follicular cell hypertrophy, diffuse	0	0	0	1	0	0	0	2	0	0	0	3
	Ultimobranchial cyst(s), focal/multifocal	0	1	2	0	4	1	4	2	4	2	6	2
	Ectopic thymic tissue, focal/multifocal	0	0	0	0	2	0	2	0	2	0	2	0
DORSAL PROSTATE	Number examined: Unremarkable:	5 5	5 4	5 5	5 3	5 5	5 4	5 5	5 4	10 10	10 8	10 10	10 7
	Hyperplasia, focal/multifocal	0	1	0	2	0	1	0	1	0	2	0	3

Table 40. Histopathological findings for females in the laboratory 3 Methyl Testosterone study

Methyl Testosterone (mg/kg/day)	Animal sex: Group dose levels Number in groups	FEMALES (subgroup A)				FEMALES (subgroup B)				FEMALES (combined)			
		Ctls	10	100	600	Ctls.	10	100	600	Ctls	10	100	600
LIVER	Number examined:	5	5	5	5	5	5	5	5	10	10	10	10
	Unremarkable:	0	0	0	0	1	1	0	0	1	1	0	0
	Intracellular glycogen storage, diffuse	0	5	3	5	1	1	4	5	1	6	7	10
	Increased cytoplasmic eosinophilia with decreased -intracellular glycogen storage, centrilobular, diffuse	0	0	2	3	0	0	4	3	0	0	6	6
	Necrotic focus(i), focal/multifocal	5	3	5	3	4	4	0	2	9	7	5	5
	Interstitial infiltration, mononuclear cell, -focal/multifocal	0	2	1	1	1	2	1	0	1	4	2	1
	Capsular fibrosis, focal/multifocal	0	0	0	0	0	0	0	1	0	0	0	1
	Misshapen lobe	0	0	0	0	0	0	0	1	0	0	0	1
KIDNEY(S)	Number examined:	5	5	5	5	5	5	5	5	10	10	10	10
	Unremarkable:	1	1	1	0	1	1	2	0	2	2	3	0
	Tubular dilatation, cortical, diffuse	0	0	0	5	0	0	0	3	0	0	0	8
	Increased hyaline droplets, corticotubular epith., multifocal/diffuse	0	0	0	0	0	0	0	0	0	0	0	0
	Basophilic tubules, cortical, focal/multifocal	2	2	2	5	0	2	2	3	2	4	4	8
	Interstitial infiltration, mononuclear cell, cortical, focal/multifocal	1	2	1	0	1	0	1	0	2	2	2	0
	Pelvic dilatation, unilateral	0	1	1	0	1	0	1	2	1	1	2	2
	Pelvic dilatation, bilateral	1	1	0	0	0	0	1	1	1	1	1	1
	Urothelial hyperplasia, pelvis, focal/multifocal	0	0	0	0	0	0	0	0	0	0	0	0
	Mineralization, corticomedullary junction, focal/multifocal	3	4	3	1	4	4	3	4	7	8	6	5
OVARY(IES)	Number examined:	5	5	5	5	5	5	5	5	10	10	10	10
	Unremarkable:	5	4	1	0	5	5	2	0	10	9	3	0
	Atrophy, interstitial cells, diffuse	0	0	4	5	0	0	2	5	0	0	6	10
	Follicular atresia, focal/multifocal	0	1	3	5	0	0	1	5	0	1	4	10
Intraovarian cyst(s)	0	0	0	0	0	0	1	0	0	0	1	0	
UTERUS	Number examined:	5	5	5	5	5	5	5	5	10	10	10	10
	Unremarkable:	1	5	0	0	4	4	1	0	5	9	1	0
	Epithelial hyperplasia, diffuse	0	0	3	5	0	1	2	5	0	1	5	10
	Epithelial vacuolation, diffuse	0	0	0	3	0	0	0	1	0	0	0	4
	Epithelial squamous metaplasia, focal/multifocal	0	0	2	0	0	0	0	0	0	0	2	0
	Secretory material, intraluminal and/or intraglandular	0	0	0	2	0	0	0	0	0	0	0	2
	Dilated uterine horn(s)	3	0	3	3	1	1	3	4	4	1	6	7
	Dilation of endometrial glands, multifocal/diffuse	4	0	4	5	0	1	4	4	4	1	8	9

Table 40 continued. Histopathological findings for females in the laboratory 3 Methyl Testosterone study

Methyl Testosterone (mg/kg/day)	Animal sex: Group dose levels Number in groups	FEMALES (subgroup A)				FEMALES (subgroup B)				FEMALES (combined)			
		Ctls	10	100	600	Ctls.	10	100	600	Ctls	10	100	600
VAGINA	Number examined: Unremarkable:	5	5	5	5	5	5	5	5	10	10	10	10
	Epithelial hypertrophy/hyperplasia, diffuse	0	0	3	5	0	0	2	5	0	0	5	10
	Epithelial mucoid metaplasia, diffuse	0	0	5	5	0	0	5	5	0	0	10	10
	Proestrus morphology	3	0	0	0	0	0	0	0	3	0	0	0
	Estrus morphology	1	0	0	0	1	1	0	0	2	1	0	0
	Met-/Diestrus morphology	1	5	0	0	4	4	0	0	5	9	0	0
MAMMARY GLAND (S)	Number examined: Unremarkable:	5	4	3	5	5	5	5	4	10	9	8	9
	Acinar secretion, multifocal/diffuse	0	0	3	5	0	0	5	4	0	0	8	9
PITUITARY GLAND	Number examined: Unremarkable:	5	0	0	5	4	0	0	5	9	0	0	10
	cyst(s), pars distalis, focal/multifocal	4	0	0	5	4	0	0	5	8	0	0	10
		1	0	0	0	0	0	0	0	1	0	0	0
ADRENAL GLAND (S)	Number examined: Unremarkable:	5	5	5	5	5	5	5	5	10	10	10	10
		5	5	0	0	5	4	2	0	10	9	2	0
	Increased vacuolation, zona fasciculata, diffuse	0	0	2	3	0	0	1	2	0	0	3	5
	Intracellular eosinophilic droplets, zona fasciculata	0	0	4	4	0	0	3	5	0	0	7	9
	Congestion and/or hemorrhage, zona. fasciculata, multifocal/diffuse	0	0	0	3	0	0	0	1	0	0	0	4
	Extracortical nodule(s)	0	0	0	0	0	1	0	0	0	1	0	0
THYROID GLAND(S)	Number examined: Unremarkable:	5	5	5	5	5	5	5	5	10	10	10	10
		2	3	1	2	3	3	3	2	5	6	4	4
	Follicular cell hypertrophy, diffuse	0	0	2	1	0	0	0	3	0	0	2	4
	Ultimobranchial cyst(s), focal/multifocal	3	2	2	1	1	2	1	1	4	4	3	2
	Ectopic thymic tissue, focal/multifocal	0	0	0	1	1	0	1	0	1	0	1	1

Laboratory 12 – Methyl Testosterone

Subgroup A findings

85. Arrest of spermatogenesis was observed in the testis of all males in the 200 mg/kg/day group; this was characterised by a striking reduction in the number of elongated spermatids and mature spermatozoa from tubules at all stages of spermatogenesis. Corresponding changes in the epididymis of these animals were absent/reduced spermatozoa and increased numbers of sperm precursor cells. No histological changes were observed in the prostate gland.

86. In females, extensive changes were observed in the reproductive tract and mammary gland of animals receiving 100 and 600 mg/kg/day. In the ovary, reduction in numbers of corpora lutea was associated with an increase in the number of medium/large follicles indicating inhibition of ovulation. Uterine changes consisting of metaplasia /hyperplasia and increased apoptosis/vacuolation of endometrial epithelium were associated with cervical epithelial hyperplasia. Minimal endometritis was also observed in a few animals at the highest dose level. The majority of animals also showed mucoid metaplasia of the vaginal epithelium, indicating disturbance of normal oestrous cyclicity. Changes in the mammary gland comprised focal or diffuse hyperplasia and, in the 600 mg/kg/day group, secretory activity.

87. A number of changes were observed in other organs.

88. Adrenal cortical vacuolation, mainly affecting the zona reticularis, was observed in males receiving 200 mg/kg/day and in females receiving 600 mg/kg/day. Thyroid follicular cell hypertrophy was also observed in animals of both sex, i.e. in males at 40 and 200 mg/kg/day and in females at 600 mg/kg/day only.

89. Other changes in males comprised an increase in the incidence and severity of intratubular microlithiasis (40 and 200 mg/kg/day) and tubular basophilia (200 mg/kg/day) in the kidney. In females there was a slight increase in the severity of intratubular microlithiasis at 100 mg/kg/day only. Other changes confined to females receiving 600 mg/kg/day were increased hepatic glycogen, thymic lymphocyte depletion and thymic epithelial cysts.

90. All other findings were considered to be spontaneous in origin.

Subgroup B findings

91. Arrest of spermatogenesis was observed in the testis of all males in the 200 mg/kg/day group; this was characterised by a striking reduction in the number of elongated spermatids and mature spermatozoa from tubules at all stages of spermatogenesis. Corresponding changes in the epididymis of these animals were absent/reduced spermatozoa and increased numbers of sperm precursor cells. No histological changes were observed in the seminal vesicles.

92. In females, extensive changes were observed in the reproductive tract and mammary gland of animals receiving 100 and 600 mg/kg/day. In the ovary, reduction in numbers of corpora lutea was associated with an increase in the number of medium/large follicles indicating inhibition of ovulation. Uterine changes consisting of metaplasia /hyperplasia and increased apoptosis/vacuolation of endometrial epithelium were associated with cervical epithelial hyperplasia. Minimal endometritis was also observed in a few animals at the highest dose level. The majority of animals also showed mucoid metaplasia of the vaginal epithelium, indicating disturbance of normal oestrous cyclicity. Changes in the mammary gland comprised focal or diffuse hyperplasia and, in the 600 mg/kg/day group, secretory activity.

93. A number of changes were observed in other organs.

94. Adrenal cortical vacuolation, mainly affecting the zona reticularis, was observed in males receiving 200 mg/kg/day and in females receiving 600 mg/kg/day. Thyroid follicular cell hypertrophy was also observed in animals of both sex, i.e. in males at 40 and 200 mg/kg/day and in females at 600 mg/kg/day only.

95. Other changes in males comprised an increase in the incidence and severity of intratubular microlithiasis (40 and 200 mg/kg/day) and tubular basophilia (200 mg/kg/day) in the kidney. In females there was a slight increase in the severity of intratubular microlithiasis and tubular basophilia at 100 mg/kg/day only. Other changes confined to females receiving 600 mg/kg/day were increased hepatic glycogen, thymic lymphocyte depletion and thymic epithelial cysts.

96. All other findings were considered to be spontaneous in origin.

Combined Subgroups findings

97. Arrest of spermatogenesis was observed in the testis of all males in the 200 mg/kg/day group; this was characterised by a striking reduction in the number of elongated spermatids and mature spermatozoa from tubules at all stages of spermatogenesis. Corresponding changes in the epididymis of these animals were absent/reduced spermatozoa and increased numbers of sperm precursor cells. No histological changes were observed in the prostate gland or seminal vesicles.

98. In females, extensive changes were observed in the reproductive tract and mammary gland of animals receiving 100 and 600 mg/kg/day. In the ovary, reduction in numbers of corpora lutea was associated with an increase in the number of medium/large follicles indicating inhibition of ovulation. Uterine changes consisting of metaplasia /hyperplasia and increased apoptosis/vacuolation of endometrial epithelium were associated with cervical epithelial hyperplasia. Minimal endometritis was also observed in a few animals at the highest dose level. The majority of animals also showed mucoid metaplasia of the vaginal epithelium, indicating disturbance of normal oestrous cyclicity. Changes in the mammary gland comprised focal or diffuse hyperplasia and, in the 600 mg/kg/day group, secretory activity.

99. A number of changes were observed in other organs.

100. Adrenal cortical vacuolation, mainly affecting the zona reticularis, was observed in males receiving 200 mg/kg/day and in females receiving 600 mg/kg/day. Thyroid follicular cell hypertrophy was also observed in animals of both sex, i.e. in males at 40 and 200 mg/kg/day and in females at 600 mg/kg/day only. Other changes in males comprised an increase in the incidence and severity of intratubular microlithiasis (40 and 200 mg/kg/day) and tubular basophilia (200 mg/kg/day) in the kidney. In females, there was a slight increase in the severity of intratubular microlithiasis and tubular basophilia at 100 mg/kg/day only, but this was not continued at the 600 mg/kg/d dose. Other changes confined to females receiving 600 mg/kg/day were increased hepatic glycogen, thymic lymphocyte depletion and thymic epithelial cysts.

101. All other findings were considered to be spontaneous and incidental in origin, i.e., not treatment related.

102. The detailed summary histopathological findings for each Subgroup are shown for males in Table 41 and for females in Table 42.

Table 41. Histopathological findings for males in the Laboratory 12 Methyl Testosterone study

Methyl Testosterone (mg/kg/day)	0		10		40		200	
Subgroups	A	B	A	B	A	B	A	B
MALES ON STUDY	5	5	5	5	5	5	5	5
ANIMALS COMPLETED	5	5	5	5	5	5	5	5
ADRENAL GLAND								
Examined.	5	5	5	5	5	5	5	5
No abnormalities detected	0	0	0	0	0	0	1	0
Cortical vacuolation, zona reticularis (Total)	0	0	0	0	0	0	4	5
minimal	0	0	0	0	0	0	0	2
slight.	0	0	0	0	0	0	2	1
moderate	0	0	0	0	0	0	2	2
EPIDIDYMIS								
Examined.	5	5	5	5	5	5	5	5
Reduced spermatozoa (Total)	0	0	0	0	0	0	5	4
moderate	0	0	0	0	0	0	5	4
Increased sperm precursor cells (Total)	0	0	0	0	0	0	5	5
slight.	0	0	0	0	0	0	1	1
moderate	0	0	0	0	0	0	4	4
Spermatozoa absent	0	0	0	0	0	0	0	1
KIDNEY								
Examined.	5	5	5	5	5	5	5	5
No abnormalities detected	2	3	0	1	2	0	0	0
Unilateral hydronephrosis (Total)	0	1	0	1	0	0	1	1
minimal	0	0	0	0	0	0	0	1
slight.	0	1	0	1	0	0	1	0
Intratubular microlithiasis (Total)	0	0	0	0	3	5	5	5
minimal	0	0	0	0	3	4	3	1
slight.	0	0	0	0	0	1	0	3
moderate							2	1
Transitional cell hyperplasia (Total)	0	0	1	0	0	0	0	0
minimal	0	0	1	0	0	0	0	0
Tubular basophilia (Total)	3	2	4	3	1	5	4	5
minimal	2	2	4	3	1	5	4	4
slight.	1	0	0	0	0	0	0	1
Tubular vacuolation (Total)	0	0	0	0	0	0	2	0
minimal	0	0	0	0	0	0	2	0
Cystic tubule/s (Total)	0	0	0	1	0	0	0	0
minimal	0	0	0	1	0	0	0	0

Table 41 continued. Histopathological findings for males in the Laboratory 12 Methyl Testosterone study

Methyl Testosterone (mg/kg/day)	0		10		40		200	
Subgroups	A	B	A	B	A	B	A	B
MALES ON STUDY	5	5	5	5	5	5	5	5
ANIMALS COMPLETED	5	5	5	5	5	5	5	5
LIVER								
Examined.	5	5	5	5	5	5	5	5
No abnormalities detected	5	5	5	5	5	5	5	3
Inflammatory cell infiltration (Total).	0	0	0	0	0	0	0	2
minimal	0	0	0	0	0	0	0	2
PROSTATE GLAND(DOR-LAT/VENT)								
Examined.	5	5	0	0	1	0	0	0
No abnormalities detected	5	4	0	0	1	0	0	0
Mononuclear cell infiltration (Total)	0	1	0	0	0	0	0	0
minimal	0	1	0	0	0	0	0	0
TESTIS								
Examined.	5	5	5	5	5	5	5	5
No abnormalities detected	4	5	5	5	5	5	5	5
Unilateral tubular degeneration (Total)	1	0	0	0	0	0	0	0
minimal	1	0	0	0	0	0	0	0
Arrested spermatogenesis (Total)	0	0	0	0	0	0	5	5
moderate	0	0	0	0	0	0	5	5
THYMUS								
Examined.	5	5	0	0	1	0	5	5
No abnormalities detected	5	5	0	0	0	0	5	3
Congestion/haemorrhage (Total)	0	0	0	0	1	0	0	1
minimal	0	0	0	0	1	0	0	1
Epithelial cyst/s	0	0	0	0	0	0	0	1
THYROID GLAND								
Examined.	5	5	5	5	5	5	5	5
No abnormalities detected	4	5	5	4	1	4	0	1
Squamous cyst/s	0	0	0	1	2	1	0	0
Follicular cell hypertrophy (Total)	1	0	0	0	2	1	5	4
minimal	1	0	0	0	2	1	5	4

Table 42. Histopathological findings for females in the Laboratory 12 Methyl Testosterone study

Methyl Testosterone mg/kg/d	0		10		100		600	
Subgroups	A	B	A	B	A	B	A	B
FEMALES ON STUDY	5	5	5	5	5	5	5	5
ANIMALS COMPLETED	5	5	5	5	5	5	5	5
ADRENAL GLAND								
Examined.	5	5	5	5	5	5	5	5
No abnormalities detected	5	5	5	5	5	5	0	0
Cortical congestion/haemorrhage (Total)	0	0	0	0	0	0	1	0
slight.	0	0	0	0	0	0	1	0
Cortical vacuolation, zona reticularis (Total)	0	0	0	0	0	0	5	5
minimal	0	0	0	0	0	0	1	1
slight.	0	0	0	0	0	0	3	1
moderate	0	0	0	0	0	0	1	3
Cortical vacuolation, zona fasciculata (Total)	0	0	0	0	0	0	1	0
slight.	0	0	0	0	0	0	1	0
CERVIX								
Examined.	5	5	5	5	5	5	5	5
No abnormalities detected	5	5	4	5	4	5	0	0
Mononuclear cell infiltration (Total)	0	0	1	0	1	0	5	5
minimal	0	0	1	0	1	0	0	0
slight.	0	0	0	0	0	0	2	5
moderate	0	0	0	0	0	0	2	0
marked.	0	0	0	0	0	0	1	0
KIDNEY								
Examined.	5	5	5	5	5	5	5	5
Eosinophilic casts (Total)	0	0	0	0	0	0	1	0
minimal	0	0	0	0	0	0	1	0
Intratubular microlithiasis (Total)	5	5	5	5	5	5	5	5
minimal	2	2	2	2	0	0	3	4
slight.	3	2	3	3	4	2	2	1
moderate	0	1	0	0	1	3	0	0
Tubular basophilia (Total)	5	5	5	5	5	5	4	4
minimal	4	4	4	4	4	1	4	4
slight.	1	1	1	1	1	4	0	0
Interstitial mononuclear cell infiltration (Total)	0	0	0	1	0	0	0	0
minimal	0	0	0	1	0	0	0	0
MAMMARY GLAND								
Examined.	5	5	5	4	4	4	5	5
MISSING..	0	0	0	1	1	1	0	0
No abnormalities detected	4	5	5	4	1	1	0	0
Keratin cyst	1	0	0	0	0	0	0	0
Secretory activity (Total)	0	0	0	0	0	0	5	5
minimal	0	0	0	0	0	0	1	3
slight.	0	0	0	0	0	0	4	2

Table 42 continued. Histopathological findings for females in the Laboratory 12 Methyl Testosterone study

Methyl Testosterone mg/kg/d	0		10		100		600	
Subgroups	A	B	A	B	A	B	A	B
FEMALES ON STUDY	5	5	5	5	5	5	5	5
ANIMALS COMPLETED	5	5	5	5	5	5	5	5
MAMMARY GLAND								
Examined.	5	5	5	4	4	4	5	5
MISSING..	0	0	0	1	1	1	0	0
Focal hyperplasia (Total)	0	0	0	0	3	2	0	0
minimal	0	0	0	0	0	2	0	0
slight.	0	0	0	0	3	0	0	0
Diffuse hyperplasia (Total)	0	0	0	0	0	1	5	5
minimal	0	0	0	0	0	1	1	1
slight.	0	0	0	0	0	0	4	4
THYMUS								
Examined.	5	5	5	5	5	5	5	5
No abnormalities detected	4	2	4	5	5	3	1	0
Congestion/haemorrhage (Total)	1	3	1	0	0	2	0	1
minimal	1	3	1	0	0	2	0	1
Epithelial cyst/s	0	0	0	0	0	0	2	1
Depleted cortical lymphocytes (Total)	0	0	0	0	0	0	4	4
minimal	0	0	0	0	0	0	1	2
slight.	0	0	0	0	0	0	2	2
moderate	0	0	0	0	0	0	1	0
THYROID GLAND								
Examined.	5	5	5	5	5	5	5	5
No abnormalities detected	5	4	4	3	3	2	0	0
Squamous cyst/s	0	1	1	2	2	3	0	0
Ectopic thymus	0	0	0	0	0	0	0	1
Follicular cell hypertrophy (Total)	0	0	0	0	0	0	5	5
minimal	0	0	0	0	0	0	5	3
slight.	0	0	0	0	0	0	0	2
OVARY								
Examined.	5	5	5	5	5	5	5	5
No abnormalities detected	0	0	0	0	5	5	5	5
Reduced corpea lutea (TOTAL)	0	0	0	0	5	5	5	5
slight	0	0	0	0	4	1	1	0
moderate	0	0	0	0	1	4	1	5
marked	0	0	0	0	0	0	3	0
Increased medium/large follicles (TOTAL)	0	0	0	0	4	5	5	5
slight	0	0	0	0	3	2	3	0
moderate	0	0	0	0	1	3	1	5
marked	0	0	0	0	0	0	1	0

Table 42 continued. Histopathological findings for females in the Laboratory 12 Methyl Testosterone study

Methyl Testosterone mg/kg/d	0		10		100		600	
	A	B	A	B	A	B	A	B
Subgroups								
FEMALES ON STUDY	5	5	5	5	5	5	5	5
ANIMALS COMPLETED	5	5	5	5	5	5	5	5
UTERUS								
Examined.	5	5	5	5	5	5	5	5
No abnormalities detected	5	4	2	5	0	0	0	0
Luminal dilatation (Total)	0	1	0	0	0	0	0	0
slight.	0	1	0	0	0	0	0	0
Metaplasia/hyperplasia - endometrial epithelium (Total)	0	0	0	0	2	2	5	4
minimal	0	0	0	0	2	2	0	0
slight.	0	0	0	0	0	0	1	4
moderate	0	0	0	0	0	0	4	0
Increased vacuolation/apoptosis - endometrial epithelium (Total)	0	0	0	0	5	5	4	5
minimal	0	0	0	0	2	1	0	2
slight.	0	0	0	0	3	4	2	3
moderate	0	0	0	0	0	0	2	0
Squamous metaplasia glands (Total)	0	0	3	0	0	1	1	0
minimal	0	0	3	0	0	1	1	0
Endometritis (Total)	0	0	0	0	0	0	2	3
minimal	0	0	0	0	0	0	2	3
VAGINA								
Examined.	5	5	5	5	5	5	5	5
Mucoid metaplasia (Total)	0	0	0	0	4	5	5	5
minimal	0	0	0	0	0	1	1	1
slight.	0	0	0	0	2	3	1	3
moderate	0	0	0	0	2	1	2	1
marked.	0	0	0	0	0	0	1	0
Stage of oestrous cycle: Dioestrus	5	3	3	5	1	0	0	0
Stage of oestrous cycle: Metoestrus	0	0	2	0				
Stage of oestrous cycle: Pro-oestrous	0	1	0	0				
Stage of oestrous cycle: Oestrous	0	1	0	0				
Stage of cycle: not ascertainable due to abnormal appearance.	0	0	0	0	4	5	5	5
Keratinisation (Total)...	0	0	0	0	0	1	0	0
slight.	0	0	0	0	0	1	0	0

Flutamide**Laboratory 2 - Flutamide**

103. A summary of the laboratory 2 histopathological findings obtained with flutamide is shown in the Table 43.

Table 43. Summary of histopathological findings

Organ system	Histopathological Findings	Subgroup A	Subgroup B
		(Flutamide mg/kg bw/d)	
liver	hepatocellular hypertrophy, cytoplasmic change single cell necrosis (M, F), increased mitotic rate (M)	100 100	100 -
testis	diffuse Leydig cell hypertrophy	from 10	from 10
epididymis	decreased tubular size/diameter, increase of interstitial tissue, increased spermatic debris	100 100	100 -
dorsolateral and ventral prostate, coagulation glands	reduction of secretory products, flattened epithelium, reduction of acinar size	100	100
seminal vesicles	reduction of secretory products, flattened epithelium, reduction of luminal size	100	100
ovaries	minimally increased incidence of increased interstitial glands	100	100
pituitary gland	increased number of intracytoplasmic inclusions and PAS-positive cells (M) hypertrophy of basophilic cells (M)	from 10 from 10 100	100 from 10 100
adrenal glands	microvesicular cytoplasmic structure of adreno- cortical cells of the zona fasciculata (M)	100	100
NOEL (M)		I	I
NOEL (F)		10	10

M male F female

105. It is clear from the Table that the histopathological results of both studies are almost identical. The most sensitive alterations observed at ≥ 10 mg/kg body weight per day were diffuse Leydig cell hypertrophy in the testis and increased numbers of intracytoplasmic inclusions and PAS-positive cells in pituitary cells of male rats.

106. All other changes were only observed in animals administered 100 mg/kg body weight per day. Centrilobular hepatocellular hypertrophy and cytoplasmic change was found in both sexes. All other changes that were found in both studies were only found in high-dose males. Hypertrophy of basophilic cells in the pituitary (adenohypophysis) and microvesicular cytoplasm of the adrenal zona fasciculata and single cell necrosis and increased mitotic rates in the liver were male specific effects seen in organs other than the accessory male reproductive organs. Reductions of secretory products and flattened epithelia were found in the prostate (dorsolateral and ventral), the coagulation glands and the seminal vesicles, while increased interstitial tissue and spermatic debris were found in the epididymides. All male accessory reproductive organs were reduced in size.

107. An effect of treatment on the ovaries, a minimal increase in interstitial glands at 100 mg/kg body weight per day, was seen. The vaginal epithelium of almost all females from all groups was in the diestrous stage (except animal nos. 30 (estrous) and 36 (proestrous) of Subgroup B).

Subgroup A from Laboratory 2 Flutamide study

105. The dose related histopathological findings for Subgroup A of the laboratory 2 flutamide study are presented in this section.

106. Liver: Centrilobular hepatocellular hypertrophy associated with cytoplasmic change was seen in all rats of both sexes given 100 mg/kg bw Flutamide. The findings were scored minimal to slight (grade 1 or 2). One single male (grade 2) and one female (grade 1) of the high dose group had hepatocytic single cell necroses. One male of the high dose group had an increased mitotic rate.

107. Testis: Diffuse hypertrophy of the Leydig cells was seen in rats at 10 mg/kg and above. The frequency of Leydig cell hypertrophy was 0/0/4/5. The severity increased in a dose related manner. It was graded minimal (grade 1) after 10 mg/kg and slight or moderate (grade 2 or grade 3) after 100 mg/kg Flutamide. The hypertrophic Leydig cells had a distinctly eosinophilic cytoplasm. With respect to the germinal epithelium, no morphological differences were found between untreated and treated males.

108. Epididymides: The epididymides were decreased in size in four out of five males given 100 mg/kg. The size and diameter of the epididymidal tubules were decreased with an apparent increase of the interstitial tissue. The severity of this finding was slight or moderate. In addition, two males of the same group showed an increased amount of spermatic debris. The amount of spermatozoa within the epididymidal tubules appeared to be similar in untreated and in treated males.

109. Dorsolateral Prostate/Ventral Prostate/Coagulating glands: The histopathological correlates for the severely diminished size of the prostate noted in all males from the high dose group were reduction of secretory products, flattening of epithelial cells, and reduction of the acinar size in all lobes. After 100 mg/kg all males and all parts of the prostate were affected. The severity varied between grade 2 and grade 4. After 10 mg/kg, a single male had a mild reduction of secretory products and acinar size in the dorsolateral prostate. In the ventral lobes of the prostate, reduction of secretory products was also found in one control male (grade 3) and one male of the low dose group (grade 2) which also revealed mild flattening of the epithelial cells.

110. Seminal vesicles: The microscopic findings in the seminal vesicles were similar to those in the prostate. Reduction of secretory products, flattening of the epithelium and the reduction of the luminal size were investigated and scored. A reduced amount of secretory products (grade 2 or 3), flattened epithelial cells, and a reduced size of the vesicle lumina were observed in all high dose males. The scores ranged from grade 2 to grade 4. No such findings occurred in controls and males of the low and mid dose group.

111. Ovaries: The number of females with an apparent increase of interstitial glands of the ovarian stroma was minimally increased in females which had received 100 mg/kg (incidence:1/2/2/3; grade 2).

112. Vagina/Estrous Cycle: The vaginal epithelium of females from all groups including controls was considered to be in the stage of diestrus.

113. Pituitary Gland: Hypertrophy of the basophilic cell population of the adenohypophysis was seen in males which had received 100 mg/kg flutamide (incidence:0/0/0/5). In addition, the number of small intracytoplasmic inclusions within the basophils was elevated in males given 10 mg/kg and above (incidence: 0/0/1/5). A periodic-acid-Schiff reaction (PAS) specific for glycoproteins, i.e. gonadotrophs, demonstrated higher numbers of PAS-positive cells in males at 10 mg/kg and above. Treatment-related findings of the pituitary gland were not observed in females.

114. Adrenal Glands: A microvesicular cytoplasmic structure of adrenocortical cells from the zona fascicula was found in all males but not in females which had received 100 mg/kg Flutamide.

115. No treatment related findings were seen in the other organs and tissues investigated (brain, pancreas, kidneys, oviducts, uterus, thymus, thyroid glands including parathyroid glands and skin/mammary gland). All findings are presented in the table of individual microscopic findings and the individual animal data sheets.

116. The detailed histopathological findings for Subgroup A are shown in Table 44.

Table 44. Histopathological findings in Laboratory 2 Flutamide study - Subgroup A

Sex	Males				Females			
	0	1	10	100	0	1	10	100
Flutamide (mg/kg/day)								
No. Animals per Dose Group	5	5	5	5	5	5	5	5
LIVER No. Examined	5	5	5	5	5	5	5	5
No Abnormalities Observed	1	3	3	-	4	2	4	-
- HYPERTROPHY	-	-	-	5	-	-	-	5
Grade 1	-	-	-	1	-	-	-	1
Grade 2	-	-	-	4	-	-	-	4
- CYTOPLASMIC CHANGE	-	-	-	5	-	-	-	5
Grade 1	-	-	-	1	-	-	-	1
Grade 2	-	-	-	4	-	-	-	4
- SINGLE CELL NECROSES	-	-	-	1	-	-	-	1
Grade 1	-	-	-	-	-	-	-	1
Grade 2	-	-	-	1	-	-	-	-
- INCREASED MITOTIC RATE	-	-	-	1	-	-	-	-
Grade 2	-	-	-	1	-	-	-	-
- FOCAL KUPFFER CELL PROLIF.	4	2	2	1	-	3	1	1
Grade 1	3	2	2	-	-	3	-	1
Grade 2	1	-	-	1	-	-	1	-
- HEMATOPOIESIS	-	-	-	-	1	-	-	-
Grade 2	-	-	-	-	1	-	-	-
LIVER (ORO) No. Examined	5	5	5	5	5	5	5	5
No Abnormalities Observed	4	2	3	4	4	3	3	5
- FATTY CHANGE	1	3	2	1	1	2	2	-
Grade 1	1	3	1	1	1	2	2	-
Grade 2	-	-	1	-	-	-	-	-
KIDNEYS No. Examined	5	-	-	5	5	-	-	5
No Abnormalities Observed	1	-	-	-	3	-	-	4
- BASOPHILIC TUBULES	2	-	-	4	1	-	-	-
Grade 1	2	-	-	4	1	-	-	1
- MONONUCLEAR CELL INFILTRATION	2	-	-	1	1	-	-	-
Grade 1	2	-	-	1	1	-	-	-
- DILATED TUBULES	2	-	-	3	-	-	-	-
Grade 1	-	-	-	2	-	-	-	-
Grade 2	2	-	-	1	-	-	-	-
TESTIS LEFT No. Examined	5	5	5	5				
No Abnormalities Observed	4	5	1	-				
- LEYDIG CELL HYPERTROPHY DIFFUSE	-	-	4	5				
Grade 1	-	-	4	-				
Grade 2	-	-	-	1				
Grade 3	-	-	-	4				

Table 44 continued. Histopathological findings in Laboratory 2 Flutamide study - Subgroup A

Sex	Males				Females			
	0	1	10	100	0	1	10	100
Flutamide (mg/kg/day)								
No. Animals per Dose Group	5	5	5	5	5	5	5	5
TESTIS LEFT No. Examined	5	5	5	5				
No Abnormalities Observed	4	5	1	-				
- DEGENERATION GERM. EPITHELIUM	1	-	-	-				
FOCAL								
Grade 1	1	-	-	-				
EPIDIDYMIDIS LEFT No. Examined	5	5	5	5				
No Abnormalities Observed	4	5	3	-				
- DECREASED TUBULAR SIZE/DIAMETER	-	-	-	4				
Grade 2	-	-	-	3				
Grade 3	-	-	-	1				
- INCREASED INTERSTITIAL TISSUE	-	-	-	4				
Grade 2	-	-	-	3				
Grade 3	-	-	-	1				
- SPERMATIC DEBRIS	-	-	-	2				
Grade 1	-	-	-	2				
- MONONUCLEAR CELL INFILTRATION	1	-	1	-				
Grade 1	1	-	1	-				
- SPERM GRANULOMA	-	-	1	1				
Grade 3	-	-	1	-				
Grade 4	-	-	-	1				
DORSOLAT.PROSTATE No. Examined	5	5	5	5				
No Abnormalities Observed	5	5	4	-				
- REDUCTION SECRETORY PRODUCTS	-	-	1	5				
Grade 2	-	-	1	2				
Grade 3	-	-	-	3				
- FLATTENED EPITHELIAL CELLS	-	-	-	3				
Grade 2	-	-	-	1				
Grade 3	-	-	-	2				
- REDUCED ACINAR SIZE	-	-	1	5				
Grade 2	-	-	1	-				
Grade 3	-	-	-	4				
Grade 4	-	-	-	1				
VENTRAL PROSTATE No. Examined	5	5	5	5				
No Abnormalities Observed	1	3	4	-				
- REDUCTION OF SECRETORY PRODUCTS	1	1	-	5				
Grade 2	-	1	-	-				
Grade 3	1	-	-	4				
Grade 4	-	-	-	1				

M = Male animal; F = Female animal

Grade 1 = Minimal / very few / very small; Grade 2 = Slight / few / small; Grade 3 = Moderate / moderate number / moderate size; Grade 4 = Marked / many / large

P = Finding present, severity not scored

= Finding unilateral in paired organs

Table 44 continued. Histopathological findings in Laboratory 2 Flutamide study - Subgroup A

Sex	Males				Females			
Flutamide (mg/kg/day)	0	1	10	100	0	1	10	100
No. Animals per Dose Group	5	5	5	5	5	5	5	5
VENTRAL PROSTATE continued	5	5	5	5				
- FLATTENED EPITHELIAL CELLS	-	1	-	5				
Grade 2	-	1	-	4				
Grade 3	-	-	-	1				
- REDUCED ACINAR SIZE	-	-	-	5				
Grade 3	-	-	-	4				
Grade 4	-	-	-	1				
- MONONUCLEAR CELL INFILTRATION	3	2	2	1				
Grade 1	3	2	-	1				
Grade 2	-	-	1	-				
COAGULATING GLANDS No. Examined	5	5	5	5				
NAD	5	5	5	-				
- REDUCTION OF SECRETORY PRODUCTS	-	-	-	5				
Grade 2	-	-	-	1				
Grade 3	-	-	-	4				
- FLATTENED EPITHELIAL CELLS	-	-	-	5				
Grade 2	-	-	-	2				
Grade 3	-	-	-	3				
- REDUCED ACINAR SIZE	-	-	-	5				
Grade 2	-	-	-	1				
Grade 3	-	-	-	4				
SEMINAL VESICLES No. Examined	5	5	5	5				
No Abnormalities Observed	5	5	5	-				
- REDUCTION OF SECRETORY PRODUCTS	-	-	-	5				
Grade 2	-	-	-	1				
Grade 3	-	-	-	4				
- FLATTENED EPITHELIAL CELLS	-	-	-	5				
Grade 2	-	-	-	2				
Grade 3	-	-	-	3				
- REDUCED LUMINAL SIZE	-	-	-	5				
Grade 2	-	-	-	1				
Grade 3	-	-	-	2				
Grade 4	-	-	-	2				
OVARIES No. Examined					5	5	5	5
No Abnormalities Observed					4	3	3	2
- INCREASED INTERSTITIAL GLANDS					1	2	2	3
Grade 2					1	2	2	3
UTERUS No. Examined					5	5	5	5
No Abnormalities Observed					5	4	4	4
- METAPLASIA SQUAM. CELL FOCAL					-	1	1	1
Grade 1					-	1	1	-
Grade 2					-	-	-	1

M = Male animal; F = Female animal

Grade 1 = Minimal / very few / very small; Grade 2 = Slight / few / small; Grade 3 = Moderate / moderate number / moderate size; Grade 4 = Marked / many / large

P = Finding present, severity not scored

= Finding unilateral in paired organs

Table 44 continued. Histopathological findings in Laboratory 2 Flutamide study - Subgroup A

Sex	Males				Females			
Flutamide (mg/kg/day)	0	1	10	100	0	1	10	100
No. Animals per Dose Group	5	5	5	5	5	5	5	5
VAGINA No. Examined					5	5	5	5
- DIESTROUS					5	5	5	5
PITUITARY GLAND No. Examined	5	5	5	5	5	5	5	5
No Abnormalities Observed	5	5	-	-	4	5	5	5
- HYPERTROPHY BASOPHILIC CELLS	-	-	-	5	-	-	-	-
Grade 1	-	-	-	1	-	-	-	-
Grade 2	-	-	-	4	-	-	-	-
- INCLUSIONS	-	-	1	5	-	-	-	-
Grade 1	-	-	1	1	-	-	-	-
Grade 2	-	-	-	4	-	-	-	-
- INCREASED PAS-POSITIVE CELLS	-	-	5	5	-	-	-	-
Grade 1	-	-	5	1	-	-	-	-
Grade 2	-	-	-	2	-	-	-	-
Grade 3	-	-	-	2	-	-	-	-
- CYST/S	-	-	-	1	1	-	-	-
Grade 2	-	-	-	1	1	-	-	-
ADRENAL GLANDS No. Examined	5	5	5	5	5	5	5	5
No Abnormalities Observed	1	3	-	-	5	5	3	5
- VACUOLATION MICROVESICULAR ZONA FASCICULATA	-	-	-	5	-	-	-	-
Grade 1	-	-	-	2	-	-	-	-
Grade 2	-	-	-	3	-	-	-	-
- VACUOLATION MIXED TYPE ZONA FASCICULATA	4	2	5	2	-	-	-	-
Grade 1	1	1	3	1	-	-	-	-
Grade 2	3	1	2	1	-	-	-	-
- SINGLE CELL NECROSES	-	1	-	-	-	-	-	-
Grade 1	-	1	-	-	-	-	-	-
- MONONUCLEAR CELL INFILTRATION	-	-	-	-	-	-	2	-
Grade 1	-	-	-	-	-	-	2	-

M = Male animal; F = Female animal

Grade 1 = Minimal / very few / very small; Grade 2 = Slight / few / small; Grade 3 = Moderate / moderate number / moderate size; Grade 4 = Marked / many / large

P = Finding present, severity not scored

= Finding unilateral in paired organs

Summary – Subgroup B in Laboratory 2 Flutamide Study

117. The dose related histopathological findings for Subgroup B of the laboratory 2 flutamide study are presented in this section.

118. Liver: Centrilobular hepatocellular hypertrophy associated with cytoplasmic change was seen in all rats of both sexes given 100 mg/kg bw flutamide. The findings were scored minimal in males and minimal to slight (grade 1 or 2) in females.

119. Testis: Diffuse hypertrophy of the Leydig cells was seen in rats at 10 mg/kg and above. The frequency of Leydig cell hypertrophy was 0/0/5/5. The severity increased in a dose related manner. It was graded minimal to slight (grade 1 or 2) after 10 mg/kg and moderate or severe (grade 3 or 4) after 100 mg/kg flutamide. The hypertrophic Leydig cells had a distinctly eosinophilic cytoplasm. With respect to the germinal epithelium, no morphological differences were found between untreated and treated males.

120. One male (no.8) from the low dose group exhibited severe diffuse degeneration of the testis associated with severe oligospermia and apparent increase of the interstitial connective tissue of the epididymidis which are considered to be of spontaneous origin.

121. Epididymidis: The epididymides were decreased in size in all males given 100 mg/kg. The size and diameter of the epididymidal tubules were decreased (grade 3) with an apparent increase of the interstitial tissue (grade 2 or 3). The amount of spermatozoa within the epididymidal tubules appeared to be similar in untreated and in treated males. Spermatic debris occur red in all groups including controls (incidence: 1/1/1/1).

122. Dorsolateral Prostate/Ventral Prostate/Coagulating glands: The histopathological correlates for the severely diminished size of the prostate noted in all males from the high dose group were reduction of secretory products, flattening of epithelial cells, and reduction of the acinar size in all lobes. After 100 mg/kg all males and all parts of the prostate were affected. The severity varied between grade 2 and grade 4. After 10 mg/kg, a single male had a mild reduction of secretory products in the ventral prostate.

123. Seminal Vesicles: The microscopic findings in the seminal vesicles were similar to those in the prostate. Reduction of secretory products, flattening of the epithelium and the reduction of the luminal size were investigated and scored. A reduced amount of secretory products (grade 3), flattened epithelial cells (grade 3), and a reduced size of the vesicle lumina (grade 3 or 4) were observed in high dose males. No such findings occurred in controls and males of the low and mid dose group.

124. Ovaries: The number of females with an apparent increase of interstitial glands of the ovarian stroma was increased in females which had received 100 mg/kg (incidence:1/2/1/5).

125. Vagina/Estrous Cycle: The vaginal epithelium of most females from all groups including controls was considered to be in the stage of diestrous. One female from the low dose group was in estrous and one female from the high dose group in proestrous.

126. Pituitary Gland: Hypertrophy of the basophilic cell population of the adenohypophysis was seen in males which had received 100 mg/kg flutamide (incidence:0/0/0/5). In addition, the number of small intracytoplasmic inclusions within the basophils was elevated in males given 100 mg/kg (incidence: 1/0/0/5). A periodic-acid-Schiff reaction (PAS) specific for glycoproteins, i.e. gonadotrophs, demonstrated higher numbers of PAS-positive cells in males at 10 mg/kg and above (0/1/5/5). Treatment-related findings of the pituitary gland were not observed in females.

127. Adrenal Glands: A microvesicular cytoplasmic structure of adrenocortical cells from the zona fascicula was found in males but not in females, which had received 100 mg/kg flutamide.

128. No treatment related findings were seen in the other organs and tissues investigated (brain, pancreas, kidneys, oviducts, uterus, thymus, thyroid glands (incl. parathyroid glands) and skin/mammary gland). All findings are presented in the table of individual microscopic findings and the individual animal data sheets.

129. The Subgroup B data from the laboratory 2 flutamide study are summarized in Table 45.

Table 45. Histopathological findings in Laboratory 2 Flutamide study - Subgroup B

Sex	Males				Females			
Flutamide (mg/kg/day)	0	1	10	100	0	1	10	100
No. Animals per Dose Group	5	5	5	5	5	5	5	5
LIVER No. Examined	5	5	5	5	5	5	5	5
No Abnormalities Observed	1	2	1	-	4	4	4	-
- HYPERTROPHY	-	-	-	5	-	-	-	5
Grade 1	-	-	-	5	-	-	-	2
Grade 2	-	-	-	-	-	-	-	3
- CYTOPLASMIC CHANGE	-	-	-	5	-	-	-	5
Grade 1	-	-	-	5	-	-	-	1
Grade 2	-	-	-	-	-	-	-	4
- FOCAL KUPFFER CELL PROLIFERATION	4	3	4	1	-	1	1	2
Grade 1	3	3	4	1	-	1	1	2
Grade 2	1	-	-	-	-	-	-	-
- FOCAL NECROSES	1	-	-	-	-	-	-	-
Grade 2	1	-	-	-	-	-	-	-
- HEMATOPOIESIS	-	-	-	-	1	-	-	-
Grade 1	-	-	-	-	1	-	-	-
LIVER (ORO) No. Examined	5	5	5	5	5	5	5	5
No Abnormalities Observed	4	1	3	5	1	3	2	5
- FATTY CHANGE	1	4	2	-	4	2	3	-
Grade 1	1	3	2	-	4	2	3	-
Grade 2	-	1	-	-	-	-	-	-
KIDNEYS No. Examined	5	-	-	5	5	-	-	5
No Abnormalities Observed	1	-	-	2	3	-	-	5
- BASOPHILIC TUBULES	4	-	-	3	-	-	-	-
Grade 1	3	-	-	3	-	-	-	-
Grade 2	1	-	-	-	-	-	-	-
TESTIS LEFT No. Examined	5	5	5	5				
No Abnormalities Observed	4	4	-	-				
- DEGENERATION GERMINAL EPITHELIUM DIFFUSE	-	1	-	-				
Grade 4	-	-	-	-				

M = Male animal; F = Female animal

Grade 1 = Minimal / very few / very small; Grade 2 = Slight / few / small; Grade 3 = Moderate / moderate number / moderate size; Grade 4 = Marked / many / large

P = Finding present, severity not scored

= Finding unilateral in paired organs

Table 45 continued. Histopathological findings in Laboratory 2 Flutamide study - Subgroup B

Sex	Males				Females			
Flutamide (mg/kg/day)	0	1	10	100	0	1	10	100
No. Animals per Dose Group	5	5	5	5	5	5	5	5
TESTIS LEFT continued	5	5	5	5				
- LEYDIG CELL HYPERTROPHY	-	-	5	5				
Grade 1	-	-	1	-				
Grade 2	-	-	4	-				
Grade 3	-	-	-	3				
Grade 4	-	-	-	2				
- DEGENERATION GERMINAL EPITHELIUM FOCAL	1	-	1	-				
Grade 1	1	-	1	-				
EPIDIDYMIDIS LEFT No. Examined	5	5	5	5				
No Abnormalities Observed	3	2	4	-				
- DECREASED TUBULAR SIZE/DIAMETER	-	1	-	5				
Grade 2	-	1	-	-				
Grade 3	-	-	-	5				
- INCREASED INTERSTITIAL TISSUE	-	1	-	5				
Grade 2	-	1	-	1				
Grade 3	-	-	-	4				
- SPERMATIC DEBRIS	1	1	1	1				
Grade 1	1	1	1	1				
- OLIGO-SPERMIA	-	1	-	-				
Grade 4	-	1	-	-				
- MONONUCLEAR CELL INFILTRATION	1	2	-	-				
Grade 1	1	2	-	-				
- SPERM GRANULOMA	-	-	-	1				
Grade 2	-	-	-	1				
DORSOLAT.PROSTATE No. Examined	5	5	5	5				
No Abnormalities Observed	5	5	5	-				
- REDUCED SECRETORY PRODUCTS	-	-	-	3				
Grade 2	-	-	-	1				
Grade 3	-	-	-	2				
- FLATTENED EPITHELIAL CELLS	-	-	-	5				
Grade 2	-	-	-	5				
- REDUCED ACINAR SIZE	-	-	-	5				
Grade 3	-	-	-	4				
Grade 4	-	-	-	1				
VENTRAL PROSTATE No. Examined	5	5	5	5				
No Abnormalities Observed	5	5	4	-				
- REDUCED SECRETORY PRODUCTS	-	-	1	5				
Grade 2	-	-	1	-				
Grade 3	-	-	-	3				
Grade 4	-	-	-	2				
- FLATTENED EPITHELIAL CELLS	-	-	-	4				
Grade 2	-	-	-	4				

M = Male animal; F = Female animal

Grade 1 = Minimal / very few / very small; Grade 2 = Slight / few / small; Grade 3 = Moderate / moderate number / moderate size; Grade 4 = Marked / many / large

P = Finding present, severity not scored

= Finding unilateral in paired organs

Table 45 continued. Histopathological findings in Laboratory 2 Flutamide study - Subgroup B

Sex	Males				Females			
	0	1	10	100	0	1	10	100
Flutamide (mg/kg/day)								
No. Animals per Dose Group	S	5	5	5	5	5	5	5
VENTRAL PROSTATE continued	.5	5	5	S				
- REDUCED ACINAR SIZE	-	-	-	5				
Grade 3	-	-	-	4				
Grade 4	-	-	-	1				
- MONONUCLEAR CELL INFILTRATION	-	-	1	-				
Grade 1	-	-	1	-				
COAGULATING GLANDS No. Examined	5	5	5	4				
NAD	5	5	5	-				
- REDUCED SECRETORY PRODUCTS	-	-	-	4				
Grade 3	-	-	-	4				
- FLATTENED EPITHELIAL CELLS	-	-	-	4				
Grade 3	-	-	-	4				
- REDUCED ACINAR SIZE	-	-	-	4				
Grade 3	-	-	-	3				
Grade 4	-	-	-	1				
SEMINAL VESICLES No. Examined	5	5	5	5				
No Abnormalities Observed	5	5	5	-				
- REDUCED SECRETORY PRODUCTS	-	-	-	4				
Grade 3	-	-	-	4				
- FLATTENED EPITHELIAL CELLS	-	-	-	5				
Grade 3	-	-	-	5				
- REDUCED LUMINAL SIZE	-	-	-	4				
Grade 3	-	-	-	1				
Grade 4	-	-	-	3				
OVARIES No. Examined					5	5	5	5
No Abnormalities Observed					4	3	4	-
- INCREASED INTERSTITIAL GLANDS					1	2	1	5
Grade 1					1	1	1	3
Grade 2					-	1	-	2
UTERUS No. Examined					5	5	5	5
No Abnormalities Observed					4	3	3	3
- DILATION					-	1	-	1
Grade 1					-	-	-	1
Grade 3					-	1	-	-
- METAPLASIA SQUAMOUS CELL FOCAL					1	1	1	1
Grade 1					1	1	1	-
Grade 2					-	-	-	1
- DILATED GLANDS					-	-	1	-
Grade 1					-	-	1	-
VAGINA No. Examined					5	5	5	5
- ESTROUS					-	1	-	-
- DIESTROUS					5	4	5	4
- PROESTROUS					-	-	-	1

M = Male animal; F = Female animal

Grade 1 = Minimal / very few / very small; Grade 2 = Slight / few / small; Grade 3 = Moderate / moderate number / moderate size; Grade 4 = Marked / many / large

P = Finding present, severity not scored

= Finding unilateral in paired organs

Table 45 continued. Histopathological findings in Laboratory 2 Flutamide study - Subgroup B

Sex	Males				Females			
	0	1	10	100	0	1	10	100
Flutamide (mg/kg/day)								
No. Animals per Dose Group	5	5	5	5	5	5	5	5
PITUITARY GLAND No. Examined	5	5	5	5	5	5	5	5
No Abnormalities Observed	3	4	-	-	5	5	5	5
- HYPERTROPHY BASOPHILIC CELLS	-	-	-	5	-	-	-	-
Grade 1	-	-	-	1	-	-	-	-
Grade 2	-	-	-	4	-	-	-	-
- INCLUSIONS	1	-	-	5	-	-	-	-
Grade 1	1	-	-	2	-	-	-	-
Grade 2	-	-	-	3	-	-	-	-
- INCREASED NUMBER OF PAS-POS. CELLS	-	1	5	5	-	-	-	-
Grade 1	-	1	5	-	-	-	-	-
Grade 2	-	-	-	5	-	-	-	-
- CYST/S	1	-	-	-	-	-	-	-
Grade 2	1	-	-	-	-	-	-	-
ADRENAL GLANDS No. Examined	5	5	5	5	5	5	5	5
No Abnormalities Observed	-	-	-	1	5	5	5	4
- VACUOLATION MICROVESICULAR	-	-	-	4	-	-	-	-
Grade 1	-	-	-	2	-	-	-	-
Grade 2	-	-	-	2	-	-	-	-
- VACUOLATION MIXED TYPE	5	5	5	-	-	-	-	-
Grade 1	3	4	4	-	-	-	-	-
Grade 2	2	1	1	-	-	-	-	-
- MONONUCLEAR CELL INFILTRATION	1	-	-	-	-	-	-	1
Grade 1	1	-	-	-	-	-	-	1

M = Male animal; F = Female animal

Grade 1 = Minimal / very few / very small; Grade 2 = Slight / few / small; Grade 3 = Moderate / moderate number / moderate size; Grade 4 = Marked / many / large

P = Finding present, severity not scored

= Finding unilateral in paired organs

Laboratory 11

1) Study A (Subgroup A – n = 5).

130. Results for Subgroup A are presented in Table 46A for males and Table 46B for females.

- In the liver, centrilobular hepatocyte hypertrophy was observed in all males and females in the 100 mg/kg group.
- In the testis, tubular atrophy was observed in 1 male (No.931) in the 100 mg/kg group and interstitial cell hyperplasia was observed in all males in the 100 mg/kg group-
- In the epididymis, decreased number of sperms was observed in 1 male (No.924) in the 10 mg/kg group and in all males in the 100 mg/kg group.
- In the prostate, atrophy was observed in 1 male (No.922) in the 10 mg/kg group and in all males in the 100 mg/kg group.
- In the coagulating gland, atrophy was observed in 1 male (No.922) in the 10 mg/kg group and in all males in the 100 mg/kg group.
- In the seminal vesicle, atrophy was observed in all males in the 100 mg/kg group.
- In the ovary, decreased number of corpora luteum and follicular cyst were observed in 1 female (No.981) in the 100 mg/kg group.
- In the mammary gland, small size of the alveolus/duct lined by basophilic epithelial cells was observed in all males in the 100 mg/kg group.
- In the pituitary, appearance of clear cell in the anterior lobe was observed in 1 male (No.922) in the 10 mg/kg group and in all males in the 100 mg/kg group.
- In the adrenal, cortical cell hypertrophy in the zona fasciculata was observed in 2 males (No.931 and 933) in the 100 mg/kg group.

2) Study B (Subgroup B – n = 5).

131. Results for Subgroup B are presented in Table 47A for males and Table 47B for females.

- In the liver, centrilobular hepatocyte hypertrophy was observed in all males and females in the 100 mg/kg group.
- In the testis, interstitial cell hyperplasia was observed in 4 males in the 100 mg/kg group and tubular dilatation was observed in 1 male (No.937) in the 100 mg/kg group.
- In the epididymis, decreased number of sperms was observed in all males in the 100 mg/kg group.
- In the prostate, atrophy was observed in all males in the 100 mg/kg group.
- In the coagulating gland, atrophy was observed in all males in the 100 mg/kg group-
- In the seminal vesicle, atrophy was observed in all males in the 100 mg/kg group.
- In the mammary gland, small size of the alveolus/duct lined by basophilic epithelial cells was observed in all males in the 100 mg/kg group.
- In the pituitary, appearance of clear cell in the anterior lobe was observed in all males in the 100 mg/kg group.

3) Study C (Combined Subgroups – n = 10).

132. Results for the combined Subgroups are presented in Table 48A for males and Table 48B for females.

- In the liver, centrilobular hepatocyte hypertrophy was observed in all males and females in the 100 mg/kg group.
- In the testis, tubular atrophy was observed in 1 male (No.931) in the 100 mg/kg group and tubular dilatation was observed in other 1 male (No.937) in the 100 mg/kg group. Interstitial cell hyperplasia was observed in 9 males in the 100 mg/kg group.
- In the epididymis, decreased number of sperms was observed in 1 male (No.924) in the 10 mg/kg group and in all males in the 100 mg/kg group.

- In the prostate, atrophy was observed in 1 male (No.922) in the 10 mg/kg group and in all males in the 100 mg/kg group.
- In the coagulating gland, atrophy was observed in 1 male (No.922) in the 10 mg/kg group and in all males in the 100 mg/kg group.
- In the seminal vesicle, atrophy was observed in all males in the 100 mg/kg group.
- In the ovary, decreased number of corpora luteum, and follicular cyst were observed in 1 female. (No.981) in the 100 mg/kg group.
- In the mammary gland, small size of the alveolus/duct lined by basophilic epithelial cells was observed in all males in the 100 mg/kg group.
- In the pituitary, appearance of clear cell in the anterior lobe was observed in 1 male (No.922) in the 10 mg/kg group and in all males in the 100 mg/kg group.
- In the adrenal, cortical cell hypertrophy in the zona fasciculata was observed in 2 males (No.931 and 933) in the 100 mg/kg group.

133. One female (No.952) in the control group macroscopically showed fracture of the maxillary incisor, however, no histological change was observed. One male (No.912) in the 1 mg/kg group macroscopically showed rough surface of the kidney, and basophilic tubule, tubular dilatation and cellular infiltration of the kidney were observed histopathologically. Since the similar changes were not observed in animals of the high dose, these changes had no relevance to the administration of flutamide.

Table 46A. Histopathological findings in Subgroup A males from Laboratory 11 Flutamide study

Sex Group and dose Number of animals Organs and findings	Male																							
	Control 6						1 mg/kg 5						10 mg/kg 5						100 mg/kg 5					
	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot
Liver	(5)						(0)						(5)						(5)					
Hypertrophy, hepatocyte, centrilobular	5	0	0	0	0	0							5	0	0	0		0	0	0	5	0	0	5**
Testis	(5)						(0)						(5)						(5)					
Atrophy, tubule	5	0	0	0	0	0							5	0	0	0	0	0	4	0	1	0	0	1
Hyperplasia, interstitial cell, diffuse	5	0	0	0	0	0							5	0	0	0	0	0	0	0	5	0	0	5**
Epididymis	(5)						(5)						(5)						(5)					
Decrease, sperm	5	0	0	0	0	0	5	0	0	0	0	0	4	1	0	0	0	1	0	0	4	1	0	5**
Prostate	(5)						(5)						(5)						(5)					
Atrophy	5	0	0	0	0	0	5	0	0	0	0	0	4	1	0	0	0	1	0	0	4	1	0	5**
Coagulating gland	(5)						(5)						(5)						(5)					
Atrophy	5	0	0	0	0	0	5	0	0	0	0	0	4	1	0	0	0	1	0	0	4	1	0	5**
Seminal vesicle	(5)						(0)						(5)						(5)					
Atrophy	5	0	0	0	0	0							5	0	0	0	0	0	0	0	5	0	0	5**
Mammary gland	(5)						(0)						(5)						(5)					
Small, alveolus/duct	5	0	0	0	0	0							5	0	0	0	0	0	0	0	5	0	0	5**
Pituitary	(5)						(5)						(5)						(5)					
Appearance, clear cell, anterior lobe, diffuse	5	0	0	0	0	0	5	0	0	0	0	0	4	1	0	0	0	1	0	0	5	0	0	5**
Adrenal	(5)						(0)						(5)						(5)					
Hypertrophy, cortical cell, zona fasciculata	5	0	0	0	0	0							5	0	0	0	0	0	3	0	2	0	0	2

** P<0.01 (significantly different from control). Grade sign: -, none; +/-, slight; +, mild; ++, moderate; +++, severe. NR; no remarkable changes, NA; not applicable. 1) The alveolus/duct was lined by basophilic epithelial cells. Figures in parentheses are number of animals with tissues examined histopathologically.

Table 46B - Histopathological findings in Subgroup A females from the laboratory 11 Flutamide study

Sex Group and dose Number of animals Organs and findings	Female																							
	Control 5						1 mg/kg 5						10 mg/kg 5						100 mg/kg 5					
	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot
Liver	(5)						(0)						(5)						(5)					
Hypertrophy, hepatocyte, centrilobular	5	0	0	0	0	0							5	0	0	0	0	0	0	0	5	0	0	5**
Kidney	(5)						(0)						(0)						(5)					
Tubule, basophilic	5	0	0	0	0	0													5	0	0	0	0	0
Dilatation, tubule	5	0	0	0	0	0													5	0	0	0	0	0
Cellular infiltration	5	0	0	0	0	0													5	0	0	0	0	0
Ovary	(5)						(0)						(5)						(5)					
Decrease, corpea luteum	5	0	0	0	0	0							5	0	0	0	0	0	4	0	1	0	0	1
Cyst, follicular	5	0	0	0	0	0							5	0	0	0	0	0	4	0	1	0	0	1
Uterus	NR (5)						(0)						(0)						NR (5)					
Vagina	NR (5)						(0)						(0)						NR (5)					
Mammary gland	(5)						(0)						(5)						(5)					
Small, alveolus/duct	5	0	0	0	0	0							5	0	0	0	0	0	5	0	0	0	0	0
Pituitary	(5)						(5)						(5)						(5)					
Appearance, clear cell, anterior lobe, diffuse	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0
Adrenal	(5)						(0)						(5)						(5)					
Hypertrophy, cortical cell, zona fasciculata	5	0	0	0	0	0							5	0	0	0	0	0	5	0	0	0	0	0

** P<0.01 (significantly different from control). Grade sign: -, none; +/-, slight; +, mild; ++, moderate; +++, severe. NR; no remarkable changes, NA; not applicable. 1) The alveolus/duct was lined by basophilic epithelial cells. Figures in parentheses are number of animals with tissues examined histopathologically.

Table 47A Histopathological findings in Subgroup B males from the laboratory 11 Flutamide study

Sex Group and dose Number of animals Organs and findings	Male																							
	Control 5						1 mg/kg						10 mg/kg						100 mg/kg 5					
	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot
Liver	(5)						(0)						(5)						(5)					
Hypertrophy, hepatocyte, centrilobular	5	0	0	0	0	0							5	0	0	0	0	0	0	0	5	0	0	5**
Testis	(5)						(0)						(5)						(5)					
Hyperplasia, interstitial cell, diffuse	5	0	0	0	0	0							5	0	0	0	0	0	1	0	4	0	0	4*
Dilatation, tubule	5	0	0	0	0	0							5	0	0	0	0	0	4	0	1	0	0	1
Epididymis	(5)						(5)						(5)						(5)					
Decrease, sperm	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	0	0	5	0	0	5**
Prostate	(5)						(5)						(5)						(5)					
Atrophy	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	0	0	5	0	0	5**
Coagulating gland	(5)						(5)						(5)						(5)					
Atrophy	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	0	0	5	0	0	5**
Seminal vesicle	(5)						(0)						(5)						(5)					
Atrophy	5	0	0	0	0	0							5	0	0	0	0	0	0	0	5	0	0	5**
Mammary gland	(5)						(0)						(5)						(5)					
Small, alveolus/duct	5	0	0	0	0	0							5	0	0	0	0	0	0	0	5	0	0	5**
Pituitary	(5)						(5)						(5)						(5)					
Appearance, clear cell, ant. lobe, diffuse	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	0	0	4	1	0	5**

** P<0.01 (significantly different from control). Grade sign: -, none; +/-, slight; +, mild; ++, moderate; +++, severe. NR; no remarkable changes, NA; not applicable. 1) The alveolus/duct was lined by basophilic epithelial cells. Figures in parentheses are number of animals with tissues examined histopathologically.

Table 47B - Histopathological findings in Subgroup B females from the laboratory 11 Flutamide study

Sex Group and dose Number of animals Organs and findings	Female																							
	Control 5						1 mg/kg 5						10 mg/kg						100 mg/kg					
	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot
Liver	(5)						(0)						(5)						(5)					
Hypertrophy, hepatocyte, centrilobular	5	0	0	0	0	0							5	0	0	0	0	0	0	0	5	0	0	5**
Ovary	NR (5)						(0)						NR (5)						NR (5)					
Uterus	NR (5)						(0)						(0)						NR (5)					
Vagina	NR (5)						(0)						(0)						NR (5)					
Mammary gland	(5)						(0)						(5)						(5)					
Small, alveolus/duct	5	0	0	0	0	0							5	0	0	0	0	0	5	0	0	0	0	0
Endocrine system	(5)						(5)						(5)						(5)					
Pituitary	(5)						(5)						(5)						(5)					
Appearance, clear cell, ant. lobe, diffuse	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0

** P<0.01 (significantly different from control). Grade sign: -, none; +/-, slight; +, mild; ++, moderate; +++, severe. NR; no remarkable changes, NA; not applicable. 1) The alveolus/duct was lined by basophilic epithelial cells. Figures in parentheses are number of animals with tissues examined histopathologically.

Table 48A. Histopathological findings in Combined Subgroup males from the laboratory 11 Flutamide study

Sex Group and dose Number of animals Organs and findings	Male																							
	Control 10						1 mg/kg 10						10 mg/kg 10						100 mg/kg 10					
	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot
Liver	(10)						(0)						(10)						(10)					
Hypertrophy, hepatocyte, centrilobular	10	0	0	0	0	0							10	0	0	0	0	0	0	0	10	0	0	10*
Kidney	(10)						(1)						(0)						(10)					
Tubule, basophilic	10	0	0	0	0	0	0	0	1	0	0	1**							10	0	0	0	0	0
Dilatation, tubule	10	0	0	0	0	0	0	0	1	0	0	1**							10	0	0	0	0	0
Cellular infiltration	10	0	0	0	0	0	0	0	1	0	0	1**							10	0	0	0	0	0
Testis	(10)						(0)						(10)						(10)					
Atrophy, tubule	10	0	0	0	0	0							10	0	0	0	0	0	9	0	1	0	0	1
Hyperplasia, interstitial cell, diffuse	10	0	0	0	0	0							10	0	0	0	0	0	1	0	9	0	0	9**
Dilatation, tubule	10	0	0	0	0	0							10	0	0	0	0	0	9	0	1	0	0	1
Epididymis	(10)						(10)						(10)						(10)					
Decrease, sperm	10	0	0	0	0	0	10	0	0	0	0	0	9	1	0	0	0	1	0	0	9	1	0	10
Prostate	(10)						(10)						(10)						(10)					
Atrophy	10	0	0	0	0	0	10	0	0	0	0	0	9	1	0	0	0	1	0	0	9	1	0	10**
Coagulating gland	(10)						(10)						(10)						(10)					
Atrophy	10	0	0	0	0	0	10	0	0	0	0	0	9	1	0	0	0	1	0	0	9	1	0	10**
Seminal vesicle	(10)						(0)						(10)						(10)					
Atrophy	10	0	0	0	0	0							10	0	0	0	0	0	0	0	10	0	0	10**
Mammary gland	(10)						(0)						(10)						(10)					
Small, alveolus/duct	10	0	0	0	0	0							10	0	0	0	0	0	0	0	10	0	0	10**
Pituitary	(10)						(10)						(10)						(10)					
Appearance, clear cell, anterior lobe, diffuse	10	0	0	0	0	0	10	0	0	0	0	0	9	1	0	0	0	1	0	0	9	1	0	10**
Adrenal	(10)						(0)						(10)						(10)					
Hypertrophy, cortical cell, zona fasciculata	10	0	0	0	0	0							10	0	0	0	0	0	8	0	2	0	0	2

** P<0.01 (significantly different from control). Grade sign: -, none; +/-, slight; +, mild; ++, moderate; +++, severe. NR; no remarkable changes, NA; not applicable. 1) The alveolus/duct was lined by basophilic epithelial cells. Figures in parentheses are number of animals with tissues examined histopathologically.

Table 48B. - Histopathological findings in Combined Subgroup females from the laboratory 11 Flutamide study

Sex Group and dose Number of animals Organs and findings	Female																								
	Control 10						1 mg/kg 10						10 mg/kg 10						100 mg/kg 10						
	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot	-	+/-	+	++	+++	Tot	
Liver	(10)						(0)						(10)						(10)						
Hypertrophy, hepatocyte, centrilobular	10	0	0	0	0	0							10	0	0	0	0	0	0	0	10	0	0	0	10**
Kidney	(10)						(0)						(0)						(10)						
Tubule, basophilic	10	0	0	0	0	0													10	0	0	0	0	0	0
Dilatation, tubule	10	0	0	0	0	0													10	0	0	0	0	0	0
Cellular infiltration	10	0	0	0	0	0													10	0	0	0	0	0	0
Ovary	(10)						(0)						(10)						(10)						
Decrease, corpus luteum	10	0	0	0	0	0							10	0	0	0	0	0	9	0	1	0	0	1	
Cyst, follicular	10	0	0	0	0	0							10	0	0	0	0	0	9	0	1	0	0	1	
Uterus	NR (10)						(0)						(0)						NR (10)						
Vagina	NR (10)						(0)						(0)						NR (10)						
Mammary gland	(10)						(0)						(10)						(10)						
Small, alveolus/duct	10	0	0	0	0	0							10	0	0	0	0	0	10	0	0	0	0	0	0
Pituitary	(10)						(10)						(10)						(10)						
Appearance, clear cell, anterior lobe, diffuse	10	0	0	0	0	0	10	0	0	0	0	0	10	0	0	0	0	0	10	0	0	0	0	0	0
Adrenal	(10)						(0)						(10)						(10)						
Hypertrophy, cortical cell, zona fasciculata	10	0	0	0	0	0							10	0	0	0	0	0	10	0	0	0	0	0	0

***p,p'*-DDE**

Laboratory 6 – *p,p'*-DDE Histopathology

134. The most extensive lesion was observed in the liver which consisted of diffuse or centrilobular hepatocellular hypertrophy. There were five high dose male rats that had mild or moderate diffuse hypertrophy while the remaining male and female rats of Group 4 had moderate to marked centrilobular hepatocellular hypertrophy. The male and female rats of the low and mid dosage groups had minimal to moderate centrilobular hepatocellular hypertrophy. The hypertrophied hepatocytes were enlarged with an increased amount of dense eosinophilic cytoplasm with histomorphology consistent with hypertrophy associated with microsomal enzyme induction and increased smooth endoplasmic reticulum (SER) proliferation. The incidence and severity of these changes occurred in a dose-related manner (Table 1).

135. Changes considered to be likely secondary to the centrilobular hepatocellular hypertrophy and enzyme induction were seen in the thyroid and pituitary. In the thyroid gland, there was a dose-related hyperplasia and hypertrophy of the follicular, epithelium in male and female rats of all treatment groups.

136. Hypertrophy of the adrenal cortical cells also occurred in several of the high dosage group male and female rats. Hypertrophy of individual cells of the pars distalis of the pituitary (probably thyrotrophy) was observed in a few mid and high dose male rats and high dose female rats.

137. Compound-related microscopic changes observed in the male sex organs of the high dosage group that were associated with the antiandrogen effect of the compound included increased residual bodies in the lumen of the seminiferous tubules of the testes in 4/10 high dosage group male rats, occasionally containing degenerate/necrotic spermatids. One of these rats also had evidence of degeneration of the seminiferous tubules of one of the affected testes. Necrotic germ cells and residual bodies were present in the lumen of the epididymal tubules of all rats with the testicular changes and one other high dose male rat. Other associated changes consisted of atrophy of the coagulating gland, epididymides, seminal vesicles and prostate which occurred in four or five of the non-surviving high dose male rats. The associated changes in the male sex organs were observed only in the high dose group.

138. The only sex-organ treatment-related effect in the female rats was minimal or mild hyperplasia of the mammary glands in the mid and high dosage groups and the incidence and severity generally occurred in a dose-related manner. There were no compound-related microscopic changes observed in the cervix, ovaries, oviducts, uterus or vagina of any of the Group 4 female rats.

139. Five Group 4 male rats and three Group 4 female rats died or were sacrificed in moribund condition prior to the scheduled terminal necropsy. The only consistent finding which likely was associated with treatment was mild or moderate centrilobular or diffuse hepatocellular hypertrophy which could have resulted in hepatic dysfunction. The neurotoxic effect of the compound could also have contributed to these deaths. Several of the male rats also had compound-related changes in the sex organs, but the effects in those organs likely were not associated with the cause of death. Atrophy of the spleen and thymus of a few of these rats also was observed, but this is a common secondary finding in animals that die in a moribund condition.

140. All other microscopic changes observed in the various organs and tissues were considered to have occurred spontaneously and to be unrelated to treatment. These changes also are listed and summarized in the attached histomorphology tables.

141. The dose related histopathological data are summarized for the laboratory 6 *p,p'*-DDE study in Table 49.

Table 49. Histopathology summary for Laboratory 6 *p,p'*-DDE study

<i>p,p'</i> -DDE (mg/kg/day)	Male				Female			
	0	12.5	50	200/ 150	0	6.5	25	100
Number of Animals/Group	10	10	10	10	10	10	10	10
LIVER:								
NO. Examined	10	10	10	10	10	10	10	10
NO. NORMAL	7	0	0	0	7	2	0	0
-congestion moderate	[0] 0	[0] 0	[0] 0	[1] 1	[0] 0	[0] 0	[0] 0	[0] 0
-hematopoiesis, extramedullary, multifocal minimal	[0] 0	[0] 0	[1] 1	[0] 0	[0] 0	[0] 0	[0] 0	[0] 0
-hypertrophy, hepatocellular, centrilobular minimal	[0] 0	[10] 7	[10] 0	[5] 0	[0] 0	[7] 7	[10] 5	[10] 0
mild	0	3	6	0	0	0	5	0
moderate	0	0	4	5	0	0	0	9
marked	0	0	0	0	0	0	0	1
-hypertrophy, hepatocellular, diffuse mild	[0] 0	[0] 0	[0] 0	[5] 1	[0] 0	[0] 0	[0] 0	[0] 0
moderate	0	0	0	4	0	0	0	0
-inflammation, chronic, focal/multifocal minimal	[3] 3	[1] 1	[2] 2	[0] 0	[1] 1	[1] 1	[1] 1	[2] 2
-necrosis, multifocal mild	[0] 0	[0] 0	[0] 0	[1] 1	[0] 0	[0] 0	[0] 0	[0] 0
-vacuolation, hepatocellular, multifocal minimal	[0] 0	[0] 0	[1] 1	[0] 0	[1] 1	[3] 2	[1] 1	[0] 0
mild	0	0	0	0	0	1	0	0
-vacuolation, hepatocellular, periportal minimal	[0] 0	[0] 0	[0] 0	[0] 0	[1] 1	[0] 0	[0] 0	[0] 0
THYROID:								
NO. Examined	10	10	10	10	10	10	10	10
NO. NORMAL	10	4	1	4	10	7	1	3
-hemorrhage moderate	[0] 0	[0] 0	[0] 0	[1] 1	[0] 0	[0] 0	[0] 0	[0] 0
-hypertrophy/hyperplasia, follicular epithelium minimal	[0] 0	[6] 1	[9] 3	[5] 0	[0] 0	[3] 2	[9] 3	[7] 1
mild	0	3	4	1	0	1	3	2
moderate	0	2	2	3	0	0	3	4
marked	0	0	0	1	0	0	0	0
PITUITARY:								
NO. Examined	10	8	10	10	10	10	10	10
NO. NORMAL	10	8	7	7	10	9	10	7
-hypertrophy, pars distalis, multifocal minimal	[0] 0	[0] 0	[3] 3	[3] 0	[0] 0	[0] 0	[0] 0	[3] 1
mild	0	0	0	3	0	0	0	2

[] = Total incidence of specified lesion, all grades.

Table 49 continued. Histopathology summary for Laboratory 6 *p,p'*-DDE study

<i>p,p'</i> -DDE (mg/kg/day)	Males				Females			
	0	12.5	50	200/ 150	0	6.5	25	100
Number of Animals/Group:	10	10	10	10	10	10	10	10
ADRENAL GLANDS:								
NO. Examined	10	10	10	10	10	10	10	10
NO. NORMAL	7	8	5	2	10	10	10	4
-congestion mild	[0] 0	[0] 0	[0] 0	[1] 1	[0] 0	[0] 0	[0] 0	[0] 0
-hypertrophy, cortical cells minimal mild moderate	[0] 0 0 0	(0) 0 0 0	(0) 0 0 0	[3] 1 2 0	[0] 0 0 0	[0] 0 0 0	[0] 0 0 0	[6] 0 5 1
-vacuolation, cortex mini ma 1 mild moderate	[3] 2 1 0	[2] 1 1 0	[5] 1 3 1	[5] 0 2 3	[0] 0 0 0	[0] 0 0 0	[0] 0 0 0	[0] 0 0 0
TESTES:								
NO. Examined	10	10	10	10				
NO. NORMAL	10	10	9	6				
-atrophy, unilateral, focal minimal	[0] 0	[0] 0	[1] 1	[0] 0				
-degeneration, unilateral, diffuse mild	[0] 0	[0] 0	[0] 0	[1] 1				
-increased residual bodies minimal mild	[0] 0 0	[0] 0 0	[0] 0 0	[4] 2 2				
EPIDIDYMIDES:								
NO. Examined	10	10	10	10				
NO. NORMAL	7	8	9	5				
-atrophy mild moderate	[0] 0 0	[0] 0 0	[0] 0 0	[4] 2 2				
-infiltration, mononuclear-cell, focal minimal	[3] 3	[2] 2	[1] 1	[1] 1				
-necrotic germ cells/residual bodies minimal mild moderate	[0] 0 0 0	[0] 0 0 0	[0] 0 0 0	[5] 3 1 1				

[] = Total incidence of specified lesion, all grades.

Table 49 continued. Histopathology summary for Laboratory 6 *p,p'*-DDE study

<i>p,p'</i> -DDE (mg/kg/day)	Males				Females			
	0	12.5	50	200/ 150	0	6.5	25	100
Number of Animals/Group:	10	10	10	10	10	10	10	10
PROSTATE:								
NO. Examined	10	10	10	10				
NO. NORMAL	5	7	8	5				
-atrophy	[0]	[0]	[0]	[5]				
mild	0	0	0	3				
moderate	0	0	0	2				
-inflammation, chronic, focal /multifocal,	[5]	[3]	[2]	[1]				
minimal	3	1	2	0				
mild	2	0	0	1				
moderate	0	2	0	0				
SEMINAL VESICLES:								
NO. Examined	10	10	10	10				
NO. NORMAL	10	10	10	5				
-atrophy	[0]	[0]	[0]	[5]				
mild	0	0	0	3				
moderate	0	0	0	2				
COAGULATING GLAND:								
NO. Examined	10	10	10	10				
NO. NORMAL	10	10	10	6				
-atrophy	[0]	[0]	[0]	[4]				
mild	0	0	0	2				
moderate	0	0	0	2				
MAMMARY GLAND:								
NO. Examined					10	10	10	9
NO. NORMAL					10	10	6	4
-hyperplasia					[0]	[0]	[4]	[5]
minimal					0	0	2	1
mild					0	0	2	4
BRAIN:								
NO. Examined	10	0	0	10	10	0	0	10
NO. NORMAL	10	0	0	5	10	0	0	9
-congestion	[0]	[0]	[0]	[4]	[0]	[0]	[0]	[1]
minimal	0	0	0	1	0	0	0	0
mild	a	0	0	3	0	0	0	1
-vacuolation, white matter, multifocal	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
mild	0	0	0	1	0	0	0	0

[] = Total incidence of specified lesion, all grades.

Laboratory 7 – *p,p'*-DDE

142. Histopathologically, the administration of DDE was found to cause observable effects in the liver, thyroid, and kidney. In the liver, the centrilobular hepatocyte hypertrophy with ground glass appearance was pronounced in males given 12.5 mg/kg and above and in females given 6.25 mg/kg and above. The degree of severity increased dose dependently. In the thyroid, the hypertrophy or hyperplasia of follicular cells was observed in males given 50 mg/kg and above and in females given 25 mg/kg and above. In the kidney, the significant increase in the eosinophilic body was detected in the 50 mg/kg and above groups in males. The reproductive organs in both males and females were not changed in any treatment group.

143. *p,p'*-DDE significantly increased liver weights and thyroid weights in this study. *p,p'*-DDE is known to induce several hepatic cytochrome P-450 (CYP) and uridine diphosphate glucuronosyltransferase (UDP-GT) enzymes. UDP-GT is a rate limiting enzyme in T4 metabolism, making the T4 glucuronide conjugate, which is then excreted into bile. As with phenobarbital, T4 metabolic clearance would decrease serum T4 levels, leading to increased TSH via the hypothalamic/pituitary/thyroid feed back mechanisms. This is consistent with the decrease in T4, but no parallel increase in TSH was observed. Continued stimulation of the thyroid would result in the observed increase in weights and the histopathological changes observed in this study.

144. The detailed histopathological data for the combined Subgroups from the laboratory 7 *p,p'*-DDE study is presented for males in Table 50A and females in Table 50B. The results for Subgroup A (Table 51A-B) and for Subgroup B (Table 52A-B) follow.

Table 50A. Histopathological findings with *p,p'*-DDE in males from Combined Subgroups of Laboratory 7 study

Groups (mg/kg)	0						12.5						50						100									
	Grade	-	-/+	+	++	+++	Pos.	[10]	-	-/+	+	++	+++	Pos.	[10]	-	-/+	+	++	+++	Pos.	[10]	-	-/+	+	++	+++	Pos.
(Liver)	[10]							[10]							[10]							[10]						
Hypertrophy, hepatocyte, centrilobular		10	0	0	0	0	0		0	7	3	0	0**	10##		0	0	3	7	0**	10##		0	0	0	5	5**	10##
Ground glass appearance, hepatocyte, centrilobular		10	0	0	0	0	0		0	6	4	0	0**	10##		0	0	3	7	0**	10##		0	0	0	4	6**	10##
Mitosis, hepatocyte		10	0	0	0	0	0		10	0	0	0	0	0		5	3	2	0	0	5#		4	6	0	0	0*	6##
Microgranuloma		7	3	0	0	0	3		9	1	0	0	0	1		8	2	0	0	0	2		7	2	1	0	0	3
Necrosis, focal		10	0	0	0	0	0		10	0	0	0	0	0		10	0	0	0	0	0		8	2	0	0	0	2
Hematopoiesis, extramedullary		10	0	0	0	0	0		9	1	0	0	0	1		10	0	0	0	0	0		10	0	0	0	0	0
Single cell necrosis		10	0	0	0	0	0		9	1	0	0	0	1		10	0	0	0	0	0		10	0	0	0	0	0
Degeneration, vacuolar, hepatocyte, midlobular		10	0	0	0	0	0		10	0	0	0	0	0		8	0	1	1	0	2		9	0	0	1	0	1
(Thyroid gland)	[10]							[10]							[10]							[10]						
Hypertrophy, follicular cell		10	0	0	0	0	0		10	0	0	0	0	0		0	10	0	0	0**	10##		0	6	4	0	0**	10##
Hyperplasia, follicular cell		10	0	0	0	0	0		10	0	0	0	0	0		3	7	0	0	0**	7##		0	5	5	0	0**	10##
Ectopic thymus		9	1	0	0	0	1		10	0	0	0	0	0		9	0	0	0	0	0		9	1	0	0	0	1
(Kidney)	[10]							[10]							[10]							[10]						
Eosinophilic body		6	2	1	1	0	4		6	3	1	0	0	4		1	4	3	2	0	9#		1	0	5	4	8*	9#
Basophilic tubule, cortex																												
Mineralization, medulla/papilla		4	6	0	0	0	6		4	6	0	0	0	6		2	6	2	0	0	0		3	6	1	0	0	7
Cyst, Cortico-medullary junction		10	0	0	0	0	0		9	1	0	0	0	1		9	1	0	0	0	1		9	1	0	0	0	1
Cast, hyalin, cortex		8	2	0	0	0	2		9	1	0	0	0	1		10	0	0	0	0	0		10	0	0	0	0	0
Cellular infiltration, lymphocyte, interstitium		10	0	0	0	0	0		10	0	0	0	0	0		9	1	0	0	0	1		10	0	0	0	0	0
Fibrosis, focal, subcapsule		9	1	0	0	0	1		10	0	0	0	0	0		9	1	0	0	0	1		10	0	0	0	0	0
(Prostate: ventral lobe)	[10]							[0]							[0]							[10]						
Cellular infiltration, lymphocyte & plasma cell, epithelium/interstitial		6	2	2	0	0	4																7	2	0	1	0	3

^a com oil, 10 mL/kg

- Negative; +, Positive; [], Number of animals examined

*, Significantly different from control $p < 0.05$ (Two-tailed Mann-Whitney U test), **, Significantly different from control $p < 0.01$ (Two-tailed Mann-Whitney U test).

#, Significantly different from control $p < 0.05$ (One-tailed Fisher exact test), ##, Significantly different from control $p < 0.01$ (One-tailed Fisher exact test).

Table 50B. Histopathological findings with *p,p'*-DDE in females from Combined Subgroups of Laboratory 7 study

Groups (mg/kg)	0						6.25						25						75															
	Grade							Grade							Grade							Grade												
	-	-/+	+	++	+++	Pos.	[10]	-	-/+	+	++	+++	Pos.	[10]	-	-/+	+	++	+++	Pos.	[10]	-	-/+	+	++	+++	Pos.							
(Liver)	[10]													[10]							[10]							[9]						
Hypertrophy, hepatocyte, centrilobular Ground glass appearance.	10	0	0	0	0	0		0	8	2	0	0**	10###		0	0	5	5	0**	10###		0	0	0	4	5**	9###							
hepatocyte, centrilobular	10	0	0	0	0	0		4	6	0	0	0*	6#		0	3	4	3	0**	10###		0	0	0	5	4**	9###							
Mitosis, hepatocyte	9	1	0	0	0	1		2	4	4	0	0**	8###		0	6	4	0	0**	10###		0	5	4	0	0**	9###							
Necrosis, hepatocyte, centrilobular, with cellular infiltration of neutrophil	10	0	0	0	0	0		10	0	0	0	0	0		10	0	0	0	0	0		8	0	0	1	0	1							
Necrosis, focal	10	0	0	0	0	0		10	0	0	0	0	0		10	0	0	0	0	0		7	2	0	0	0	2							
Microgranuloma	9	1	0	0	0	1		10	0	0	0	0	0		10	0	0	0	0	0		9	0	0	0	0	0							
(Thyroid gland)	[10]						[9]							[10]							[9]													
Hypertrophy, follicular cell	10	0	0	0	0	0		9	0	0	0	0	0		7	3	0	0	0	3		3	3	0	0	0*	6###							
Hyperplasia, follicular cell	10	0	0	0	0	0		9	0	0	0	0	0		10	0	0	0	0	0		7	2	0	0	0	2							
Ectopic thymus	10	0	0	0	0	0		8	1	0	0	0	1		10	0	0	0	0	0		9	0	0	0	0	0							
(Kidney)	[10]						[0]							[0]							[9]													
Basophilic tubule, cortex	3	6	1	0	0	7																8	1	0	0	0*	1#							
Mineralization, medulla/papilla	7	3	0	0	0	3																8	1	0	0	0	1							
Cyst, cortico-medullary junction	9	1	0	0	0	1																9	0	0	0	0	0							
Vacuolation, proximal tubule, cortex	10	0	0	0	0	0																8	0	0	1	0	1							
Dilatation, diffuse, distal tubule, cortex	10	0	0	0	0	0																8	0	1	0	0	1							
(Adrenal gland)	[10]						[0]							[0]							[9]													
Hypertrophy, fascicular zone, cortex	10	0	0	0	0	0																7	0	2	0	0	2							

^a com oil, 5 mL/kg

-, Negative; +/-, Very slight; +, Slight; ++, Moderate; +++ Severe; Pos., Total of positive grade

[], Number of animals examined

*, Significantly different from control $p < 0.05$ (Two-tailed Mann-Whitney U test); **, Significantly different from control $p < 0.01$ (Two-tailed Mann-Whitney U test).

#, Significantly different from control $p < 0.05$ (One-tailed Fisher exact test); ##, Significantly different from control $p < 0.01$ (One-tailed Fisher exact test).

Table 51A. Histopathological findings with *p,p'*-DDE in males from Subgroup A of Laboratory 7 study

Groups (mg/kg)	0 ^a						12.5						50						100					
	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.
(Liver)	[5]						[5]						[5]						[5]					
Hypertrophy, hepatocyte centrilobular	5	0	0	0	0	0	0	2	3	0	0**	5##	0	0	1	4	0**	5##	0	0	0	3	2**	5##
Ground glass appearance, hepatocyte, centrilobular	5	0	0	0	0	0	0	2	3	0	0**	5##	0	0	1	4	0**	5##	0	0	0	3	2**	5##
Mitosis, hepatocyte	5	0	0	0	0	0	5	0	0	0	0	0	3	2	0	0	0	2	3	2	0	0	0	2
Microgranuloma	3	2	0	0	0	2	4	1	0	0	0	1	4	1	0	0	0	1	4	0	1	0	0	1
Necrosis, focal	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	4	1	0	0	0	1
Hematopoiesis, extramedullary	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0
Single colli necrosis	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0
Degeneration, vacuolar hepatocyte, midlobular	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	4	0	0	1	0	1
(Thyroid gland)	[5]						[5]						[5]						[5]					
Hypertrophy, follicular cell	5	0	0	0	0	0	5	0	0	0	0	0	0	5	0	0	0**	5##	0	3	2	0	0**	5##
Hyperplasia, follicular cell	5	0	0	0	0	0	5	0	0	0	0	0	2	3	0	0	0	3	0	2	3	0	0**	5##
Ectopic thymus	4	1	0	0	0	1	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0
(Kidney)	[5]						[5]						[5]						[5]					
Eosinophilic body	5	0	0	0	0	0	3	1	1	0	0	2	1	2	1	1	0	4#	1	0	2	2	0	4##
Basophilic tubule, cortex	2	3	0	0	0	3	1	4	0	0	0	4	1	4	0	0	0	4	2	3	0	0	0	3
Mineralization, medulla/papilla	5	0	0	0	0	0	4	1	0	0	0	1	4	1	0	0	0	1	4	1	0	0	0	1
Cyst, cortico-medullary junction	3	2	0	0	0	2	4	1	0	0	0	1	5	0	0	0	0	0	5	0	0	0	0	0
Cast hyaline, cortex	5	0	0	0	0	0	5	0	0	0	0	0	4	1	0	0	0	1	5	0	0	0	0	0
Cellular infiltration, lymphocyte, interstitium	4	1	0	0	0	1	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0
Fibrosis, focal, subcapsule	5	0	0	0	0	0	4	1	0	0	0	1	5	0	0	0	0	0	5	0	0	0	0	0
(Spleen)	[5]						[0]						[0]						[5]					
Hematopoiesis, extramedullary	0	4	1	0	0	5												0	5	0	0	0	5	
Inflammation, focal, capsule	4	1	0	0	0	1												5	0	0	0	0	0	
(Lung & Bronchus)	[5]						[0]						[0]						[5]					
Accumulation, foam cell	2	3	0	0	0	3													1	2	2	0	0	4
Cellular infiltration, eosinophil around artery & bronchus	5	0	0	0	0	0													4	1	0	0	0	1
Mineralization, artery	5	0	0	0	0	0													5	0	0	0	0	0
(Heart)	[5]						[0]						[0]						[5]					
Degeneration/fibrosis, myocardium, focal	5	0	0	0	0	0													5	0	0	0	0	0
(Pancreas)	[5]						[0]						[0]						[5]					
Atrophy, acinar cell, focal, with ductal proliferation	5	0	0	0	0	0													5	0	0	0	0	0
(Pituitary gland)	[5]						[0]						[0]						[5]					
Cyst, Rathke's cleft	4	1	0	0	0	1													5	0	0	0	0	0

^a corn oil, 5 mL/kg

- Negative; +/- Very slight; +, Slight; ++, Moderate; +++, Severe; Pos., Total of positive grade; []. Number of animals examined

*, Significantly different from control $p < 0.05$ (Two-tailed Mann-Whitney U test); **, Significantly different from control $p < 0.01$ (Two-tailed Mann-Whitney U test)#, Significantly different from control $p < 0.05$ (One-tailed Fisher exact test); ##, Significantly different from control $p < 0.01$ (One-tailed Fisher exact test)

Table 51A continued. Histopathological findings with *p,p'*-DDE in males from Subgroup A of Laboratory 7 study

Groups (mg/kg)	0 ^a						12.5						50						100						
	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.	
(Epididymis)	[5]						[0]						[0]						[5]						
Cellular infiltration, lymphocyte, interstitium	5	0	0	0	0	0													5	0	0	0	0	0	0
(Prostate; ventral lobe)	[5]						[0]						[0]						[5]						
Cellular infiltration, lymphocyte & plasma cell, epithelium/interstitium	4	1	0	0	0	1													3	2	0	0	0	0	2
(Harderian gland)	[5]						[0]						[0]						[5]						
Cellular infiltration, lymphocyte, interstitium	5	0	0	0	0	0													5	0	0	0	0	0	0
(Thymus)	[5]						[0]						[0]						[5]						
No remarkable change																									
(Parathyroid gland)	[5]						[0]						[0]						[5]						
No remarkable change																									
(Adrenal gland)	[5]						[0]						[0]						[5]						
No remarkable change																									
(Testis)	[5]						[0]						[0]						[5]						
No remarkable change																									
(Prostate; dorsolateral lobe)	[5]						[0]						[0]						[5]						
No remarkable change																									
(Seminal vesicle & coagulating gland)	[5]						[0]						[0]						[5]						
No remarkable change																									
(Brain)	[5]						[0]						[0]						[5]						
No remarkable change																									
(Mammary gland)	[5]						[0]						[0]						[5]						
No remarkable change																									

^a corn oil, 5 mL/kg
 - Negative; +/- Very slight; +, Slight; ++, Moderate; +++, Severe; Pos., Total of positive grade
 []. Number of animals examined

Table 51B. Histopathological findings with *p,p'*-DDE in females from Subgroup A of Laboratory 7 study

Groups (mg/kg)	0 ^a						6.25						25						75					
Grade	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.
(Liver)	[5]						[5]						[5]						[5]					
Hypertrophy, hepatocyte, centrilobular	5	0	0	0	0	0	0	4	1	0	0**	5##	0	0	3	2	0**	5##	0	0	0	3	2**	5##
Ground glass appearance, hepatocyte, centrilobular	5	0	0	0	0	0	3	2	0	0	0	2	0	3	1	1	0**	5##	0	0	0	4	1**	5##
Mitosis, hepatocyte	4	1	0	0	0	1	0	2	0	0	0*	5#	0	4	1	0	0*	5#	0	2	3	0	0*	5#
Necrosis, hepatocyte, centrilobular, with cellular infiltration of neutrophil	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0
Necrosis, local	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	3	2	0	0	0	2
Microgranuloma	4	1	0	0	0	1	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0
(Thyroid gland)	[5]						[4]						[5]						[5]					
Hypertrophy, follicular cell	5	0	0	0	0	0	4	0	0	0	0	0	4	1	0	0	0	1	2	1	2	0	0	3
Hyperplasia, follicular cell	5	0	0	0	0	0	4	0	0	0	0	0	5	0	0	0	0	0	4	1	0	0	0	1
Ectopic thymus	5	0	0	0	0	0	3	1	0	0	0	1	5	0	0	0	0	0	5	0	0	0	0	0
(Kidney)	[5]						[0]						[0]						[5]					
Basophilic tubule, cortex	0	5	0	0	0	5													4	1	0	0	0	1#
Mineralization, medulla/papilla	4	1	0	0	0	1													4	1	0	0	0	1
Cyst, cortico-medullary junction	4	1	0	0	0	1													5	0	0	0	0	0
Vacuolation, proximal tubule, cortex	5	0	0	0	0	0													5	0	0	0	0	0
Dilatation, diffuse, distal tubule, cortex	5	0	0	0	0	0													5	0	0	0	0	0
(Spleen)	[5]						[0]						[0]						[5]					
Hematopoiesis, extramedullary	0	5	0	0	0	5													0	4	1	0	0	5
Deposit, pigment, brown	0	5	0	0	0	5													0	5	0	0	0	5
Atrophy, white pulp	5	0	0	0	0	0													5	0	0	0	0	0
(Lung & Bronchus)	[5]						[0]						[0]						[5]					
Alveolitis, focal	5	0	0	0	0	0													5	0	0	0	0	0
Accumulation, foam cell	4	1	0	0	0	1													1	3	1	0	0	4
Cellular infiltration, eosinophil, around artery & bronchus	5	0	0	0	0	0													4	1	0	0	0	1
Hemorrhage, focal	5	0	0	0	0	0													5	0	0	0	0	0
Metaplasia, osseous	5	0	0	0	0	0													5	0	0	0	0	0
(Adrenal gland)	[5]						[0]						[0]						[5]					
Hypertrophy, fascicular zone, cortex	5	0	0	0	0	0													5	0	0	0	0	0
(Thymus)	[5]						[0]						[0]						[5]					
Atrophy	5	0	0	0	0	0													5	0	0	0	0	0
(Harderian gland)	[5]						[0]						[0]						[5]					
Atrophy	5	0	0	0	0	0													5	0	0	0	0	0
(Ovary)	[5]						[0]						[0]						[5]					
Cyst, follicular	4	1	0	0	0	1													5	0	0	0	0	0

^a corn oil. 5 mL/kg

ENV/JM/MONO(2006)26/ANN

- Negative; +/- Very slight; +, Slight; ++, Moderate; +++, Severe; Pos., Total of positive grade; []. Number of animals examined

*, Significantly different from control p<0.05 (Two-tailed Mann-Whitney U test); **, Significantly different from control p<0.01 (Two-tailed Mann-Whitney U test)

#, Significantly different from control p<0.05 (One-tailed Fisher exact test); ##, Significantly different from control p<0.01 (One-tailed Fisher exact test)

Table 51B continued. Histopathological findings with *p,p'*-DDE in females from Subgroup A of Laboratory 7 study

Groups (mg/kg) Grade	0 ^a					6.25					25					75									
		/-		+	++	os.		/-		+	++	os.		/-		+	++	os.		/-		+	++	os.	
(Heart)	[5]						[0]						[0]						[5]						
No remarkable change																									
(Pancreas)	[5]						[0]						[0]						[5]						
No remarkable change																									
(Pituitary gland)	[5]						[0]						[0]						[5]						
No remarkable change																									
(Parathyroid gland)	[3]						[0]						[0]						[5]						
No remarkable change																									
(Oviduct)	[5]						[0]						[0]						[5]						
No remarkable change																									
(Uterus)	[5]						[0]						[0]						[5]						
No remarkable change																									
(Vagina)	[5]						[0]						[0]						[5]						
No remarkable change																									
(Brain)	[5]						[0]						[0]						[5]						
No remarkable change																									
(Mammary gland)	[5]						[0]						[0]						[5]						
No remarkable change																									
(Duodenum)	[0]						[1]						[0]						[0]						
No remarkable change																									
(Jejunum)	[0]						[1]						[0]						[0]						
No remarkable change																									
(Ileum)	[0]						[1]						[0]						[0]						
No remarkable change																									

^a corn oil. 5 mL/kg

- Negative; +/- Very slight; +, Slight; ++, Moderate; +++, Severe; Pos., Total of positive grade

[]. Number of animals examined

Table 52A. Histopathological findings with p,p'-DDE in males from Subgroup B of Laboratory 7 study

Groups (mg/kg)	0 ^a						12.5						50						100						
	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.	
(Liver)	[5]						[5]						[5]						[5]						
Hypertrophy, hepatocyte, centrilobular	5	0	0	0	0	0	0	5	0	0	0**	5	0	0	2	3	0**	5###	0	0	0	2	3**	5##	
Ground glass appearance, hepatocyte, centrilobular	5	0	0	0	0	0	0	4	1	0	0**	5	0	0	2	3	0**	5###	0	0	0	1	4**	5##	
Mitosis, hepatocyte	5	0	0	0	0	0	5	0	0	0	0	0	2	1	2	0	0	3	1	4	0	0	0	4#	
Microgranuloma	4	1	0	0	0	1	5	0	0	0	0	0	4	1	0	0	0	1	3	2	0	0	0	2	
Necrosis, focal	5	0	0	0	0	0	5	0	0	0	0		5	0	0	0	0	0	4	1	0	0	0	1	
Hematopoiesis, extramedullary	5	0	0	0	0	0	4	1	0	0	0		5	0	0	0	0	0	5	0	0	0	0	0	
Single cell necrosis	5	0	0	0	0	0	4	1	0	0	0		5	0	0	0	0	0	5	0	0	0	0	0	
Degeneration, vacuolar, hepatocyte, midlobular	5	0	0	0	0	0	5	0	0	0	0	0	3	0	1	1	0	2	5	0	0	0	0	0	
(Thyroid gland)	[5]						[5]						[5]						[5]						
Hypertrophy, follicular cell	5	0	0	0	0	0	5	0	0	0	0	0	0	5	0	0	0**	5###	0	3	2	0	0**	5##	
Hyperplasia, follicular cell	5	0	0	0	0	0	5	0	0	0	0	0	1	4	0	0	0	4#	0	3	2	0	0**	5##	
Ectopic thymus	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	4	1	0	0	0	1	
(Kidney)	[5]						[5]						[5]						[5]						
Eosinophilic body	1	2	1	1	0	4	3	2	0	0	0	2	2	2	1	0	0	3	0	0	3	2	0	5	
Basophilic tubule, cortex	2	3	0	0	0	3	3	2	0	0	0	2	1	2	2	0	0	4	1	3	1	0	0	4	
Mineralization, medulla/papilla	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	
Cyst, Cortico-medullary junction	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	
Cast hyaline, cortex	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	
Cellular infiltration, lymphocyte, interstitium	5	0	0	0	0	0	5	0	0	0	0	0	4	1	0	0	0	1	5	0	0	0	0	0	
Fibrosis, focal, subcapsule	5	0	0	0	0	0	5	0	0	0	0	0	4	0	1	0	0	1	5	0	0	0	0	0	
(Spleen)	[5]						[0]						[0]						[5]						
Hematopoiesis, extramedullary	0	5	0	0	0	5													0	3	2	0	0	5	
Inflammation, focal, capsule	5	0	0	0	0	0													5	0	0	0	0	0	
(Lung & Bronchus)	[5]						[0]						[0]						[5]						
Accumulation, foam cell	2	3	0	0	0	3													2	3	0	0	0	3	
Cellular infiltration, eosinophil around artery & bronchus	5	0	0	0	0	0													4	0	1	0	0	1	
Mineralization, artery	4	1	0	0	0	1													5	0	0	0	0	0	
(Heart)	[5]						[0]						[0]						[5]						
Degeneration/fibrosis, myocardium, focal	4	1	0	0	0	1													5	0	0	0	0	a	
(Pancreas)	[5]						[0]						[0]						[5]						
Atrophy, acinar cell, focal, with ductal proliferation	4	1	0	0	0	1													5	0	0	0	0	0	
(Pituitary gland)	[5]						[0]						[0]						[5]						
Cyst Rathke's cleft	5	0	0	0	0	0													4	1	0	0	0	1	

^a corn oil, 5 mL/kg - Negative; +/- Very slight; +, Slight; ++, Moderate; +++, Severe; Pos., Total of positive grade; []. Number of animals examined
 *, Significantly different from control p<0.05 (Two-tailed Mann-Whitney U test); **, Significantly different from control p<0.01 (Two-tailed Mann-Whitney U test)
 #, Significantly different from control p<0.05 (One-tailed Fisher exact test); ##, Significantly different from control p<0.01 (One-tailed Fisher exact test)

Table 52A continued. Histopathological findings with *p,p'*-DDE in males from Subgroup B of Laboratory 7 study

Groups (mg/kg)	0 ^a						12.5						50						100					
(Epididymis)	[5]						[0]						[0]						[5]					
Cellular infiltration, lymphocyte, interstitium	5	0	0	0	0	0													4	1	0	0	0	1
(Prostate; ventral lobe)	[5]						[0]						[0]						[5]					
Cellular infiltration, lymphocyte & plasma cell, epithelium/interstitium	2	1	2	0	0	3													4	0	0	1	0	1
(Harderian gland)	[5]						[0]						[0]						[5]					
Cellular infiltration, lymphocyte, interstitium	4	1	0	0	0	1													4	1	0	0	0	1
(Thymus)	[5]						[0]						[0]						[5]					
No remarkable change																								
(Parathyroid gland)	[5]						[0]						[0]						[5]					
No remarkable change																								
(Adrenal gland)	[5]						[0]						[0]						[5]					
No remarkable change																								
(Testis)	[5]						[0]						[0]						[5]					
No remarkable change																								
(Prostate; dorsolateral lobe)	[5]						[0]						[0]						[5]					
No remarkable change																								
(Seminal vesicle & coagulating gland)	[5]						[0]						[0]						[5]					
No remarkable change																								
(Brain)	[5]						[0]						[0]						[5]					
No remarkable change																								
(Mammary gland)	[5]						[0]						[0]						[5]					
No remarkable change																								

^a corn oil. 5 mL/kg

- Negative; +/- Very slight; +, Slight; ++, Moderate; +++, Severe; Pos., Total of positive grade

[]. Number of animals examined

Table 52B. Histopathological findings with *p,p'*-DDE in females from Subgroup B of Laboratory 7 study

Groups (mg/kg)	0 ^a						6.25						25						75					
	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.
(Liver)	[5]						[5]						[5]						[4]					
Hypertrophy, hepatocyte, centrilobular	5	0	0	0	0	0	0	4	1	0	0**	5##	0	0	2	3	0**	5##	0	0	0	1	3**	4##
Ground glass appearance.																								
hepatocyte, centrilobular	5	0	0	0	0	0	1	4	0	0	0	4#	0	0	3	2	0**	5##	0	0	0	1	3**	4##
Mitosis, hepatocyte	5	0	0	0	0	0	2	2	1	0	0	3	0	2	3	0	0**	5##	0	3	1	0	0**	4##
Necrosis, hepatocyte centrilobular, with cellular infiltration of neutrophil	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	3	0	0	1	0	1
Necrosis, focal	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	4	0	0	0	0	0
Microgranuloma	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	4	0	0	0	0	0
(Thyroid gland)	[5]						[3]						[5]						[4]					
Hypertrophy, follicular cell	5	0	0	0	0	0	5	0	0	0	0	0	3	2	0	0	0	2	1	2	1	0	0	3
Hyperplasia, follicular cell	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	3	1	0	0	0	1
Ectopic thymus	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	4	0	0	0	0	0
(Kidney)	[5]						[0]						[0]						[4]					
Basophilic tubule, cortex	3	1	1	0	0	2													4	0	0	0	0	0
Mineralization medulla/papillae	3	2	0	0	0	2													4	0	0	0	0	0
Cyst cortico-medullary junction vacuolation.	5	0	0	0	0	0													4	0	0	0	0	0
proximal tubule, cortex	5	0	0	0	0	0													3	0	0	1	0	1
Dilatation, diffuse, distal tubule, cortex	5	0	0	0	0	0													3	0	1	0	0	1
(Spleen)	[5]						[0]						[0]						[4]					
Hematopoiesis, extramedullary	0	5	0	0	0	5													1	2	1	0	0	3
Deposit, pigment brown	0	5	0	0	0	5													0	2	2	0	0	4
Atrophy, white pulp	5	0	0	0	0	0													3	0	0	0	1	1
(Lung & Bronchus)	[5]						[0]						[0]						[4]					
Alveolitis, focal	5	0	0	0	0	0													3	0	1	0	0	1
Accumulation, foam cell	3	2	0	0	0	2													2	0	1	1	0	2
Cellular infiltration, eosinophil, wound artery & bronchus	4	1	0	0	0	1													3	0	1	0	0	1
Hemorrhage, focal	4	1	0	0	0	1													4	0	0	0	0	0
Metaplasia, osseus	3	2	0	0	0	2													4	0	0	0	0	0
(Adrenal gland)	[5]						[0]						[0]						[4]					
Hypertrophy fascicular zone, cortex	5	0	0	0	0	0													2	0	2	0	0	2
(Thymus)	[5]						[0]						[0]						[4]					
Atrophy	5	0	0	0	0	0													3	0	0	0	1	1
(Harderian gland)	[5]						[0]						[0]						[4]					
Atrophy	5	0	0	0	0	0													3	0	0	1	0	1
(Ovary)	[5]						[0]						[0]						[4]					
Cyst follicular	5	0	0	0	0	0													3	1	0	0	0	1

^a corn oil, 5 mL/kg

- Negative; +/- Very slight; +, Slight; ++, Moderate; +++, Severe; Pos., Total of positive grade; []. Number of animals examined

*, Significantly different from control $p < 0.05$ (Two-tailed Mann-Whitney U test); **, Significantly different from control $p < 0.01$ (Two-tailed Mann-Whitney U test)#, Significantly different from control $p < 0.05$ (One-tailed Fisher exact test); ##, Significantly different from control $p < 0.01$ (One-tailed Fisher exact test)

Table 52B continued. Histopathological findings with *p,p'*-DDE in females from Subgroup B of Laboratory 7 study

Groups (mg/kg)	0 ^a						6.25						25						75						
	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.	-	+/-	+	++	+++	Pos.	
(Heart)	[5]						[0]						[0]						[4]						
No remarkable change																									
(Pancreas)	[5]						[0]						[0]						[4]						
No remarkable change																									
(Pituitary gland)	[5]						[0]						[0]						[4]						
No remarkable change																									
(Parathyroid gland)	[5]						[0]						[0]						[4]						
No remarkable change																									
(Oviduct)	[5]						[0]						[0]						[1]						
No remarkable change																									
(Uterus)	[5]						[0]						[0]						[4]						
No remarkable change																									
(Vagina)	[5]						[0]						[0]						[4]						
No remarkable change																									
(Brain)	[5]						[0]						[0]						[4]						
No remarkable change																									
(Mammary gland)	[5]						[0]						[0]						[4]						
No remarkable change																									
(Duodenum)	[0]						[1]						[0]						[0]						
No remarkable change																									
(Jejunum)	[0]						[1]						[0]						[0]						
No remarkable change																									
(Ileum)	[0]						[1]						[0]						[0]						
No remarkable change																									

^a corn oil, 5 mL/kg

- Negative; +/- Very slight; +, Slight; ++, Moderate; +++, Severe; Pos., Total of positive grade

[]. Number of animals examined

Propylthiouracil

Laboratory 1 - Propylthiouracil

145. There was an increase of incidence and severity of follicular cell hyperplasia and hypertrophy of the thyroid glands from low dose group to high dose group in all subsets. This was correlated with the macroscopic enlargement in both Subgroups A and B. This was regarded to be treatment related, and the thyroid data are presented in the combined Subgroups in Table 53.

Table 53. Thyroid histopathological observations in the Laboratory 1 propylthiouracil study

Incidence – Combined Subgroups	0.1 mg/kg/d PTU		1 mg/kg/d PTU		10 mg/kg/d PTU	
	6/10 (M)	2/10 (F)	10/10 (M)	10/10 (F)	10/10 (M)	10/10 (F)
Follicular cell hyperplasia/hypertrophy						
Severity						
Grade 1	4	1	0	0	0	0
Grade 2	2	1	0	1	0	0
Grade 3	0	0	7	8	0	2
Grade 4	0	0	3	1	10	8

M – male; F - female

146. There was a positive correlation between the relative weights of the pituitary gland in the dose groups 2 (female: + 20% compared to control) and 3 (male and female: +33.3% and 40.0% compared to control) and the microscopic changes (pars distalis of the pituitary glands: Diffuse hyperplasia of chromophobe and basophilic cells with a loss of acidophilic cells). This was also regarded as a substance effect.

147. The significant decrease or increase of the other mean absolute or relative organ weights did not correspond with microscopic correlates. In particular, the significant increase of the weights of testes, epididymides, seminal vesicles and prostate gland showed no morphological correspondence. Most of the grossly noted lesions were correlated with a meaningful microscopic finding, but these findings were all regarded as of spontaneous origin, i.e., they were not judged to be treatment related. The grossly noted focal constriction (diameter 20 mm) of the liver in animal #42 (female, control group) due to diaphragmatic herniation of the left medial lobe and led histologically to a fibrotic area within the liver capsule. The adhesion within the abdominal cavity in one mid dose female was a focal fibroplastic peritonitis.

148. The grossly noted retractions in one kidney lacked a microscopic correlate. In animal no.75 (female, group 3) the grossly noted pelvic dilation of the kidney could be correlated to a cyst, and the dilation of the ureter in the same animal was apparent as well in gross as in histological investigation. A spermatogenic granuloma was detected in the epididymis of one animal of the low dose group macroscopically and microscopically. The cyst in one ovary of the mid dose group was histologically acknowledged.

149. All other microscopic findings recorded were either single observations, or they occurred in control animals only, or they were recorded at comparable incidence and graded severity in control and high dose males and/or females.

150. The detailed histopathological data for the combined Subgroups from the laboratory 1 propylthiouracil study is presented in Table 54.

Table 54. Histopathology findings in the Laboratory 1 propylthiouracil study– Combined Subgroups

Sex Propylthiouracil (mg/kg/day)		M				F			
		0	0.1	1	10	0	0.1	1	10
Animals in selected Group		10	10	10	10	10	10	10	10
Adrenal cortex		10			10	10			10
- Cyst(s)	P				1 1	1 1			
Coagulation glands		10			10				
Kidneys		10	1		10	10			10
- Fibrosis, focal	1				1 1				
- Nephropathy, chronic	1	2 2			2 2	1 1			
- Cyst(s)	P	1 1							2 2
Liver		10			10	10			10
- Lymphoid infiltr.	1 2	2 2			1 1				2 1 1
- Capsule fibrosis	P								
Ovaries						10		1	10
- Cyst(s)	P							1 1	
Oviducts						10			10
Pituitary gland		10	10	9	10	10	10	10	10
- Hyperpl. chromoph.	1 2 3 4			9 5 4	10 1			10 10	10 7 3
- Cyst(s)	P	1 1	1 1	1 1					1 1
- Cyst of Rathke	P	3 3	5 5	1 1		4 4	2 2	1 1	1 1
Prostate Gland		10			10				-
Seminal vesicle		10			10				
Testes		10			10				
Thyroid glands		10	10	10	10	10	10	10	10
- Hyperpl./Hypertrophy	1 2 3 4		6 4 2	10	10		2 1 1	10 1	10 2 8
- Ectopic thymus	P	1 1	1 1				1 1		
- Cyst(s), brachioge.	P	4 4					1 1		1 1
Uterus						10			10
Vagina						10			10

Laboratory 10 – Propylthiouracil

151. Although detailed histopathological data were not originally reported, upon the specific request of the Secretariat, laboratory 10 provided an overall summary of the histopathological findings with propylthiouracil, and provided detailed summaries and grades for the primary target tissues, the thyroid. For this PTU study, no abnormal findings were reported by this laboratory in the following tissues: salivary gland, esophagus, parathyroid gland, heart, kidney, thymus, adrenal gland, pancreas, hypophysis, stomach, intestine, peripheral nerve, femur, brain, spleen, testis, epididymis, prostate gland, ovary, and uterus.

Sex PTU (mg/kg/day). Animals per Group	male				female			
	10	1	0.1	0	10	1	0.1	0
	6	6	6	6	6	6	6	6
Liver								
lymphocyte infiltration	1		2		1			
Lung								
interstitial pneumonia		2	1		1	1		1
peribronchiolar lymphoid hyperplasia			1		1		1	
Trachea								
tracheitis	1	1	2	1			2	1

Histopathological Thyroid gland changes in SD rats treated with PTU for 28 days

PTU (mg/kg/day)	Males				Females			
	0	0.1	1	10	0	0.1	1	10
normal	6	3			6	4	2	
grade1		3	5			2	3	
grade2			1	3			1	4
grade3				3				2

grade 1, mild ; grade 2, moderate ; grade 3, severe.

I-Thyroxine

Laboratory 9 - I-thyroxine

151. Changes considered attributable to the treatment were found in thyroid, heart, spleen, and liver of both sexes, in kidney and mammary gland of males, and in bone marrow and adrenal of females. The data are summarized in Table 55 and as follows:

152. In thyroid, atrophy of follicular epithelium was found in 3 males and 8 females of the 0.1 mg/kg group, and all males and females of the 1.0 mg/kg group. Follicular epithelium of bilateral thyroid was monotonously flattened, and follicular cavity was enlarged being filled with colloid.

153. In heart, hypertrophy of myocardial fiber was found in 9 males and 7 females of the 1.0 mg/kg group. Further, in the right ventricular wall of 5 males of 1.0 mg/kg group, more advanced myocardial degeneration/fibrosis were found.

154. In spleen, increase in erythrocytic hematopoietic cell was found in 4 males and 3 females of the 1.0 mg/kg group. In addition, as the changes in blood or hematopoietic system, increase in hematopoietic cell in bone marrow in 5 females and extramedullary hematopoiesis in liver in 4 males were observed in the 1.0 mg/kg group, respectively.

155. In liver, diffuse hypertrophy of hepatocyte in all males and 3 females, and increased mitotic figure in hepatocyte in all males and 5 females, and hypertrophy of Kupffer cell in 8 males and 2 females were found in 1.0 mg/kg group. In addition in the females, centrilobular inflammatory cell infiltration was found in 3, 2, 4, and 8 cases in the control, 0.01, 0.1, and 1.0 mg/kg group, respectively. Although the majority of them sporadically showed the nests of infiltration, advanced grade of lesion was observed in 5 cases of the 1.0 mg/kg group because inflammatory cell infiltration was seen in the most of lobules. In addition in these advanced cases, the lesions were frequently accompanied by single cell necrosis of hepatocyte.

156. In kidney, accelerated occurrence of hyaline droplet in proximal tubular epithelium was found in 5 males of the 1.0 mg/kg group. Many hyaline droplets with pale eosinophilic and relatively large appearance were seen in these cases. These droplets showed positive PAS reaction.

157. Dilatation of duct of mammary gland was found in a male of the 0.1 mg/kg group and 5 males of the 1.0 mg/kg group. The duct was lined by low cuboidal epithelium, and its lumen was extended. Moreover, a thick layer of hyperplastic connective tissue surrounded the dilated duct. Budding acinus was also lined by cuboidal cells, surrounding connective tissue was also thickened.

158. In adrenal, hypertrophy cortical cell in fascicular zone was found in 4 females of the 1.0 mg/kg group.

159. The other incidental and spontaneous changes seen in this study that were not judged to be treatment related were focal hemorrhage in thymus, accumulation of macrophage in lung, focal or periportal fatty change of hepatocyte and microgranuloma in liver, focal atrophy of acinus and focal inflammatory cell infiltration in pancreas, basophilic tubule, cyst, dilatation of pelvis, focal infiltration of lymphocyte in interstitium, and mineralization of cortico-medullary junction in kidney, focal atrophy of seminiferous tubules in testis, spermatic granuloma, cell debris in lumen, and focal inflammatory cell infiltration in epididymis, inflammatory cell infiltration in ventral prostate, dilatation of lumen in uterus, cyst in intermediate lobe, and cyst-like lesion in anterior and intermediate lobe of pituitary, ectopic thymic tissue, focal infiltration of lymphocyte, and ultimobranchial remnant in thyroid, increase in lipid droplet in

cortical cell of fascicular zone in adrenal, and focal inflammatory cell infiltration in adrenal, atrophy of hair follicle, and focal inflammatory cell infiltration in skin.

Table 55. Histopathological findings in the Combined Subgroups in the Laboratory 9 l-thyroxine study

Organ/tissue Findings	Sex I-Thyroxine (mg/kg/day)	Male				Female			
		0	0.01	0.1	1.0	0	0.01	0.1	1.0
	grade								
Thyroid		<10>	<10>	<10>	<10>	<10>	<10>	<10>	<10>
Atrophy, follicular epithelium	(1)	0	0	3	10	0	0	8	10
Heart		<10>	<10>	<10>	<10>	<10>	<10>	<10>	<10>
Hypertrophy, myocardial fiber	(1)	0	0	0	9	0	0	0	7
Myocardial degeneration/fibrosis, focal	(1) (2)	3 0	0 0	3 0	1 5	2 0	0 0	1 0	0 0
Spleen		<10>	<10>	<10>	<10>	<10>	<10>	<10>	<10>
Increase in hematopoietic cell, erythrocyte	(1)	0	0	0	4	0	0	0	3
Bone marrow (femur)		<10>	<10>	<10>	<10>	<10>	<10>	<10>	<10>
Increase in hematopoietic cell	(1)	0	0	0	0	0	0	0	5
Liver		<10>	<10>	<10>	<10>	<10>	<10>	<10>	<10>
Hypertrophy, hepatocyte, diffuse	(1)	0	0	0	10	0	0	0	3
Increase in mitotic figure, hepatocyte	(1)	0	0	0	10	0	0	0	5
Hypertrophy, Kupffer cell	(1)	0	0	0	8	0	0	0	2
Inflammatory cell infiltration, centrilobular	(1) (2)	0 0	0 0	0 0	0 0	3 0	2 0	4 0	3 5
Extramedullary hematopoiesis	(1)	0	0	0	4	0	0	0	0
Kidney		<10>	<10>	<10>	<10>	<10>	<0>	<2>	<10>
Hyaline droplet, tubular epithelium, proximal	(1) (2)	10 0	10 0	10 0	5 5	0 0	· ·	0 0	0 0
Mammary gland		<10>	<10>	<10>	<10>	<10>	<0>	<0>	<10>
Dilatation, duct	(1)	0	0	1	5	0	·	·	0
Adrenal		<10>	<10>	<10>	<10>	<10>	<10>	<10>	<10>
Hypertrophy, cortical cell, fascicular zone	(1)	0	0	0	0	0	0	0	4

() Grade

< > Number examined

Laboratory 13 - l-Thyroxine

160. Dose related observations were made in several tissues after the administration of l-thyroxine. The incidences of dose related histopathological findings are summarized in the Table 56A for males and Table 56B for females.

161. Adrenal glands: Cortical fatty change was slightly less frequent and had slightly lower average grading in male groups 4 (1.0 mg/kg) and 3 (0.1 mg/kg) than in male groups 1 or 2. Such effect was not observed in females, where the occurrence of cortical fatty change is normally rare.

162. Heart: Inflammation with fibrosis was found in 7/10 males of group 4. Although the features of this lesion were similar to those occasionally observed as spontaneous change in aging, especially male rat heart, its exclusive presence in the high dose group substantiated a relationship to the treatment. Minimal inflammation with fibrosis found in the heart of a single female of group 3 was considered incidental, owing to its low incidence, grading, and absence in female high dose group.

163. Kidneys: Corticomedullary mineralization, which normally occurs especially in female rats, had decreased grading in female groups 4 and 3. Lesions probably associated with corticomedullary mineralization, such as tubular atrophy or tubular basophilia, has decreased incidence and grading in female groups 4 and 3 as well. This effect was not observed in males, where corticomedullary mineralization occurs only rarely.

164. Liver: Deposition of glycogen in the hepatocytes was decreased in all male and female treated groups, with incidence and grading decreasing in dose-related manner. In high dose groups, cytoplasmic clearings characteristic of the presence of hepatocellular glycogen were virtually absent. Dissociation of hepatocytes was observed in all males and 7/10 females of group 4. This finding consisted of separation of single hepatocytes by clear spaces. Although not well known or recognized as a lesion, this finding was present only in high dose groups, despite that, liver specimens of all animals were processed by the same technician. Therefore, it is considered to represent an effect of the treatment.

165. Pituitary gland: Hypertrophy of cells in pars distalis was slightly increased with respect to incidence and grading in male group 4. Microscopic feature of this finding was formation of vacuolar area, probably containing secretory material, in the cytoplasm. It is unclear whether this finding actually resulted from an increased hormone production or decreased hormone release from such cells, probably thyrotropes.

166. Spleen: Extramedullary hematopoietic activity occurred in all male and female animals on study. Its grading was increased in male and female groups 4 and 3. Hemosiderosis had slightly decreased incidence and grading in male groups 4 and 3 and female group 4.

167. Thyroid gland: Hypertrophy of thyroid follicular cells is normally present in control male rats of this colony. This change was decreased in male group 2 (0.01 mg/kg) and absent in male groups 4 and 3. In all animals of male groups 4 and 3, the follicles were totally inactive, i.e. distended and lined by flattened cells. In control female rats of this colony, spontaneous hypertrophy of the follicular cells is rare. Features of total inactivity were observed in all females of groups 4 and 3 and 2/10 females of group 2.

168. Incidental microscopic findings: Additionally, a variety of other changes was found in this study. They commonly occur in laboratory rats of this strain, age, and type of experiment. Neither their incidences nor their distribution or morphologic appearance gave any indication of a treatment-related association.

169. The detailed microscopic findings are summarized for males in Table 53A and for females in Table 53B.

Table 56A. Histopathologic findings for males in the Laboratory 13 l-thyroxine study

l-Thyroxine (mg/kg/day)	0	0.01	0.1	1.0
NO. ANIMALS:	10	10	10	10
ADRENAL GLANDS:	10	10	10	10
- cortical fatty change				
total affected:	9	9	5	7
mean grading:	1.2	1.2	1.0	1.1
HEART:	10	10	10	10
- inflammation with fibrosis				
total affected:	-	-	-	7
mean grading:	-	-	-	2.3
KIDNEYS:	10	10	10	10
- tubular atrophy				
total affected:	-	-	1	-
mean grading:	-	-	1.0	-
- tubular basophilia				
total affected:	-	1	1	-
mean grading:	-	1.0	1.0	-
- corticomedullary mineralization				
total affected:	-	-	-	-
mean grading:	1.0	-	-	-
LIVER:	10	10	10	10
- deposition of glycogen				
total affected:	10	9	7	-
mean grading:	1.5	1.3	1.1	-
- hepatocellular dissociation				
total affected:	-	-	-	10
mean grading:	-	-	-	2.0
PITUITARY GLAND:	10	10	10	10
- hypertrophy pars distalis				
total affected:	5	7	6	9
mean grading:	1.2	1.0	1.2	1.8
SPLEEN:	10	10	10	10
- extramedullary hematopoiesis				
total affected:	10	10	10	10
mean grading:	1.7	1.8	2.1	3.0
- hemosiderosis				
total affected:	9	10	8	8
mean grading:	1.1	1.1	1.0	1.0
THYROID GLAND:	10	10	10	10
- follicular cell hypertrophy				
total affected:	8	4	-	-
mean grading:	1.3	1.0	-	-
- inactivity (present)				
total affected:	-	-	10	10

Grade 1	Minimal/very few/very small.
Grade 2	Slight/few/small.
Grade 3	Moderate/moderate number/moderate size.
Grade 4	Marked/many/large.
Grade 5	Massive/extensive number/extensive size.
Code P	Finding present, grading not scored.

Table 56B. Histopathologic findings for females in the Laboratory 13 l-thyroxine study

I-Thyroxine (mg/kg/day)	0	0.01	0.1	1.0
NO. ANIMALS:	10	10	10	10
ADRENAL GLANDS:	10	10	10	10
- cortical fatty change				
total affected:	-	-	1	-
mean grading:	-	-	1.0	-
HEART:	10	10	10	10
- inflammation with fibrosis				
total affected:	-	-	1	-
mean grading:	-	-	1.0	-
KIDNEYS:	10	10	10	10
- tubular atrophy				
total affected:	10	9	6	7
mean grading:	1.6	1.6	1.2	1.3
- tubular basophilia				
total affected:	8	9	6	5
mean grading:	1.5	1.7	1.2	1.4
- corticomedullary mineralization				
total affected:	10	10	10	10
mean grading:	3.4	3.1	2.6	2.5
LIVER:	10	10	10	10
- deposition of glycogen				
total affected:	8	6	5	-
mean grading:	1.5	1.5	1.4	-
- hepatocellular dissociation				
total affected:	-	-	-	7
mean grading:	-	-	-	1.0
PITUITARY GLAND:	10	10	10	10
- hypertrophy pars distalis				
total affected:	-	-	1	1
mean grading:	1.0	-	1.0	1.0
SPLEEN:	10	10	10	10
- extramedullary hematopoiesis				
total affected:	10	10	10	10
mean grading:	2.4	2.1	2.5	2.7
- hemosiderosis				
total affected:	9	10	9	8
mean grading:	1.3	1.3	1.6	1.1
THYROID GLAND:	10	10	10	10
- follicular cell hypertrophy				
total affected:	-	-	-	-
mean grading:	1.0	-	-	-
- inactivity (present)				
total affected:	-	2	10	10

Grade 1 Minimal/very few/very small.
Grade 2 Slight/few/small.
Grade 3 Moderate/moderate number/moderate size.
Grade 4 Marked/many/large.
Grade 5 Massive/extensive number/extensive size.
Code P Finding present, grading not scored.

ANNEX 7

Thyroid Hormone Data Generated for the Updated TG 407 Validation Studies

1. The purposes of this Annex are to record for the thyroid hormones:
 - the methodologies used by each lab to analyze the T₃, T₄, and TSH values during the updated TG 407 studies, where available from the final reports;
 - the basic statistical findings for each sex from each Subgroups and the combined Subgroups, when these data were available from the final reports; and
 - the means and standard deviations for each sex from each Subgroup and the combined Subgroups, when these data were available from the final reports.

2. Additional purposes of this Annex are to compare the reproducibility and reliability of the experimental findings for the thyroid hormones:
 - in regards to a dose response or possible random findings;
 - between Subgroups in the same lab;
 - the added value and power of combined the Subgroups (i.e., a group size of five versus ten animals per sex); and
 - between the two laboratories studying each chemical.

3. This Annex is organized in the following manner:
 - each of the ten chemicals tested in the updated TG 407 validation program has its own individual section. The chemicals are roughly grouped as:
 - Estrogens: ethinyl oestradiol, genistein, and nonylphenol.
 - Antiestrogens: tamoxifen
 - Aromatase inhibitors: CGS 18320B
 - Androgens: methyl testosterone
 - Antiandrogens: flutamide and p,p'-DDE
 - Thyroid toxicants and hormones: propylthiouracil and l-thyroxine
 - in each section, the thyroid hormone methods and data of both laboratories testing the chemical are presented with the laboratories in alphabetical order;
 - a summary table is presented for each laboratory with the male data in the top half and the female data in the bottom half;
 - for each sex, the T₃, T₄, and TSH data for the combined Subgroups is presented first, then for the two individual Subgroups;
 - the dose groups left to right are the vehicle control, the low dose, the mid-dose, and the high dose; and
 - the achievement of statistical significance, either an increase or a decrease in the values, is denoted with an asterisk.

4. Overall integration and interpretation of the thyroid hormone data with other findings are noted in the body of this report, not this or other Annexes. Likewise, discussion, recommendations, and conclusions are in the body of this report, not this or other Annexes.

Ethinyl Estradiol**Laboratory 2 – Ethinyl Estradiol**

5. **Methods:** Using serum obtained at necropsy, T₃ was determined by a triiodothyronine radioimmunoassay, Sorin T₃ RIA, from Messrs. Dia Sorin; T₄ was determined by thyroid total chemiluminescence enzyme immunological test, DPC IMMULITE T₄ assay systems from Messrs. DPC Biermann GmbH, Bad Nauheim, and TSH was determined by thyroid stimulating hormone chemiluminescence enzyme immunometric test, DPC IMMULITE rat serum thyroid stimulating hormone assay system from Messrs. DPC Biermann GmbH, Bad Nauheim.

6. **Results:** The means and standard deviations for thyroid hormones from the laboratory 2 EE studies are shown in Table 1. The TSH values were significantly increased in females at all doses and in males at the mid- and high doses. The T₄ values were significantly increased in females at the mid- and high doses, with no evidence from the absolute values of any change in the males. The T₃ values showed no trends in either sex.

Table 1. Thyroid Hormone Results for EE in the Laboratory 2 Study

Ethinyl Estradiol Dose (µg/kg/day)	Control 0	Low 10	Middle 50	High 200
Males Combined Subgroups (n=10)				
T ₃ (nmol/l)	2.31 ± 0.28	*3.16 ± 0.63	2.71 ± 0.68	2.56 ± 0.51
T ₄ (nmol/l)	93 ± 13.5	101 ± 8.7	96 ± 8.5	105 ± 18.2 ⁹
TSH (µg/l)	4.31 ± 1.76 ⁸	6.27 ± 2.52	*9.23 ± 5.44	*7.30 ± 2.08
Subgroup A (n=5)				
T ₃ (nmol/l)	2.27 ± 0.26	3.28 ± 0.83	2.83 ± 0.90	2.57 ± 0.39
T ₄ (nmol/l)	92 ± 12.5	108 ± 1.5	92 ± 7.6	⁺ 102 ± 11.5
TSH (µg/l)	5.20 ± 2.16 ⁴	⁺ 5.10 ± 1.47	⁺ 11.46 ± 6.41	⁺ 7.16 ± 1.38
Subgroup B (n=5)				
T ₃ (nmol/l)	2.34 ± 0.32	3.04 ± 0.41	2.59 ± 0.43	2.54 ± 0.66
T ₄ (nmol/l)	93 ± 16.0	95 ± 7.7	99 ± 8.8	107 ± 23.4
TSH (µg/l)	3.43 ± 0.70 ⁴	⁺ 7.44 ± 2.94	⁺ 7.00 ± 3.62	⁺ 7.44 ± 2.80
Females Combined Subgroups (n=10)				
T ₃ (nmol/l)	1.53 ± 0.37	1.46 ± 0.30	1.52 ± 0.44	1.64 ± 0.44
T ₄ (nmol/l)	62 ± 11.1	74 ± 14.4	*81 ± 17.3	**88 ± 11.3
TSH (µg/l)	2.50 ± 0.85	*3.05 ± 0.63	*4.36 ± 2.18	*4.34 ± 1.60
Subgroup A (n=5)				
T ₃ (nmol/l)	1.44 ± 0.30	1.29 ± 0.08	1.45 ± 0.19	1.56 ± 0.16
T ₄ (nmol/l)	55 ± 9.1	72 ± 19.8	*86 ± 19.4	*85 ± 11.5
TSH (µg/l)	2.66 ± 0.93	3.24 ± 0.77	*5.58 ± 2.57	4.16 ± 1.40
Subgroup B (n=5)				
T ₃ (nmol/l)	1.62 ± 0.45	1.63 ± 0.36	1.58 ± 0.62	1.71 ± 0.63
T ₄ (nmol/l)	69 ± 7.9	75 ± 8.2	77 ± 15.7	92 ± 11.3
TSH (µg/l)	2.34 ± 0.82	2.86 ± 0.46	3.14 ± 0.60	4.52 ± 1.93

*, **, Significantly different from control (p<0.05 and p<0.01, respectively) ⁺ statistics were not calculated by the lab where only 4 values were available in the control group.

7. In the male individual Subgroups, the laboratory did not calculate statistics for TSH, as the vehicle control groups were reduced to four animals in both cases. The absolute TSH values in the mid- and high doses of Subgroup A were 50-100% above the vehicle control group and all dose groups in Subgroup B were at least double the vehicle control.

8. In the female Subgroups, Subgroup A increased T_4 values achieved statistical agreement with the combined Subgroups at the mid- and high doses. TSH was significantly increased at the mid-dose, but not the high dose in Subgroup A. Although the absolute T_4 and TSH values increased, none of the Subgroup B values achieved statistical significance.

Laboratory 5 – Ethinyl Estradiol

9. Methods: Using serum obtained at necropsy, T_3 and T_4 were determined using the automatic immunoassay system (IMX, Abbott laboratories), and TSH was measured with a microplate reader (UV max, Molecular Devices).

10. Results: The means and standard deviations for thyroid hormones from the laboratory 5 EE studies are shown in Table 2. The T_3 values were significantly decreased in females at the high dose only. The T_4 values were significantly increased in males at the high dose only. Absolute TSH values were slightly decreased in both sexes at the high dose, but TSH was significantly increased in females at the low dose.

Table 2. Thyroid Hormone Results for EE in the Laboratory 5 Study

	Ethinyl Estradiol Dose ($\mu\text{g}/\text{kg}/\text{day}$)	Control 0	Low 10	Middle 50	High 200
Males					
Combined Subgroups (n=10)					
T_3	(ng/dl)	39 \pm 10	43 \pm 16	33 \pm 13	37 \pm 17
T_4	($\mu\text{g}/\text{dl}$)	5.23 \pm 0.96	5.29 \pm 1.13	5.51 \pm 0.83	*6.31 \pm 0.66
TSH	(ng/ml)	19.2 \pm 4.7	20.0 \pm 4.0	16.6 \pm 3.0	17.9 \pm 5.9
Subgroup A (n=5)					
T_3	(ng/dl)	35 \pm 3	33 \pm 11	28 \pm 11	40 \pm 16
T_4	($\mu\text{g}/\text{dl}$)	4.81 \pm 0.74	4.51 \pm 0.99	5.01 \pm 0.87	6.01 \pm 0.77
TSH	(ng/ml)	17.4 \pm 5.2	19.3 \pm 5.1	16.9 \pm 3.2	17.9 \pm 4.2
Subgroup B (n=5)					
T_3	(ng/dl)	43 \pm 14	52 \pm 13	37 \pm 14	34 \pm 19
T_4	($\mu\text{g}/\text{dl}$)	5.64 \pm 1.04	6.07 \pm 0.61	6.01 \pm 0.42	6.61 \pm 0.38
TSH	(ng/ml)	20.9 \pm 4.0	20.7 \pm 3.0	16.4 \pm 3.2	17.8 \pm 7.7
Females					
Combined Subgroups (n=10)					
T_3	(ng/dl)	55 \pm 6	48 \pm 8	47 \pm 10	*44 \pm 7
T_4	($\mu\text{g}/\text{dl}$)	4.74 \pm 0.78	4.96 \pm 0.90	5.28 \pm 0.96	5.03 \pm 0.83
TSH	(ng/ml)	20.1 \pm 3.9	*26.5 \pm 6.2	23.6 \pm 6.4	17.7 \pm 3.9
Subgroup A (n=5)					
T_3	(ng/dl)	53 \pm 3	43 \pm 7	43 \pm 9	47 \pm 6
T_4	($\mu\text{g}/\text{dl}$)	4.84 \pm 0.49	5.29 \pm 0.82	5.79 \pm 0.61	4.82 \pm 0.80
TSH	(ng/ml)	22.1 \pm 2.0	27.5 \pm 3.5	23.4 \pm 8.2	17.1 \pm 4.3
Subgroup B (n=5)					
T_3	(ng/dl)	56 \pm 7	54 \pm 5	51 \pm 10	*42 \pm 6
T_4	($\mu\text{g}/\text{dl}$)	4.65 \pm 1.06	4.63 \pm 0.92	4.76 \pm 1.02	5.23 \pm 0.90
TSH	(ng/ml)	18.1 \pm 4.5	25.6 \pm 8.5	23.7 \pm 5.1	18.3 \pm 3.9

*, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively).

11. The significant increase in male T_4 values in the combined Subgroups was not significant in either Subgroup A or B, and the overall absolute increase was modest compared to TSH values in many other of the updated TG 407 studies. The significant decrease in female T_3 values of the combined Subgroups was also significant in Subgroup A, but not in Subgroup B.

Summary – Ethinyl Oestrodial

12. The only finding that was consistent among the two studies was an increase in TSH in females at the lowest EE doses. The significant decrease in female T₃ at the high dose in the laboratory 5 study contrasts with the significant increase in female both T₄ and TSH in the laboratory 2 study. Combined with the findings in other studies, this suggests some variability in the methods.

Genistein**Laboratory 4 – Genistein**

13. Methods: Animals were not fasted prior to necropsy. Blood samples were collected from the abdominal aorta, and the serum was obtained by centrifugation. An automated EIA System IMMULIZE (Diagnostic Products Corporation, U.S.A.) was employed for T₃ (DPC IMMULIZE Total T3), T₄ (DPC IMMULIZE Total T3), and TSH (DPC IMMULIZE Total T3).

14. Results: The means and standard deviations for thyroid hormones from the laboratory 4 genistein studies are shown in Table 3. There were no dose related statistically significant differences observed for the thyroid hormones. One observation is the large absolute differences in the TSH control values between the male Subgroups A and B. The low value of the control in Subgroup B may be the source of the statistical significance for the mid-dose group in Subgroup B, and may not be treatment-related.

Table 3. Thyroid Hormone Results for Genistein in the Laboratory 4 Study

Genistein Dose (mg/kg/day)		Control 0	Low 120	Middle 400	High 1000
Males		Combined Subgroups (n=10)			
T ₃	(ng/dl)	116.1 ± 20.0	119.7 ± 14.3	118.2 ± 16.5	119.8 ± 13.3
T ₄	(µg/dl)	7.2 ± 1.0	7.7 ± 1.7	6.7 ± 1.4	7.4 ± 1.5
TSH	(ng/ml)	6.48 ± 5.27	5.22 ± 0.10	7.29 ± 4.19	5.83 ± 3.91
		Subgroup A (n=5)			
T ₃	(ng/dl)	106.1 ± 19.0	111.5 ± 12.3	112.2 ± 18.1	114.3 ± 14.0
T ₄	(µg/dl)	6.7 ± 0.9	6.8 ± 0.6	6.4 ± 1.2	6.6 ± 1.4
TSH	(ng/ml)	10.31 ± 4.78	6.40 ± 4.25	5.81 ± 2.07	7.52 ± 4.04
		Subgroup B (n=5)			
T ₃	(ng/dl)	126.2 ± 17.0	127.8 ± 11.9	124.2 ± 14.0	125.2 ± 11.3
T ₄	(µg/dl)	7.8 ± 0.9	8.6 ± 2.1	7.0 ± 1.6	8.3 ± 1.1
TSH	(ng/ml)	2.64 ± 1.72	4.03 ± 2.28	*8.77 ± 5.45	4.15 ± 3.32
Females		Combined Subgroups (n=10)			
T ₃	(ng/dl)	129.1 ± 16.6	108.8 ± 19.7	114.8 ± 25.2	121.1 ± 26.9
T ₄	(µg/dl)	5.0 ± 0.9	4.4 ± 0.8	4.3 ± 1.6	4.6 ± 1.2
TSH	(ng/ml)	2.67 ± 1.70	4.40 ± 2.95	4.02 ± 1.97	3.22 ± 2.56
		Subgroup A (n=5)			
T ₃	(ng/dl)	134.4 ± 18.4	99.3 ± 18.0	107.9 ± 21.6	114.2 ± 36.0
T ₄	(µg/dl)	4.7 ± 0.7	4.2 ± 0.6	3.8 ± 1.3	3.8 ± 0.6
TSH	(ng/ml)	3.55 ± 2.09	4.66 ± 2.74	3.98 ± 2.08	3.73 ± 2.79
		Subgroup B (n=5)			
T ₃	(ng/dl)	123.8 ± 14.6	118.4 ± 18.0	121.7 ± 29.0	128.0 ± 14.8
T ₄	(µg/dl)	5.2 ± 1.1	4.5 ± 1.0	4.9 ± 1.9	5.3 ± 1.3
TSH	(ng/ml)	1.78 ± 0.47	4.15 ± 3.46	4.07 ± 2.09	3.70 ± 2.52

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

15. The TSH values were variable with the vehicle values of the two Subgroups being nearly four-fold different in the males and two-fold in the females. In the male Subgroup B, the low vehicle value resulted

in statistical significance in the mid-dose group, which had the lowest value among the vehicle and the treated groups in Subgroup A. The coefficients of variation for the TSH values were often 50 or more.

Laboratory 12 – Genistein

16. **Methods:** No description of the methods used for thyroid hormone analyses was found in report.

17. **Results:** The means and standard deviations for thyroid hormones from the laboratory 12 genistein studies are shown in Table 4. The TSH values were significantly increased in females at the high dose. No other changes were evident.

Table 4. Thyroid Hormone Results for Genistein in the Laboratory 12 Study

Genistein Dose (mg/kg/day)		Control 0	Low 120	Middle 400	High 1000
Males		Combined Subgroups (n=10)			
T ₃	(nmol/l)	1.52 ± 0.35 ⁹	1.44 ± 0.19 ⁹	1.36 ± 0.20 ⁹	1.51 ± 0.17
T ₄	(nmol/l)	64.3 ± 8.5 ⁹	63.8 ± 15.2 ⁹	65.6 ± 10.1 ⁹	64.9 ± 6.8
TSH	(ng/ml)	0.11 ± 0.06 ⁹	0.13 ± 0.10 ⁹	0.07 ± 0.03 ⁸	0.14 ± 0.07 ⁹
		Subgroup A (n=5)			
T ₃	(nmol/l)	1.50 ± 0.31	1.36 ± 0.19	1.42 ± 0.19	1.60 ± 0.12
T ₄	(nmol/l)	67.7 ± 5.5	63.1 ± 15.5	62.3 ± 11.1	64.9 ± 7.5
TSH	(ng/ml)	0.10 ± 0.05	0.12 ± 0.07	0.08 ± 0.04 ⁴	0.17 ± 0.06
		Subgroup B (n=5)			
T ₃	(nmol/l)	1.55 ± 0.44 ⁴	1.55 ± 0.13 ⁴	1.28 ± 0.21 ⁴	1.42 ± 0.16
T ₄	(nmol/l)	60.0 ± 10.3 ⁴	64.7 ± 17.0 ⁴	69.6 ± 8.4 ⁴	62.8 ± 6.0
TSH	(ng/ml)	0.12 ± 0.08 ⁴	0.15 ± 0.14 ⁴	0.07 ± 0.03 ⁴	0.10 ± 0.05 ⁴
Females		Combined Subgroups (n=10)			
T ₃	(nmol/l)	1.30 ± 0.20	A	1.39 ± 0.20 ⁹	1.38 ± 0.19 ⁸
T ₄	(nmol/l)	54.4 ± 16.2	A	57.7 ± 14.9 ⁸	67.4 ± 11.4 ⁸
TSH	(ng/ml)	0.06 ± 0.04	A	0.11 ± 0.08 ⁸	**0.16 ± 0.06 ⁸
		Subgroup A (n=5)			
T ₃	(nmol/l)	1.36 ± 0.23	1.45 ± 0.13 ⁴	1.35 ± 0.19 ⁴	1.36 ± 0.24
T ₄	(nmol/l)	59.7 ± 16.6	67.9 ± 9.8 ⁴	50.5 ± 13.0 ³	66.9 ± 14.6
TSH	(ng/ml)	0.05 ± 0.03	0.10 ± 0.06 ³	*0.16 ± 0.13 ³	*0.17 ± 0.06
		Subgroup B (n=5)			
T ₃	(nmol/l)	1.24 ± 0.17	NA	1.42 ± 0.22	1.40 ± 0.10 ³
T ₄	(nmol/l)	49.0 ± 15.8	NA	62.0 ± 15.7	68.1 ± 5.1 ³
TSH	(ng/ml)	0.07 ± 0.04	NA	0.09 ± 0.05	0.14 ± 0.07 ³

NA – Not available, samples were lost due to analytical instrument malfunction.

A – only data from one Subgroup available.

Numerical superscripts indicate the number of animals or samples in a Combined Subgroup or a Subgroup.

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

18. In the females, statistically significant increases in TSH in the combined Subgroups were seen at the mid- and the high dose in Subgroup A. Absolute TSH values were increased at the same doses in Subgroup B, but did not achieve statistical significance.

Summary – Genistein

19. None of the statistically significant findings in one study were replicated in the other. For example, there was no trend to increased female absolute TSH values in the laboratory 4 study compared to statistical significance at the high dose in the laboratory 12 study, taking into account the potentially spurious low control value in the female Subgroup B.

Nonylphenol

Laboratory 1 – Nonylphenol

20. **Methods:** Total triiodothyronine (T₃) nmol/l, was determined by direct, competitive radioimmunoassay, Diagnostic Products Corp. (Los Angeles, CA). Total thyroxine (T₄) nmol/l, was by determined direct, competitive radioimmunoassay, Diagnostic Systems Laboratories (Webster, TX). Thyroid stimulating hormone (TSH) µg/ml, was determined by direct, competitive radioimmunoassay, Amersham Pharmacia biotech (Freiburg, Germany).

21. **Results:** The means and standard deviations for thyroid hormones from the laboratory 1 nonylphenol studies are shown in Table 5. The T₃ values were significantly increased in males at the high dose, and the T₄ values were significantly increased in males at the mid- and high doses. No other statistically significant differences were observed in either Subgroup. There were no apparent changes or trends in the females.

Table 5. Thyroid Hormone Results for Nonylphenol in the Laboratory 1 Study

	Nonylphenol Dose (mg/kg/day)	Control 0	Low 20	Middle 80	High 200/150
Males					
Combined Subgroups (n=10)					
T ₃	(nmol/l)	1.40 ± 0.25	1.28 ± 0.22	1.51 ± 0.26	**1.73 ± 0.20
T ₄	(nmol/l)	47.33 ± 5.19	50.05 ± 7.35	**56.01 ± 6.44	**60.68 ± 7.54
TSH	(µg/l)	7.44 ± 1.96	6.73 ± 1.68	7.10 ± 0.94	6.94 ± 1.57
Subgroup A (n=5)					
T ₃	(nmol/l)	1.33 ± 0.31	1.34 ± 0.22	1.42 ± 0.21	*1.80 ± 0.21
T ₄	(nmol/l)	46.63 ± 6.47	52.91 ± 6.40	57.08 ± 5.80	59.89 ± 9.68
TSH	(µg/l)	7.00 ± 2.05	7.56 ± 1.92	6.83 ± 0.72	7.66 ± 2.00
Subgroup B (n=5)					
T ₃	(nmol/l)	1.47 ± 0.18	1.21 ± 0.22	1.60 ± 0.29	1.66 ± 0.19
T ₄	(nmol/l)	48.02 ± 4.19	47.20 ± 7.75	54.93 ± 7.54	**61.46 ± 5.72
TSH	(µg/l)	7.89 ± 1.98	5.91 ± 1.00	7.38 ± 1.14	6.21 ± 0.51
Females					
Combined Subgroups (n=10)					
T ₃	(nmol/l)	1.75 ± 0.23	1.73 ± 0.32	1.84 ± 0.38	1.74 ± 0.35
T ₄	(nmol/l)	42.01 ± 9.39	42.72 ± 10.52	42.84 ± 4.82	44.01 ± 9.98
TSH	(µg/l)	5.58 ± 1.02	6.25 ± 0.95	6.28 ± 0.94	6.66 ± 0.56
Subgroup A (n=5)					
T ₃	(nmol/l)	1.72 ± 0.29	1.79 ± 0.39	1.66 ± 0.44	1.62 ± 0.44
T ₄	(nmol/l)	41.09 ± 9.65	42.67 ± 8.23	40.22 ± 3.04	38.83 ± 8.30
TSH	(µg/l)	5.54 ± 0.73	6.54 ± 0.97	5.97 ± 1.16	6.97 ± 0.74
Subgroup B (n=5)					
T ₃	(nmol/l)	1.77 ± 0.18	1.67 ± 0.26	2.01 ± 0.24	1.83 ± 0.28
T ₄	(nmol/l)	42.94 ± 10.16	42.77 ± 13.47	45.46 ± 5.08	48.16 ± 9.95
TSH	(µg/l)	5.62 ± 1.34	5.96 ± 0.94	6.58 ± 0.63	6.41 ± 0.19

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

22. The increase in male T₃ values at the high dose in the combined Subgroups was statistically significant only in Subgroup A, while the increase in male T₄ values at the mid- and high doses in the combined Subgroups was statistically significant only at the high dose in Subgroup B. Trends in absolute numbers, however, did occur in the corresponding Subgroup in both cases.

Laboratory 6 – Nonylphenol

23. **Methods:** A description of the methods was not found in the final report of the study other than to say that the measurement was conducted by radioimmunoassay.

24. **Results:** The means and standard deviations for thyroid hormones from the laboratory 6 nonylphenol studies are shown in Table 6. There was no statistically significant difference in T₃, T₄, or TSH between the vehicle control group and any test substance group in the combined Subgroups for either sex.

Table 6. Thyroid Hormone Results for Nonylphenol in the Laboratory 6 Study

Nonylphenol Dose(mg/kg/day)		Control 0	Low 12.5	Middle 50	High 300/250
Males		Combined Subgroups (n=10)			
T ₃	(ng/dl)	70.6 ± 7.8	73.20 ± 9.1	60.8 ± 72.3	72.3 ± 14.9
T ₄	(µg/dl)	5.2 ± 0.8	5.3 ± 1.0	4.6 ± 1.0	4.7 ± 1.4
TSH	(ng/ml)	8.3 ± 2.1	8.7 ± 3.7	8.6 ± 1.7	9.3 ± 2.7
		Subgroup A (n=5)			
T ₃	(ng/dl)	72.6 ± 7.1	70.8 ± 8.6	58.0 ± 11.3	76.0 ± 11.2
T ₄	(µg/dl)	5.3 ± 0.7	4.5 ± 0.7	4.8 ± 1.4	5.6 ± 0.9
TSH	(ng/ml)	8.6 ± 2.6	8.9 ± 4.7	9.9 ± 0.9	9.7 ± 1.5
		Subgroup B (n=5)			
T ₃	(ng/dl)	68.6 ± 8.7	75.6 ± 9.9	63.0 ± 11.6	69.4 ± 18.1
T ₄	(µg/dl)	5.1 ± 0.9	6.0 ± 0.6	4.4 ± 0.6	4.1 ± 1.4
TSH	(ng/ml)	8.1 ± 1.7	8.5 ± 2.8	7.5 ± 1.3	8.9 ± 3.5
Females		Combined Subgroups (n=10)			
T ₃	(ng/dl)	75.6 ± 12.1	75.9 ± 12.2	69.9 ± 8.9	66.3 ± 8.0
T ₄	(µg/dl)	2.6 ± 0.4	2.9 ± 0.8	2.5 ± 0.6	3.4 ± 0.5
TSH	(ng/ml)	6.4 ± 1.6	5.9 ± 1.1	6.2 ± 1.8	6.8 ± 2.0
		Subgroup A (n=5)			
T ₃	(ng/dl)	80.6 ± 14.1	77.8 ± 13.5	70.4 ± 10.9	70.3 ± 9.3
T ₄	(µg/dl)	2.5 ± 0.4	2.8 ± 0.2	2.6 ± 0.5	**3.8 ± 0.3
TSH	(ng/ml)	6.7 ± 2.0	5.9 ± 1.6	7.3 ± 1.9	5.9 ± 1.7
		Subgroup B (n=5)			
T ₃	(ng/dl)	70.6 ± 8.4	74.0 ± 12.0	69.4 ± 7.6	63.2 ± 5.9
T ₄	(µg/dl)	2.6 ± 0.5	2.9 ± 1.2	2.4 ± 0.7	3.0 ± 0.4
TSH	(ng/ml)	6.1 ± 1.1	5.8 ± 0.6	5.2 ± 1.0	7.5 ± 2.2

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

25. The T₄ values in the female Subgroup A were significantly increased at the high dose, and were not replicated in Subgroup B or the combined Subgroups. The TSH values are divergent at the high dose in the individual Subgroups.

Summary – Nonylphenol

26. Neither study observed statistically significant effects in females in thyroid hormones at any dose. The findings in male T₃ and T₄ values in the laboratory 1 study were not replicated in the laboratory 6 study, where, in fact, absolute values were similar at all doses. Additionally, the laboratory 6 study had the greater high dose compared to the laboratory 1 study.

Tamoxifen**Laboratory 3 – Tamoxifen**

27. Methods: TSH, T₃ and T₄ levels were measured on plasma samples by radio-immunoassay. Specific radio-immunoassay kits were supplied by Amersham for TSH and by Johnson and Johnson for T₃ and T₄. The RIASTAR program for the counter was provided by Packard (Cambera Company, Meridien, U.S.A.).

28. Results: The means and standard deviations for thyroid hormones from the laboratory 3 studies are shown in Table 7. The T₄ values were significantly increased in females at the high dose. TSH values were variable with large coefficients of variation in the male dose groups.

Table 7. Thyroid Hormone Results for Tamoxifen in the Laboratory 3 Study

	Tamoxifen Dose (µg/kg/day)	Control 0	Low 5	Middle 30	High 200
Males					
Combined Subgroups (n=10)					
T ₃	(ng/ml)	0.35 ± 0.16	0.28 ± 0.12	0.25 ± 0.07	0.36 ± 0.07 ⁹
T ₄	(ng/ml)	25.59 ± 4.24	22.13 ± 4.86	22.80 ± 4.58	28.40 ± 3.44 ⁹
TSH	(ng/ml)	3.53 ± 1.83	4.50 ± 2.41	5.30 ± 2.77	2.96 ± 1.67 ⁹
Subgroup A (n=5)					
T ₃	(ng/ml)	0.35 ± 0.21	0.29 ± 0.15	0.25 ± 0.09	0.34 ± 0.04 ⁴
T ₄	(ng/ml)	26.14 ± 6.06	20.65 ± 3.78	20.80 ± 4.95	27.19 ± 2.31 ⁴
TSH	(ng/ml)	4.29 ± 2.07	4.50 ± 2.71	4.22 ± 1.37	2.86 ± 1.96 ⁴
Subgroup B (n=5)					
T ₃	(ng/ml)	0.35 ± 0.11	0.27 ± 0.11	0.26 ± 0.05	0.37 ± 0.09
T ₄	(ng/ml)	25.04 ± 1.71	23.61 ± 5.78	24.81 ± 3.56	29.37 ± 4.13
TSH	(ng/ml)	2.76 ± 1.34	4.50 ± 2.39	6.39 ± 3.33	3.04 ± 1.64
Females					
Combined Subgroups (n=10)					
T ₃	(ng/ml)	0.44 ± 0.13 ⁹	0.40 ± 0.09 ⁹	0.46 ± 0.07 ⁹	0.52 ± 0.17 ⁷
T ₄	(ng/ml)	16.93 ± 7.69 ⁹	16.87 ± 3.23 ⁹	22.81 ± 4.30 ⁹	**32.01 ± 7.27 ⁸
TSH	(ng/ml)	4.51 ± 2.34 ⁸	3.17 ± 1.52 ⁹	4.31 ± 1.73 ⁹	2.47 ± 1.35 ⁷
Subgroup A (n=5)					
T ₃	(ng/ml)	0.45 ± 0.07 ⁴	0.41 ± 0.10	0.46 ± 0.05	0.50 ± 0.16 ⁴
T ₄	(ng/ml)	13.92 ± 3.71 ⁴	17.89 ± 4.02	*24.27 ± 5.00	**28.71 ± 7.38 ⁴
TSH	(ng/ml)	4.10 ± 2.77 ⁴	3.17 ± 1.87	4.39 ± 2.06	3.32 ± 1.18 ⁴
Subgroup B (n=5)					
T ₃	(ng/dl)	0.43 ± 0.17	0.38 ± 0.08 ⁴	0.46 ± 0.09 ⁴	0.56 ± 0.20 ³
T ₄	(ng/ml)	19.35 ± 9.56	15.58 ± 1.54 ⁴	21.63 ± 3.79 ⁴	35.31 ± 6.30 ⁴
TSH	(ng/ml)	4.93 ± 2.15	3.17 ± 1.24 ⁴	4.25 ± 1.67 ⁴	*1.35 ± 0.24 ³

*, **, Significantly different from control (p<0.05 and p<0.01, respectively). Values were expressed as mean ± SD. Superscript indicates number of animals, if not 10 for combined Subgroups or 5 for individual Subgroups.

29. The significant increase in female T₄ values seen in the combined Subgroups at the high dose was also observed in Subgroup A at both mid- and the high doses, but not in Subgroup B. However, absolute values approximately doubled in both Subgroups. Simultaneously, the TSH values were significantly decreased in female Subgroup B at the high dose (about 75% in absolute terms), but the values were only modestly lower in Subgroup A.

Laboratory 10 – Tamoxifen

30. Methods: No methods for the thyroid hormones were found in the final report from this laboratory.

31. Results: The means and standard deviations for thyroid hormones from the laboratory 10 tamoxifen studies are shown in Table 8. The T₃ values were significantly increased in males at the high dose, and the T₄ values were significantly increased in females at the mid- and high doses.

Table 8. Thyroid Hormone Results for Tamoxifen in the Laboratory 10 Study

	Tamoxifen Dose (µg/kg/day)	Control 0	Low 5	Middle 30	High 200
Males		Group (n=6)			
T ₃	(ng/dl)	110.40 ± 3.88 ^{a)}	99.77 ± 13.50	114.43 ± 7.33	*119.94 ± 5.09
T ₄	(ng/dl)	4.01 ± 0.40	3.94 ± 0.60	4.39 ± 0.12	4.17 ± 0.85
TSH	(µIU/ml)	0.02 ± 0.02	0.01 ± 0.00	0.02 ± 0.02	0.03 ± 0.02
Females		Group (n=6)			
T ₃	(ng/dl)	124.70 ± 8.06	111.80 ± 9.21	117.09 ± 8.91	113.76 ± 14.84
T ₄	(ng/dl)	2.55 ± 0.22	2.45 ± 0.85	*3.83 ± 0.65	*5.26 ± 0.75
TSH	(µIU/ml)	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.00

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

32. This laboratory used only a total of six animals and did not perform the Subgroup experiments. The CVs are among the lowest for the T₃ and T₄ values in the updated TG407 studies. The TSH values in contrast appear to have large CVs, but this may be impacted by having only two significant figures and the resulting rounding of the numbers.

Summary – Tamoxifen

33. Both studies observed a significant increase in female T₄ values at the high dose. The significant increase in male T₃ values at the high dose in the laboratory 10 study was not reproduced in the laboratory 3 study. When the antiestrogen tamoxifen results are compared with the EE studies, laboratory 3 also observed an increase in T₄ values, making it difficult to interpret the results. Compared to the aromatase inhibitor CGS 18302B studies, one laboratory there had a significant increase in T₄ values, but the other did not.

CGS 18320B**Laboratory 8 – CGS 18320B**

34. Methods: Animals were not fasted prior to killing. Under ether anaesthesia, blood was obtained from abdominal aorta using untreated syringes. A part of the blood samples was heparinised, plasma samples obtained by centrifugation, and samples for hormone analyses were stored at -80°C until use. For the determination of treatment effects on endocrine-mediated functions, serum levels of the thyroid hormones total tri-iodothyronine (T₃) (Coat-A-Count[®] Total T3, Diagnostic Products Corporation, CA, USA) and total thyroxine (T₄) (Coat-A-Count[®] Total T4, Diagnostic Products Corporation), and thyroid stimulating hormone (TSH) (Rat Thyroid Stimulating Hormone (rTSH) [¹²⁵I] Assay System, Amersham) were measured by means of radioimmunoassay.

35. Results: The means and standard deviations for thyroid hormones from the laboratory 8 CGS 18320B studies are shown in Table 9. The T₃ values were significantly decreased in males at all doses and in females at the high dose. The T₄ values were significantly decreased in males at all doses, but absolute values were actually higher in females at all doses. Absolute TSH values surprisingly decreased (up to

about 30%) in a dose related manner in the males. This was even though both T₃ and T₄ values decreased, which would lead to a simplistic expectation of an increased TSH response in compensation, unless the thyroid is being down-regulated at the hypothalamic level. However, absolute values rose slightly in females.

Table 9. Thyroid Hormone Results for CGS 18320B in the Laboratory 8 Study

CGS 18320B		Control	Low	Middle	High
Dose (mg/kg/day)		0	0.3	3	30
Males		Combined Subgroups (n=10)			
T ₃	(ng/ml)	0.753 ± 0.047	**0.632 ± 0.100	**0.640 ± 0.075	**0.620 ± 0.088
T ₄	(ng/ml)	44.0 ± 5.1	**31.5 ± 6.5	**32.6 ± 4.2	**28.1 ± 6.6
TSH	(ng/ml)	11.18 ± 4.98	9.35 ± 3.95	8.76 ± 4.56	7.49 ± 2.44
		Subgroup A (n=5)			
T ₃	(ng/ml)	0.770 ± 0.055	**0.588 ± 0.101	0.661 ± 0.092	0.676 ± 0.029
T ₄	(ng/ml)	46.9 ± 5.1	**31.6 ± 6.5	**32.2 ± 3.8	**29.7 ± 1.9
TSH	(ng/ml)	12.27 ± 6.02	10.36 ± 5.08	6.04 ± 0.64	6.52 ± 2.16
		Subgroup B (n=5)			
T ₃	(ng/ml)	0.736 ± 0.036	0.677 ± 0.084	*0.618 ± 0.055	**0.564 ± 0.095
T ₄	(ng/ml)	41.1 ± 3.4	31.4 ± 7.3	32.9 ± 5.0	*26.5 ± 9.6
TSH	(ng/ml)	10.10 ± 4.08	8.33 ± 2.59	11.48 ± 5.27	8.46 ± 2.59
Females		Combined Subgroups (n=10)			
T ₃	(ng/ml)	0.656 ± 0.072	0.556 ± 0.115	0.589 ± 0.106	**0.501 ± 0.109
T ₄	(ng/ml)	20.4 ± 6.1	25.9 ± 7.1	26.3 ± 6.1	25.6 ± 8.0
TSH	(ng/ml)	5.77 ± 0.81	**7.80 ± 1.71	5.89 ± 1.00	6.55 ± 0.94
		Subgroup A (n=5)			
T ₃	(ng/ml)	0.704 ± 0.059	**0.514 ± 0.097	0.605 ± 0.054	*0.548 ± 0.085
T ₄	(ng/ml)	21.2 ± 8.2	28.5 ± 7.3	27.5 ± 7.4	27.0 ± 8.1
TSH	(ng/ml)	5.99 ± 0.47	**8.38 ± 1.47	6.10 ± 1.26	6.84 ± 0.91
		Subgroup B (n=5)			
T ₃	(nmol/l)	0.608 ± 0.049	0.597 ± 0.128	0.572 ± 0.147	0.454 ± 0.117
T ₄	(nmol/l)	19.6 ± 3.8	23.3 ± 6.5	25.0 ± 5.1	24.1 ± 8.5
TSH	(ng/ml)	5.54 ± 1.06	7.21 ± 1.88	5.68 ± 0.74	6.27 ± 0.99

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

36. In males, the significant decreases of T₃ values at all doses in the combined Subgroups were seen only at the low dose in Subgroup A and the mid- and high doses in Subgroup B. The other absolute values were decreased, but did not achieve statistical significance. The significant decreases of T₄ values at all doses in the combined Subgroups were seen at all doses in Subgroup A, but only the high dose in Subgroup B. In females, a significant decrease of T₃ values at the high dose was observed in Subgroup A, but not Subgroup B. Additionally, a significant decrease of T₃ values was observed at the low dose in Subgroup A only. TSH values were significantly increased at the low dose in the female Subgroup A, but not Subgroup B.

Laboratory 13 – CGS 18320B

37. Methods: The methods used by this laboratory are described in the thyroxine section.

38. Results: The means and standard deviations for thyroid hormones from the laboratory 13 CGS 18320B studies are shown in Table 10. The T₃ values were significantly increased in both sexes at the high dose. The T₄ values were significantly increased in females at all doses, and absolute values were somewhat lower in males at all doses.

Table 10. Thyroid Hormone Results for CGS 18320B in the Laboratory 13 Study

CGS 18320B Dose (mg/kg/day)		Control 0	Low 0.3	Middle 3	High 30
Males		Group (n=10)			
T ₃	(nmol/l)	1.96 ± 0.36	1.94 ± 0.43	2.50 ± 0.64	**2.84 ± 0.81
T ₄	(nmol/l)	93.1 ± 0.36	83.5 ± 8.1	85.9 ± 13.3	85.5 ± 16.5
TSH	(ng/ml)	22.31 ± 6.01	22.68 ± 4.20	20.57 ± 5.46	22.77 ± 4.54
Females		Group (n=10)			
T ₃	(nmol/l)	2.78 ± 0.90	3.39 ± 0.64	3.50 ± 0.87	*3.85 ± 0.85
T ₄	(nmol/l)	69.2 ± 10.45	**104.4 ± 9.72	**106.0 ± 14.62	**109.6 ± 18.09
TSH	(ng/ml)	29.02 ± 7.73	33.22 ± 9.28	29.83 ± 10.60	40.27 ± 11.46

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

39. This laboratory did not divide its experiment into Subgroups, but did have an overall group size of 10.

Summary – CGS 18320B

40. The results of the two studies were contradictory. T₃ values significantly decreased in both sexes in the laboratory 8 study and increased in the laboratory 13 study. Similarly, the T₄ values significantly decreased in males in the laboratory 8 study and significantly increased in females in the laboratory 13 study. The male TSH absolute values declined in the laboratory 8 study and were stable in the laboratory 13 study. The female TSH absolute values were higher at the lowest dose in the laboratory 8 study, but this was not seen in the laboratory 13 study.

Methyl Testosterone

Laboratory 3 – Methyl Testosterone

41. Methods: Same methods were used as those with laboratory 3 tamoxifen study.

42. Results: The means and standard deviations for thyroid hormones from the laboratory 3 methyl testosterone studies are shown in Table 11. The TSH values were significantly increased in males at all doses with the absolute values more than doubling at the high dose. In females, TSH values were significantly increased at the mid- and high doses with the absolute values more than tripling at the high dose. There was a significant increase in T₄ values in males at the high dose and, in females, at the mid- and high doses. In contrast, the T₃ values were significantly decreased in females at all doses. This implies a strong methyl testosterone effect, potentially on the metabolism of the T₃ and T₄ that would require strong TSH compensation to stimulate thyroid hormone production. There were modest but not significant increases in the absolute and relative thyroid weights in both sexes at the high dose, and there was modest evidence of thyroid hypertrophy in some animals in males at the high dose and females at the mid- and high dose upon histological examination. These overall findings are then consistent.

Table 11. Thyroid Hormone Results for Methyl Testosterone in the Laboratory 3 Study

Methyl Testosterone Dose (mg/kg/day)		Control 0	Low 10	Middle 40	High 200
Males		Combined Subgroups (n=10)			
T ₃	(ng/ml)	0.31 ± 0.09	0.32 ± 0.11	0.36 ± 0.11	0.34 ± 0.13
T ₄	(ng/ml)	24.74 ± 4.22	24.60 ± 4.65	28.26 ± 4.42	*30.52 ± 3.90
TSH	(ng/ml)	4.92 ± 2.50	*8.58 ± 2.92	*8.81 ± 3.72	**11.97 ± 3.18
		Subgroup A (n=5)			
T ₃	(ng/ml)	0.30 ± 0.03	0.33 ± 0.08	0.33 ± 0.07	0.32 ± 0.14
T ₄	(ng/ml)	22.50 ± 4.31	25.66 ± 4.91	29.14 ± 3.97	29.22 ± 4.22
TSH	(ng/ml)	3.75 ± 1.33	*8.49 ± 3.21	*7.97 ± 2.78	**13.00 ± 2.06
		Subgroup B (n=5)			
T ₃	(ng/ml)	0.32 ± 0.13	0.32 ± 0.14	0.39 ± 0.15	0.36 ± 0.13
T ₄	(ng/ml)	26.99 ± 2.97	23.54 ± 4.67	27.37 ± 5.11	31.83 ± 3.48
TSH	(ng/ml)	6.08 ± 2.98	8.68 ± 2.97	9.65 ± 4.65	10.93 ± 3.97
Methyl Testosterone Dose (mg/kg/day)		Control 0	Low 10	Middle 100	High 600
Females		Combined Subgroups (n=10)			
T ₃	(ng/ml)	0.49 ± 0.15 ⁹	*0.35 ± 0.10	*0.34 ± 0.10 ⁹	**0.28 ± 0.06
T ₄	(ng/ml)	21.59 ± 3.66 ⁹	23.56 ± 3.54	**28.58 ± 3.74 ⁹	**27.54 ± 3.23
TSH	(ng/ml)	4.42 ± 1.96 ⁹	4.13 ± 1.79	**12.19 ± 3.06 ⁹	**17.99 ± 5.84
		Subgroup A (n=5)			
T ₃	(ng/ml)	0.46 ± 0.17	0.31 ± 0.09	0.35 ± 0.12	0.30 ± 0.03
T ₄	(ng/ml)	21.27 ± 4.62	21.96 ± 2.58	*28.67 ± 3.17	*27.76 ± 4.49
TSH	(ng/ml)	4.64 ± 2.30	3.40 ± 1.85	*13.13 ± 3.24	**18.75 ± 6.70
		Subgroup B (n=5)			
T ₃	(ng/dl)	0.54 ± 0.12 ⁴	0.39 ± 0.10	*0.33 ± 0.10 ⁴	**0.27 ± 0.09
T ₄	(ng/ml)	21.98 ± 2.63 ⁴	25.15 ± 3.89	*28.47 ± 4.89 ⁴	27.33 ± 1.78
TSH	(ng/ml)	4.13 ± 1.72 ⁴	4.87 ± 1.55	*11.01 ± 2.76 ⁴	**17.22 ± 5.52

*, **, Significantly different from control (p<0.05 and p<0.01, respectively). Values were expressed as mean ± SD. Superscript indicates number of animals, if not 10 for combined Subgroups or 5 for individual Subgroups.

43. The significant increase in male T₄ values at the high dose in the combined Subgroups was not achieved in either individual Subgroup, although absolute trends are consistent in both. The significant increases in male TSH at all doses in the combined Subgroups were also observed in Subgroup A at all doses, but not Subgroup B. Here, the role of the different control mean values in Subgroups A and B need to be considered. The significant decrease in female T₃ values in the combined Subgroups were seen at the mid- and high doses in Subgroup B, but at no dose in Subgroup A. Significant increases in female T₄ at the mid- and high doses in the combined Subgroups were seen at the mid and high dose in Subgroup A, at the mid-dose in Subgroup B, but not at the high dose in Subgroup B. The increase in female TSH values at the mid- and high doses was also observed in both individual Subgroups.

Laboratory 12 – Methyl Testosterone

44. Methods: No description of the methods used for thyroid hormone analyses were found in the final report.

45. Results: The means and standard deviations for thyroid hormones from the laboratory 12 methyl testosterone studies are shown in Table 12. The TSH values were significantly increased in both sexes at the high dose. There was a significant increase in T₄ values in females at all doses, but no similar change was observed in males. In support, both male and female thyroid absolute and relative weights were increased in the laboratory 12 study. Thyroid follicular cell hypertrophy was also observed in males at the mid- and high doses and in the females at the high dose, supporting the overall observations.

Table 12. Thyroid Hormone Results for Methyl Testosterone in the Laboratory 12 Study

Methyl Testosterone Dose (mg/kg/day)		Control 0	Low 10	Middle 40	High 200
Males					
Combined Subgroups (n=10)					
T ₃	(nmol/l)	1.65 ± 0.30	1.46 ± 0.34	1.67 ± 0.18	1.59 ± 0.19
T ₄	(nmol/l)	67.8 ± 6.9	70.6 ± 16.7	73.9 ± 7.8	72.0 ± 11.0
TSH	(ng/ml)	0.22 ± 0.11	0.13 ± 0.08	0.24 ± 0.16	*0.42 ± 0.24
Subgroup A (n=5)					
T ₃	(nmol/l)	1.52 ± 0.36	1.50 ± 0.34	1.80 ± 0.14	1.62 ± 0.20
T ₄	(nmol/l)	64.3 ± 4.0	73.9 ± 20.1	75.7 ± 8.7	72.6 ± 6.6
TSH	(ng/ml)	0.23 ± 0.14	0.14 ± 0.09	0.30 ± 0.12	0.35 ± 0.25
Subgroup B (n=5)					
T ₃	(nmol/l)	1.78 ± 0.18	*1.40 ± 0.39	1.54 ± 0.11	1.56 ± 0.18
T ₄	(nmol/l)	71.3 ± 7.8	66.4 ± 12.8	72.2 ± 7.2	71.5 ± 15.1
TSH	(ng/ml)	0.22 ± 0.09	0.13 ± 0.08	0.19 ± 0.19	*0.50 ± 0.23
Methyl Testosterone Dose (mg/kg/day)		Control 0	Low 10	Middle 100	High 600
Females					
Combined Subgroups (n=10)					
T ₃	(nmol/l)	1.54 ± 0.26	1.58 ± 0.20	1.46 ± 0.16	1.61 ± 0.26
T ₄	(nmol/l)	51.5 ± 10.0	*63.7 ± 9.4	**76.2 ± 13.5	**71.6 ± 11.6
TSH	(ng/ml)	0.08 ± 0.04	0.12 ± 0.04	0.12 ± 0.06	*0.51 ± 0.48
Subgroup A (n=5)					
T ₃	(nmol/l)	1.70 ± 0.25	1.50 ± 0.21	1.44 ± 0.11	1.54 ± 0.21
T ₄	(nmol/l)	55.3 ± 7.3	65.0 ± 10.2	**80.6 ± 15.1	*75.1 ± 10.1
TSH	(ng/ml)	0.08 ± 0.05	0.11 ± 0.03	0.14 ± 0.07	**0.30 ± 0.21
Subgroup B (n=5)					
T ₃	(nmol/l)	1.38 ± 0.15	1.66 ± 0.18	1.48 ± 0.22	*1.68 ± 0.30
T ₄	(nmol/l)	47.7 ± 11.6	62.4 ± 9.5	*71.9 ± 11.7	*68.1 ± 13.0
TSH	(ng/ml)	0.08 ± 0.04	0.14 ± 0.05	0.09 ± 0.06	**0.76 ± 0.64

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

46. The significant increase in TSH in both sexes at the high dose was also observed in males at the high dose in Subgroup B, but not Subgroup A, and in females in both individual Subgroups. The significant increases for female T₄ values were observed in both individual Subgroups at the mid- and the high doses, but in neither Subgroup at the low dose.

Summary – Methyl Testosterone

47. The studies reproduced the significant increase of the T₄ values in females at the mid- and high doses and the significant increase of TSH in both sexes at the high dose. The absolute TSH mean values increase about 2- to 3-fold in the laboratory 3 study at the high dose depending upon sex, and about 2- to 5-fold in the laboratory 12 study. However, the laboratory 3 significant increase of TSH in males at the low and mid-doses, significant increase in T₄ values in males at the high dose, and the significant decreases of T₃ in females at all doses were not observed in the laboratory 12 study. Thyroid weights and histology were consistent with increased TSH serum levels and stimulation in both studies.

Flutamide**Laboratory 2 – Flutamide**

48. Methods were not described in this report. The apparent methods page in the final report of this study (page 162) was blank.

49. Results: The means and standard deviations for thyroid hormones from the laboratory 2 flutamide studies are shown in Table 13. The T₃ and the T₄ values were significantly decreased in males at the high dose. No significant effects in the females were observed.

Table 13. Thyroid Hormone Results for Flutamide in the Laboratory 2 Study

	Flutamide Dose (mg/kg/day)	Control 0	Low 1	Middle 10	High 100
Males Combined Subgroups (n=10)					
T ₃	(nmol/l)	1.87 ± 0.091	1.86 ± 0.0160	1.88 ± 0.144	*1.66 ± 0.100
T ₄	(nmol/l)	49 ± 5.5	54 ± 5.0	54 ± 6.9	**39 ± 5.6
TSH	(µg/l)	2.93 ± 2.83	3.23 ± 2.35	3.00 ± 1.55	3.64 ± 3.36
Subgroup A (n=5)					
T ₃	(nmol/l)	1.89 ± 0.050	1.87 ± 0.203	1.87 ± 0.178	1.66 ± 0.104
T ₄	(nmol/l)	52 ± 4.3	51 ± 4.2	57 ± 6.3	*38 ± 6.7
TSH	(µg/l)	1.64 ± 0.79	2.44 ± 0.86	3.56 ± 1.95	5.24 ± 4.30
Subgroup B (n=5)					
T ₃	(nmol/l)	1.85 ± 0.124	1.85 ± 0.127	1.89 ± 0.120	1.67 ± 0.107
T ₄	(nmol/l)	46 ± 5.3	56 ± 5.2	51 ± 6.7	38 ± 5.0
TSH	(µg/l)	4.22 ± 3.63	4.02 ± 3.19	2.44 ± 0.93	2.04 ± 0.73
Females Combined Subgroups (n=10)					
T ₃	(nmol/l)	1.95 ± 0.179	1.93 ± 0.158	1.96 ± 0.160	1.91 ± 0.261
T ₄	(nmol/l)	36 ± 6.4	35 ± 9.5	35 ± 5.8	32 ± 5.7
TSH	(µg/l)	1.77 ± 0.69	2.32 ± 1.36	1.64 ± 0.64	2.26 ± 0.90
Subgroup A (n=5)					
T ₃	(nmol/l)	1.86 ± 0.110	1.85 ± 0.084	1.89 ± 0.125	1.87 ± 0.201
T ₄	(nmol/l)	38 ± 6.4	30 ± 9.8	33 ± 5.4	30 ± 4.7
TSH	(µg/l)	1.62 ± 0.82	1.66 ± 0.46	1.30 ± 0.49	2.50 ± 1.11
Subgroup B (n=5)					
T ₃	(nmol/l)	2.04 ± 0.195	2.02 ± 0.172	2.04 ± 0.168	1.98 ± 0.323
T ₄	(nmol/l)	35 ± 7.0	41 ± 5.3	37 ± 6.1	35 ± 5.6
TSH	(µg/l)	1.92 ± 0.58	2.98 ± 1.69	1.98 ± 0.64	2.02 ± 0.67

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

50. The significant decrease in male T₃ values was not observed in either Subgroup. The significant decrease in T₄ was observed in Subgroup A, but not Subgroup B. TSH trends were opposite between the Subgroups, increasing with dose in Subgroup A to over twice the vehicle control value at the high dose, and decreasing in Subgroup B by one third at the high dose.

Laboratory 11 – Flutamide

51. Methods: TSH (ng/ml) was determined with Amersham Pharmacia Biotech LTD. Kit – rat TSH [125I] assay system, double antibody RIA method. T₃ (ng/dl) was determined with Diagnostic Products Corp. kit – coat-a-count canine T₃ kit, solid-phase RIA method. T₄ (µg/dl) was determined with Diagnostic Products Corp. kit – DPC Total T4 kit, solid phase RIA method.

52. **Results:** The means and standard deviations for thyroid hormones from the laboratory 11 flutamide studies are shown in Table 14. The T₃ values were significantly increased in males at the high dose, and the TSH values were significantly increased in males at the high dose. In contrast, the absolute T₄ values were lower in males at the high dose, indicating a possible lack of concordance. In females, the T₄ values achieved were significantly decreased in Subgroup B at the high dose, while absolute values of T₃ and TSH were increased, indicating a possible lack of concordance.

Table 14. Thyroid Hormone Results for Flutamide in the Laboratory 11 Study

Flutamide Dose (mg/kg/day)		Control 0	Low 1	Middle 10	High 100
Males		Combined Subgroups (n=10)			
T ₃	(ng/dl)	110.8 ± 11.1	120.3 ± 18.9	121.8 ± 14.1	*128.3 ± 10.9
T ₄	(µg/dl)	4.57 ± 0.49	4.66 ± 0.96	4.62 ± 0.71	4.22 ± 0.67
TSH	(ng/ml)	7.98 ± 1.78	10.61 ± 4.76	9.23 ± 1.93	*10.34 ± 2.32
		Subgroup A (n=5)			
T ₃	(ng/dl)	108.0 ± 9.3	124.5 ± 27.4	117.5 ± 18.1	125.6 ± 14.5
T ₄	(µg/dl)	4.61 ± 0.52	5.05 ± 1.06	4.65 ± 0.85	4.20 ± 0.89
TSH	(ng/ml)	8.19 ± 2.28	8.74 ± 4.73	8.58 ± 1.81	10.46 ± 3.27
		Subgroup B (n=5)			
T ₃	(ng/dl)	113.6 ± 13.0	116.0 ± 3.1	126.0 ± 8.7	*130.9 ± 6.3
T ₄	(µg/dl)	4.52 ± 0.52	4.26 ± 0.76	4.40 ± 0.61	4.24 ± 0.47
TSH	(ng/ml)	7.76 ± 1.18	12.48 ± 4.46	9.88 ± 2.02	*10.22 ± 1.19
Females		Combined Subgroups (n=10)			
T ₃	(ng/dl)	110.9 ± 18.2	106.9 ± 22.0	111.4 ± 18.2	122.0 ± 17.7
T ₄	(µg/dl)	3.14 ± 1.02	2.56 ± 0.70	2.67 ± 0.69	2.24 ± 1.05
TSH	(ng/ml)	7.12 ± 1.63	6.96 ± 1.47	6.44 ± 0.85	8.62 ± 3.35
		Subgroup A (n=5)			
T ₃	(ng/dl)	111.8 ± 18.2	118.4 ± 23.7	112.0 ± 17.7	127.6 ± 13.6
T ₄	(µg/dl)	2.70 ± 0.56	2.67 ± 0.61	2.60 ± 0.68	2.54 ± 1.40
TSH	(ng/ml)	6.63 ± 0.96	6.50 ± 1.65	6.60 ± 1.11	7.15 ± 1.64
		Subgroup B (n=5)			
T ₃	(ng/dl)	109.9 ± 20.3	95.4 ± 13.9	110.8 ± 20.7	116.4 ± 21.0
T ₄	(µg/dl)	3.58 ± 1.25	2.46 ± 0.83	2.78 ± 0.78	*1.95 ± 0.54
TSH	(ng/ml)	7.60 ± 2.12	7.43 ± 1.27	6.28 ± 0.58	10.09 ± 4.14

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

53. In the males, significant increases in T₃ and TSH values were seen in Subgroup B, but not in Subgroup A for either value. In the females, Subgroup B had a statistically significant decrease in T₄ values, which was not seen in either the combined Subgroups or Subgroup A. In fact, there was no apparent dose related trend in the absolute values in Subgroup A.

Summary – Flutamide

54. In males, the T₃ value observations were in conflict, significantly decreasing at the high dose in the laboratory 2 study and significantly increasing in the laboratory 11 study. The significant decrease in male T₄ in the laboratory 2 study and increase in male TSH in the laboratory 11 study were not observed in the other study. The two studies were in agreement in the lack of any statistically significant effect in females when the combined Subgroups are compared.

p,p'-DDELaboratory 6 –*p,p'*-DDE

55. Methods: The methods were not described in the final report other than to indicate that radioimmunoassays were used to measure the hormones.

56. Results: The means and standard deviations for thyroid hormones from the laboratory 6 *p,p'*-DDE studies are shown in Table 15. The TSH values were significantly increased in males at all doses. Although absolute values were higher, only the mid-dose was significantly increased in females. The T₃ values were significantly increased in males at the high dose, while the T₄ values were significantly decreased in males at the high dose. Despite mortalities reducing the combined Subgroups to a total of five animals, the male T₃, T₄, and TSH values still all achieved significance at the high dose. The female combined Subgroup size was reduced to seven, and no statistical significance was observed at the high dose. The greatly increased liver weights suggests increased metabolism and compensation by increased TSH levels. This is supported by a statistically significant increase in relative thyroid weights in males and an increased absolute trend in females.

Table 15. Thyroid Hormone Results for *p,p'*-DDE in the Laboratory 6 Study

<i>p,p'</i> -DDE Dose (mg/kg/day)		Control 0	Low 12.5	Middle 50	High 200/150
Males		Combined Subgroups (n=10)			
T ₃	(ng/dl)	90.3 ± 10.5	85.0 ± 7.5 ⁹	96.9 ± 15.1	**115.0 ± 19.8 ³
T ₄	(µg/dl)	5.5 ± 0.6	5.1 ± 0.8 ⁹	4.9 ± 0.9	**3.6 ± 0.6 ⁵
TSH	(ng/ml)	8.6 ± 2.4	*13.9 ± 5.5 ⁹	*13.5 ± 2.7	*18.2 ± 8.8 ⁵
		Subgroup A (n=5)			
T ₃	(ng/dl)	95.6 ± 8.7	86.6 ± 9.6	103.0 ± 17.4	122.0 ± 24.6 ³
T ₄	(µg/dl)	5.6 ± 0.8	4.7 ± 0.5	4.9 ± 0.9	*3.6 ± 0.7 ³
TSH	(ng/ml)	7.6 ± 1.5	*16.4 ± 5.0	12.5 ± 2.0	**22.2 ± 9.1 ³
		Subgroup B (n=5)			
T ₃	(ng/dl)	85.0 ± 10.0	83.0 ± 4.1 ⁴	90.8 ± 10.9	104.5 ± 0.7 ²
T ₄	(µg/dl)	5.3 ± 0.5	5.5 ± 0.9 ⁴	4.9 ± 0.9	3.6 ± 0.7 ²
TSH	(ng/ml)	9.6 ± 2.9	10.7 ± 4.7 ⁴	14.5 ± 3.1	12.2 ± 4.9 ²
<i>p,p'</i> -DDE		Control 0	Low 6.5	Middle 25	High 100
Females		Combined Subgroups (n=10)			
T ₃	(ng/dl)	84.6 ± 12.5	94.8 ± 13.1	91.2 ± 15.6	97.9 ± 14.9 ⁷
T ₄	(µg/dl)	3.5 ± 0.5	3.8 ± 1.0	3.8 ± 1.1	3.1 ± 0.9 ⁷
TSH	(ng/ml)	5.3 ± 1.3	6.1 ± 1.9	*7.5 ± 1.9	7.3 ± 2.0 ⁷
		Subgroup A (n=5)			
T ₃	(ng/dl)	92.0 ± 11.3	89.6 ± 15.5	85.5 ± 19.5	99.3 ± 15.5 ³
T ₄	(µg/dl)	3.7 ± 0.4	3.4 ± 0.5	3.8 ± 0.8	3.3 ± 0.3 ³
TSH	(ng/ml)	5.8 ± 1.0	6.4 ± 2.5	8.1 ± 2.4	8.1 ± 1.9 ³
		Subgroup B (n=5)			
T ₃	(ng/dl)	77.2 ± 9.2	*100.0 ± 8.7	*96.6 ± 9.6	96.8 ± 16.7 ⁴
T ₄	(µg/dl)	3.3 ± 0.5	4.2 ± 1.2	3.9 ± 1.5	3.0 ± 1.3 ⁴
TSH	(ng/ml)	4.9 ± 1.4	5.8 ± 1.2	6.9 ± 1.3	6.8 ± 2.1 ⁴

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).
numerical superscripts are the actual group size due to mortalities

57. The significant number of mortalities at the high dose impair the comparison of the power of the combined Subgroups and the individual Subgroups. In the males, only three animals survived in Subgroup A, where T₄ and TSH values did achieve statistical significance. Only two animals survived in Subgroup

B. In the females, three animals survived in Subgroup A and four in Subgroup B. The TSH values at the mid-dose were significantly increased in the combined Subgroups, but in neither individual Subgroup. Within the Subgroups, T₃ values were significantly increased in Subgroup B at the mid- and low doses, but the low vehicle control value may have contributed to the significance and not a treatment related effect.

Laboratory 7 – *p,p'*-DDE

58. **Methods:** T₃, T₄, and TSH were determined using the enzyme immunoassay system (Diagnostic Systems Laboratories for T₃ and T₄, Amersham Biosciences Co. for TSH).

59. **Results:** The means and standard deviations for thyroid hormones from the laboratory 7 *p,p'*-DDE studies are shown in Table 16. The T₃ values were significantly increased in males at all doses and in females at the high dose. The T₄ values were significantly decreased in males at the high dose. TSH absolute values were modestly increased.

Table 16. Thyroid Hormone Results for *p,p'*-DDE in the Laboratory 7 Study

<i>p,p'</i> -DDE Dose(mg/kg/day)		Control 0	Low 12.5	Middle 50	High 100
Combined Subgroups (n=10)					
T ₃	(ng/ml)	0.7 ± 0.1	*1.1 ± 0.3	**1.3 ± 0.2	**1.7 ± 0.4
T ₄	(ng/ml)	105.2 ± 12.1	110.7 ± 22.0	89.1 ± 9.7	**74.8 ± 9.3
TSH	(ng/ml)	25.7 ± 8.5	29.2 ± 7.5	27.0 ± 7.6	31.7 ± 4.0
Subgroup A (n=5)					
T ₃	(ng/dl)	0.6 ± 0.1	1.2 ± 0.3	1.2 ± 0.2	**1.6 ± 0.2
T ₄	(ng/dl)	103.8 ± 12.9	102.4 ± 14.8	94.2 ± 4.7	**72.5 ± 10.4
TSH	(ng/ml)	27.4 ± 12.4	27.0 ± 4.5	30.0 ± 9.4	32.1 ± 1.5
Subgroup B (n=5)					
T ₃	(ng/dl)	0.8 ± 0.0	*1.0 ± 0.1	*1.0 ± 0.2	**1.8 ± 0.5
T ₄	(ng/dl)	106.6 ± 12.5	118.9 ± 26.4	84.0 ± 11.1	*77.1 ± 8.5
TSH	(ng/ml)	23.9 ± 1.2	31.5 ± 9.7	24.1 ± 4.4	31.2 ± 5.8
<i>p,p'</i> -DDE		Control 0	Low 6.5	Middle 25	High 75
Females					
Combined Subgroups (n=10)					
T ₃	(ng/ml)	0.9 ± 0.1	0.9 ± 0.2	1.0 ± 0.1	**1.4 ± 0.5
T ₄	(ng/ml)	99.6 ± 20.3	109.5 ± 29.6	101.1 ± 20.0	75.4 ± 33.9
TSH	(ng/ml)	31.3 ± 10.3	36.3 ± 12.5	34.8 ± 5.7	36.0 ± 16.2
Subgroup A (n=5)					
T ₃	(ng/dl)	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	*1.1 ± 0.2
T ₄	(ng/dl)	90.0 ± 9.2	91.7 ± 14.8	97.4 ± 8.0	89.5 ± 34.6
TSH	(ng/ml)	25.5 ± 7.6	*37.9 ± 4.7	34.7 ± 3.7	35.8 ± 9.0
Subgroup B (n=5)					
T ₃	(ng/dl)	0.9 ± 0.1	0.9 ± 0.2	1.0 ± 0.2	*1.7 ± 0.7
T ₄	(ng/dl)	109.2 ± 24.7	127.3 ± 30.9	104.7 ± 28.4	*61.4 ± 29.9
TSH	(ng/ml)	37.1 ± 9.9	34.6 ± 18.0	34.8 ± 7.8	36.1 ± 22.5

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

60. In the males, significant increases of T₄ values at the high dose occurred in the combined Subgroups and in both individual Subgroups. Significant increases in T₃ values at all doses in the combined Subgroups, at all doses in Subgroup B, and at the high dose only in Subgroup A. In the females, significant increases in T₃ values at the high dose in the combined Subgroups and were also observed in both individual Subgroups. A significant increase in T₄ values was observed at the high dose in Subgroup B, but not in Subgroup A or the combined Subgroups.

Summary – *p,p'*-DDE

61. The significant increase in male T₃ values and significant decrease in male T₄ values at the high dose was observed in both DDE studies despite the difference in doses employed. The significant increase in male TSH values at all doses in the laboratory 6 study was not observed in the laboratory 7 study. In females, a significant increase in female T₃ values at the high dose of 75 mg/kg/d was not reproduced in the laboratory 6 study at 100 mg/kg/d, although absolute values were increased.

Propylthiouracil**Laboratory 1 – Propylthiouracil**

62. Methods: The same methods were used as described for this laboratory in the nonylphenol section.

63. Results: The means and standard deviations for thyroid hormones from the Laboratory 1 propylthiouracil studies are shown in Table 17. The TSH values were significantly increased in both sexes at the mid- and high doses with corresponding significant decreases in both T₃ values in both sexes at the high dose and T₄ values in males at the mid- and high doses and in females at all doses. The changes in absolute values were very marked. The TSH increases were approximately ten-fold in both sexes, and the T₃ and T₄ declines were over 75%.

Table 17. Thyroid Hormone Results for Propylthiouracil in the Laboratory 1 Study

Propylthiouracil Dose(mg/kg/day)		Control 0	Low 0.1	Middle 1	High 10
Males		Combined Subgroups (n=10)			
T ₃	(nmol/l)	0.98 ± 0.17	1.09 ± 0.48	1.14 ± 0.34	**0.27 ± 0.18
T ₄	(nmol/l)	49.2 ± 4.04	54.4 ± 9.80	**20.4 ± 6.13	**8.0 ± 1.24
TSH	(µg/l)	6.47 ± 2.30	7.77 ± 2.06	**30.7 ± 27.58	**58.4 ± 10.06
		Subgroup A (n=5)			
T ₃	(nmol/l)	1.01 ± 0.20	1.19 ± 0.43	1.04 ± 0.19	**0.28 ± 0.07
T ₄	(nmol/l)	48.74 ± 5.02	56.1 ± 12.60	**19.1 ± 4.01	**8.3 ± 1.65
TSH	(µg/l)	5.52 ± 0.85	*7.97 ± 2.03	**33.3 ± 34.08	**53.9 ± 8.35
		Subgroup B (n=5)			
T ₃	(nmol/l)	0.94 ± 0.15	1.00 ± 0.56	1.24 ± 0.44	**0.26 ± 0.27
T ₄	(nmol/l)	49.7 ± 3.33	52.6 ± 7.08	**21.6 ± 8.03	**7.8 ± 0.74
TSH	(µg/l)	7.43 ± 2.98	7.56 ± 2.31	*28.1 ± 23.07	**62.9 ± 10.36
Females		Combined Subgroups (n=10)			
T ₃	(nmol/l)	1.37 ± 0.38	1.25 ± 0.45	1.26 ± 0.33	**0.41 ± 0.15
T ₄	(nmol/l)	44.5 ± 9.20	*36.2 ± 5.28	**18.5 ± 6.67	**9.8 ± 1.05
TSH	(µg/l)	5.80 ± 0.85	6.70 ± 1.61	**20.6 ± 14.02	**64.2 ± 12.64
		Subgroup A (n=5)			
T ₃	(nmol/l)	1.44 ± 0.26	1.34 ± 0.37	1.17 ± 0.29	**0.46 ± 0.12
T ₄	(nmol/l)	46.5 ± 9.45	36.0 ± 4.67	**15.5 ± 3.39	**9.8 ± 0.97
TSH	(µg/l)	5.39 ± 0.91	7.04 ± 1.21	**20.8 ± 15.93	**68.6 ± 14.93
		Subgroup B (n=5)			
T ₃	(nmol/l)	1.30 ± 0.49	1.15 ± 0.55	1.35 ± 0.37	*0.37 ± 0.19
T ₄	(nmol/l)	42.6 ± 9.57	36.3 ± 6.40	*21.5 ± 8.15	**9.7 ± 1.23
TSH	(µg/l)	6.22 ± 0.62	6.36 ± 2.02	20.4 ± 13.73	**59.8 ± 9.43

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

64. In the males, the results were fully consistent at the mid- and high doses for all values between the combined Subgroups and both individual Subgroups. At the low dose, the TSH values were significantly increased in Subgroup A, but not in the combined Subgroups or Subgroup B. In the females, the T₃ and

T4 results were consistent at the mid- and high doses between the combined Subgroups and both individual Subgroups. The TSH values were significantly increased at both the mid- and the high doses in the combined Subgroups and Subgroup A, but only at the high dose in Subgroup B. At the low dose, T₄ values were significantly decreased in the combined Subgroups, but a decrease in absolute values did not achieve statistical significance in either individual Subgroup.

Laboratory 10 – Propylthiouracil

65. Methods: No methods for the thyroid hormones were found in the final report from this laboratory.

66. Results: The means and standard deviations for thyroid hormones from the laboratory 10 propylthiouracil studies are shown in Table 18. The thyroid stimulating hormone values were significantly increased in females at the high dose with apparent absolute, but not statistically significant (n=6 in this study), decreases in both T₃ and T₄ values. Both T₃ and T₄ values were significantly decreased in males at the high dose with an apparent absolute, but not statistically significant, increase in TSH values.

Table 18. Thyroid Hormone Results for Propylthiouracil in the Laboratory 10 Study

	Propylthiouracil Dose (mg/kg/day)	Control 0	Low 0.1	Middle 1.0	High 10
Males		Group (n=6)			
T ₃	(ng/dl)	112.21 ± 11.58 ^{a)}	107.35 ± 1.75	123.85 ± 29.67	*75.16 ± 28.06
T ₄	(ng/dl)	4.09 ± 0.65	3.75 ± 0.52	3.37 ± 1.17	*0.09 ± 0.17
TSH	(μIU/ml)	0.018 ± 0.019	0.029 ± 0.024	0.057 ± 0.078	0.027 ± 0.025
Females		Group (n=6)			
T ₃	(ng/dl)	111.70 ± 10.64	106.28 ± 7.22	133.68 ± 40.36	87.94 ± 21.47
T ₄	(ng/dl)	4.19 ± 0.48	3.65 ± 0.99	3.24 ± 1.48	1.41 ± 1.84
TSH	(μIU/ml)	0.020 ± 0.017	0.011 ± 0.003	0.010 ± 0.000	*0.038 ± 0.014

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

67. This laboratory used only a total of six animals and did not perform the Subgroup experiments.

Summary – Propylthiouracil

68. The response to PTU on thyroid hormonal parameters was present, but considerably weaker in the laboratory 10 study than in the laboratory 1 study. TSH increases were less than two-fold in the laboratory 10 study. The T₄ changes were almost as marked in absolute terms, but even a 60% decline did not achieve statistical significance in the laboratory 10 study in the females. Given the robust and consistent response in the laboratory 1 Subgroups, this does not appear to be a function of the group size of six in the laboratory 10 study, but may reflect methodologies and coefficients of variation.

L-Thyroxine

Laboratory 9 – L-Thyroxine

69. Methods: The final reports states only that radioimmunoassay methods were used for determining thyroid hormones (see section 6.6 of materials and methods).

70. Results: The means and standard deviations for thyroid hormones from the laboratory 9 l-thyroxine studies are shown in Table 19. The TSH values were significantly decreased in both sexes at the high dose, indicating a very sharp down-regulation of pituitary activity. There was no dose related change in either T₃ and T₄ values, although the T₃ were significantly decreased in both sexes at the mid-dose with an absolute value higher than the vehicle control at the high doses in both sexes.

Table 19. Thyroid Hormone Results for L-Thyroxine in the Laboratory 9 Study

L-Thyroxine Dose (mg/kg/day)		Control 0	Low 0.01	Middle 0.1	High 1
Males		Combined Subgroups (n=10)			
T₃	(ng/dl)	76.21 ± 6.11	68.30 ± 10.32	**51.03 ± 11.07	92.10 ± 16.54
T₄	(µg/dl)	5.14 ± 0.92	5.49 ± 0.74	5.39 ± 1.28	5.08 ± 1.47
TSH	(ng/ml)	13.41 ± 2.68	10.09 ± 4.90	8.66 ± 4.37	**2.84 ± 0.65
		Subgroup A (n=5)			
T₃	(ng/dl)	76.98 ± 3.26	73.12 ± 8.44	*56.02 ± 12.55	95.16 ± 22.44
T₄	(µg/dl)	5.90 ± 0.84	5.90 ± 0.84	5.30 ± 1.60	5.20 ± 1.15
TSH	(ng/ml)	13.92 ± 3.38	**8.08 ± 1.18	**8.32 ± 2.48	**2.74 ± 0.93
		Subgroup B (n=5)			
T₃	(ng/dl)	75.44 ± 8.48	63.48 ± 10.51	**46.04 ± 7.48	89.04 ± 9.43
T₄	(µg/dl)	5.12 ± 1.28	5.08 ± 0.34	5.48 ± 1.05	4.96 ± 1.87
TSH	(ng/ml)	12.90 ± 2.02	12.10 ± 6.53	9.00 ± 6.04	**2.94 ± 0.23
Females		Combined Subgroups (n=10)			
T₃	(ng/dl)	67.13 ± 19.20	58.69 ± 9.56	*47.84 ± 11.11	61.60 ± 7.76
T₄	(µg/dl)	3.21 ± 0.78	3.19 ± 0.91	*4.41 ± 1.15	3.40 ± 0.71
TSH	(ng/ml)	5.11 ± 0.78	5.04 ± 1.02	4.73 ± 1.74	**2.97 ± 0.78
		Subgroup A (n=5)			
T₃	(ng/dl)	62.70 ± 17.46	59.26 ± 6.83	44.58 ± 12.21	62.98 ± 9.56
T₄	(µg/dl)	3.38 ± 0.97	3.02 ± 0.80	4.22 ± 0.41	3.76 ± 0.45
TSH	(ng/ml)	4.88 ± 0.55	4.78 ± 0.83	5.54 ± 1.94	*3.42 ± 0.47
		Subgroup B (n=5)			
T₃	(ng/dl)	71.56 ± 21.81	58.12 ± 12.58	51.10 ± 10.10	60.22 ± 6.26
T₄	(µg/dl)	30.4 ± 0.59	3.36 ± 1.07	4.60 ± 1.66	3.04 ± 0.79
TSH	(ng/ml)	5.34 ± 0.97	5.30 ± 1.22	3.92 ± 1.18	**2.52 ± 0.80

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

71. A significant decrease of TSH values in the combined Subgroups in both sexes at the high dose was observed in both respective individual Subgroups. In males, significant decreases of TSH values were also seen at the low and the mid-doses in Subgroup A, but not in the combined Subgroups or in Subgroup B. A significant decrease of T3 values in the combined Subgroups in both sexes was observed in the mid-dose group of both sexes. This was seen in both male Subgroups, but in neither female Subgroup.

Laboratory 13 – L-Thyroxine

72. Methods: Thyroid stimulating hormone (TSH) concentrations were determined with an EIA kit (Amersham Pharmacia Biotech code RPN 2564). T₃ concentrations were determined with an EIA kit (Biomerica code No. 7013). T₄ concentrations were determined with an EIA kit (Biomerica code No. 7012).

73. Results: The means and standard deviations for thyroid hormones from the laboratory 13 l-thyroxine studies are shown in Table 20. The TSH values were significantly decreased in males at the high dose. Both T₃ and T₄ values were significantly increased in both sexes at the mid- and the high doses.

Table 20. Thyroid Hormone Results for l-Thyroxine in the Laboratory 13 Study

l-Thyroxine Dose (mg/kg/day)		Control 0	Low 0.01	Middle 0.1	High 1
Males		Group (n=10)			
T₃	(nmol/l)	1.81 ± 0.27	2.06 ± 0.34	*2.46 ± 0.27	**5.18 ± 1.48
T₄	(nmol/l)	84.50 ± 8.10	101.2 ± 8.46	**129.3 ± 11.98	**172.8 ± 19.46
TSH	(ng/ml)	28.42 ± 4.26	27.13 ± 3.75	26.78 ± 3.64	*20.09 ± 6.99
Females		Group (n=10)			
T₃	(nmol/l)	2.52 ± 0.26	2.43 ± 0.49	*2.95 ± 0.40	**4.64 ± 0.36
T₄	(nmol/l)	52.4 ± 6.59	61.3 ± 10.59	**89.2 ± 17.25	**113.9 ± 51.12
TSH	(ng/ml)	35.53 ± 5.41	35.91 ± 4.70	33.55 ± 6.49	34.33 ± 5.45

*, **, Significantly different from control (p<0.05 and p<0.01, respectively).

74. This laboratory did not divide its experiment into Subgroups, having an overall group size of 10.

Summary – Thyroxine

75. A significant decrease in male TSH values replicated at the high dose in both studies. The laboratory 9 TSH values indicate a sharp down-regulation in both sexes, while the laboratory 13 TSH changes were modest in males and not evident in females. In contrast, the laboratory 9 study did not observe the significant increase of T₄ values in both sexes at the mid- and high dose in the laboratory 13 study, and also did not observe the significant increase in the T₃ values at the high dose. At the mid-dose, the T₃ results were in direct conflict, significantly increasing in the laboratory 13 study and significantly decreasing in the laboratory 9 study.

ANNEX 8

Sperm Analysis Data Generated for the Updated TG 407 Validation Studies

1. The purposes of this Annex are to record for the sperm analyses:
 - the methodologies used by each lab to analyze the sperm number and morphology values during the updated TG 407 studies, where the methods are available from the final reports;
 - the basic statistical findings from each Subgroups and the combined Subgroups, when these data were available from the final reports; and
 - the means and standard deviations for each sex from each Subgroup and the combined Subgroups, when these data were available from the final reports.

2. Additional purposes of this Annex are to compare the reproducibility and reliability of the experimental findings for the sperm analyses:
 - in regards to a dose response or possible random findings;
 - between Subgroups in the same lab;
 - the added value and power of combined the Subgroups (i.e., a group size of five versus ten animals per sex); and
 - between the two laboratories studying each chemical.

3. This Annex is organized in the following manner:
 - each of the ten chemicals tested in the updated TG 407 validation program has its own individual section. The chemicals are roughly grouped as:
 - Estrogens: ethinyl oestradiol, genistein, and nonylphenol.
 - Antiestrogens: tamoxifen
 - Aromatase inhibitors: CGS 18320B
 - Androgens: methyl testosterone
 - Antiandrogens: flutamide and *p,p'*-DDE
 - Thyroid toxicants and hormones: propylthiouracil and l-thyroxine
 - in each section, the sperm analysis methods and data of both laboratories testing the chemical are presented with the laboratories in alphabetical order;
 - a summary table is presented for each laboratory;
 - data for the combined Subgroups is presented first, then for the two individual Subgroups, where available;
 - the dose groups left to right are the vehicle control, the low dose, the mid-dose, and the high dose; and
 - the achievement of statistical significance, either an increase or a decrease in the values, is denoted with one or more asterisks, depending upon the level of significance tested by each laboratory.

4. Overall integration and interpretation of the sperm analysis data with other findings are noted in the body of this report, not this or other Annexes. Likewise, discussion, recommendations, and conclusions are in the body of this report, not this or other Annexes.

Ethinyl Estradiol

Laboratory 2 – Ethinyl Estradiol

5. **Sperm number methods:** After mincing the right cauda epididymis, spermatozoa were suspended in 0.9% NaCl, briefly heated to $>70^{\circ}\text{C}$, and counted in a hemocytometer, calculating the sperm number per g epididymis.

6. **Sperm morphology methods:** Sperm were taken from the right cauda epididymis and suspended and stained in a formalin citrate Eosin G solution. Morphological changes of the head, upper and middle tail were evaluated on 200 sperm from each individual animal using a light microscope.

7. **Results:** The laboratory 2 EE study sperm analyses are shown in Table 1. Mean numbers were reported only for groups of $n=5$. No statistical differences were observed between the vehicle control group and any test substance group in either Subgroup A or B.

Table 1. Sperm count and morphology results for Ethinyl Estradiol in the Laboratory 2 study

Ethinyl Estradiol Dose	Control	Low	Middle	High
	0	10 $\mu\text{g}/\text{kg}/\text{d}$	50 $\mu\text{g}/\text{kg}/\text{d}$	200 $\mu\text{g}/\text{kg}/\text{d}$
Sperm Count (10^6 per g cauda epididymis)				
Subgroup A	715.3 \pm 159.9	664.3 \pm 87.8	618.3 \pm 209.9	742.6 ^a \pm 239.4
Subgroup B	553.3 \pm 152.7	496.3 \pm 189.0	733.4a \pm 213.6	798.8 \pm 249.0
Morphology				
% Normal				
Subgroup A	99.4	98.4	99.9	98.75 ^a
Subgroup B	99.8	98.6	99.25 ^a	99.6
% Abnormal				
Subgroup A	0.6	1.6	0.1	1.25 ^a
Subgroup B	0.2	1.4	0.75 ^a	0.4

^a A single aspermic male was omitted from the Subgroup calculations.

8. No statistical difference was observed for sperm number or morphology at any dose in either Subgroup. The variation between groups was large in Subgroup B with a trend to higher sperm counts with dose. The coefficients of variations (CVs) were relatively large, over 30% on several occasions and in one instance approaching 40%.

Laboratory 5-Ethinyl Estradiol

9. **Methods:** At termination, sperm were collected from the right cauda epididymis, and sperm number and morphological changes were examined.

10. **Results:** The laboratory 5 EE study sperm analyses are shown in Table 2. No statistical differences were detected. This should be compared to the histopathological findings indicating degenerating spermatocytes were observed in the testes.

Table 2. Sperm count and morphology results for Ethinyl Estradiol in the Laboratory 5 study

Ethinyl Estradiol Dose	Control	Low	Middle	High
	0	10 µg/kg/d	50 µg/kg/d	200 µg/kg/d
Sperm Count (10 ⁶ per g cauda epididymis)				
Combined (n=10)	540.4 ± 85.2	545.2 ± 64.8	495.2 ± 82.6	527.4 ± 72.7
Subgroup A	514.8 ± 90.0	536.0 ± 73.7	491.4 ± 76.0	578.8 ± 50.7
Subgroup B	566.0 ± 81.2	554.6 ± 61.6	499.0 ± 97.6	476.0 ± 52.3
Morphology				
% Normal	99.7	99.7	99.6	99.6
% Abnormal				
Combined (n=10)	0.3 ± 0.3	0.3 ± 0.5	0.4 ± 0.4	0.4 ± 0.4
Subgroup A	0.5 ± 0.4	0.4 ± 0.7	0.1 ± 0.2	0.2 ± 0.3
Subgroup B	0.1 ± 0.2	0.2 ± 0.4	0.6 ± 0.4	0.5 ± 0.5

Mean ± Standard Deviation

11. No statistical difference was observed for sperm number or morphology at any dose in either Subgroup. The sperm count CVs were relatively low in this lab: 11-19%.

Summary

12. The results of the two studies are consistent. There were no findings for any effects by EE on sperm counts or sperm morphology in any treatment group.

Genistein

Laboratory 4 – Genistein

13. At the time of necropsy, the right epididymis of each male was removed, weighed and sperm analysis was done.

14. **Sperm number methods:** The caudal epididymis sample remaining after morphological evaluation was frozen below -4°C until examination. On the day of examination, the frozen organ was allowed to stand at room temperature. Then, a sperm (head) suspension was prepared by mincing and homogenizing the caudal epididymis in the phosphate-buffered saline. The sperm suspension was treated with an ultrasonicator, and the number of sperms (heads) was counted using a erythrocytometer under a light microscope. The count was performed 3 times per sample, and the sperm number per 1g caudal epididymis was calculated based on the mean of the three counts.

15. **Sperm morphology methods:** After dissecting and weighing the caudal epididymis, the mass of sperm was collected from the caudal epididymis. The mass of sperm was suspended in Hanks' solution containing 0.5 w/v% bovine calf serum albumin. Sperm suspension was stained by adding 1 w/v% Eosin Y solution and smear specimens were prepared. After differentiating excessive eosin from smear specimens with 1% acetic acid, the sperm morphology were evaluated under the microscope according to the following categories: normal, no hook, excessive hook, amorphous, pin head, two heads, two tails and short tail. Two hundred sperms per sample were evaluated, and the percentage of abnormal sperm rate was then calculated.

16. **Results:** The laboratory 4 genistein study sperm analyses are shown in Table 3. There was one statistically significant difference between the vehicle control group and any test substance group, at the mid-dose in Subgroup A. However, this appeared random and not dose-related.

Table 3. Sperm count and morphology results for Genistein in the Laboratory 4 study

Genistein Dose	Control	Low	Middle	High
	0	120 mg/kg/d	400 mg/kg/d	1000 mg/kg/d
Sperm Count (10^6 per g cauda epididymis)				
Combined (n=10)	1147.6 ± 132.4	1282.0 ± 245.8	1273.7 ± 216.3	1069.3 ± 173.2
Subgroup A	1184.0 ± 46.5	1344.7 ± 181.0	*1413.4 ± 112.7	1068.3 ± 145.0
Subgroup B	1111.2 ± 184.3	1219.4 ± 305.6	1133.9 ± 209.2	1070.3 ± 215.5
Morphology				
% Normal	99.5	99.8	99.5	99.4
% Abnormal				
Combined (n=10)	0.5 ± 0.4	0.2 ± 0.2	0.5 ± 0.5	0.6 ± 0.7
Subgroup A	0.3 ± 0.4	0.1 ± 0.2	0.4 ± 0.4	0.4 ± 0.5
Subgroup B	0.6 ± 0.4	0.2 ± 0.3	0.6 ± 0.5	0.8 ± 0.8

Mean ± Standard Deviation. *, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively).

17. There was statistically significant increase in sperm counts at the mid-dose of genistein in Subgroup A, which was not observed in either the combined Subgroups or Subgroup B. There was no evidence for a dose relationship in this result, and it is judged to be spurious. Except for one group, the CVs for sperm counts were ≤ 20 .

Laboratory 12 – Genistein

18. Sperm number methods: The right cauda epididymis was removed from all males and weighed. The distal end of the right cauda epididymis was cut twice with a scalpel, placed in a 20 ml vial containing 10 ml medium 199E, minced with a scissors and a sample of minced cauda was stained with IDENT, a DNA specific stain. Stained samples were placed into a slide chamber, and the number of sperm in the sample were counted using the CASA fluorescent mode.

19. Sperm morphology methods: Duplicate samples of minced right cauda epididymis were placed on labelled microscopic slides, which were dried, fixed and stained using a multicoloured single step stain (0.8% trypan blue, 0.4% naphthol yellow, 0.2% eosin Y in 1% acetic acid). Two slides were prepared from each male and up to 200 sperm per slide were examined on each slide.

20. Results: The laboratory 12 genistein study sperm analyses are shown in Table 4. There was no statistically significant difference between the vehicle control group and any test substance group.

Table 4. Sperm count and morphology results for Genistein in the Laboratory 12 study

Genistein Dose	Control	Low	Middle	High
	0	120 mg/kg/d	400 mg/kg/d	1000 mg/kg/d
Sperm Count (10^6 per g cauda epididymis)				
Combined (n=10)	417 ± 96	426 ± 118	426 ± 120	436 ± 101
Subgroup A	375 ± 107	426 ± 128	424 ± 74	395 ± 64
Subgroup B	458 ± 69	426 ± 122	428 ± 164	478 ± 121
Morphology				
% Normal	96.5	96.2	95.6	96.1
% Abnormal				
Combined (n=10)	4.2 ± 2.3	4.2 ± 2.5	4.2 ± 2.3	4.5 ± 2.8
Subgroup A	4.2 ± 2.3	4.2 ± 2.5	4.2 ± 2.3	4.5 ± 2.8
Subgroup B	2.7 ± 1.2	3.5 ± 2.3	4.5 ± 1.9	3.3 ± 0.6

Mean ± Standard Deviation

21. No statistical difference was observed for sperm number or morphology at any dose in either Subgroup. The sperm count CVs in this lab ranged from 15-38.

Summary

22. The results of the two studies are consistent. There were no findings for any effects by genistein on sperm counts or sperm morphology in any treatment group.

Nonylphenol

Laboratory 1 – Nonylphenol

23. Immediately after necropsy and organ weight determination, the cauda epididymis was taken from all male animals. The following parameters were determined: Sperm morphology and sperm head count (cauda epididymis). Sperm morphology and sperm head counts (cauda epididymis) were evaluated from the animals of the control and high dose group, only.

24. Sperm number methods: Sperm heads (10⁶/g cauda epididymis) were determined by microscopic evaluation using a MAKLER chamber after homogenization using a laboratory modification of the procedure of Feuston et al. (1989).

25. Sperm morphology methods: The percent abnormal sperm was determined after vital staining with eosin, using microscopic evaluation by the method of Feuston et al., Toxicology and Applied Pharmacology 100, 145-161 (1989).

26. Results: The laboratory 1 nonylphenol study sperm analyses are shown in Table 5. There was no statistically significant difference between the vehicle control group and any test substance group.

Table 5. Sperm count and morphology results for Nonylphenol in the laboratory 1 study

Nonylphenol Dose	Control	Low	Middle	High
	0	20 mg/kg/d	80 mg/kg/d	200/150 mg/kg/d
Sperm Count (10 ⁶ per g cauda epididymis)				
Combined (n=10)	610 ± 181.4	Not done	Not done	556 ± 108.7
Morphology				
% Normal	94.6	Not done	Not done	94.9
% Abnormal				
Combined (n=10)	5.4 ± 3.10	Not done	Not done	5.1 ± 3.98

Mean ± Standard Deviation

27. Sperm data was not reported for separate Subgroups A and B in the final report. The CVs for the two reported analyses, when rounded, were 20 and 30

Laboratory 6 – Nonylphenol

28. Sperm number methods: The sample was collected from the left cauda by slicing the weighed cauda and allowing the sliced cauda to sit in 10 mls of warmed (37 °C) Dulbecco's Phosphate Buffered Saline (DPBS) with calcium and magnesium, supplemented with 1.0% w/v bovine serum albumin for 15 minutes. One milliliter was added to a centrifuge tube containing one drop of 10% neutral buffered formalin, 100 µl of sample was then added to a DNA stain and distilled water, and 6 µl of this solution was placed on a microcell® slide. Counts were performed using the Hamilton Thorne Research IVOS (Integrated Visual Optical System). All settings are described in the final report. The total counts are the mean of three individual analysis runs.

29. Sperm morphology methods: The sample was collected from the left cauda by slicing the weighed cauda and allowing the sliced cauda to sit in 10 mls of warmed media for 15 minutes. Two slides were prepared with the suspension after it had sat for the 15 minutes. The slides were stained with Papanicolaou stain. Epididymal sperm morphology was performed. The stained slides were evaluated manually by phase contrast microscopy at a minimum power of 200x, and the slides were archived for any future re-evaluations. Five hundred sperm were evaluated (Filler, R., (1993). Male Reproductive Toxicology, Methods in Toxicology, Volume 3A, Methods of Evaluation of Rat Epididymal Sperm Morphology, pg 334-343.) from each animal and the percent normal sperm calculated: $((500 \text{ sperm counted} - \text{total sperm with abnormalities}) \div 500) * 100$.

30. Results: The laboratory 6 nonylphenol study sperm analyses are shown in Table 6. The sperm numbers from the mid- and high dose samples were significantly decreased, but not in a dose responsive manner.

Table 6. Sperm count and morphology results for Nonylphenol in the Laboratory 6 study

Nonylphenol Dose	Control	Low	Middle	High
	0	20 mg/kg/d	80 mg/kg/d	300/250 mg/kg/d
Sperm Count (10^6 per g cauda epididymis)				
Combined (n=10)	673.2 ± 328.0	419.5 ± 328.9	**195.1 ± 215.3	*341.5 ± 232.2
Subgroup A	709.6 ± 280.1	338.9 ± 292.5	*151.3 ± 144.8	*321.6 ± 185.6
Subgroup B	636.9 ± 400.4	500.0 ± 376.6	250.0 ± 297.4	361.4 ± 293.1
Nonylphenol Dose	Control	Low	Middle	High
	0	20 mg/kg/d	80 mg/kg/d	300/250 mg/kg/d
Morphology				
% Normal				
Combined (n=10)	54.5 ± 25.2	42.6 ± 23.2	53.1 ± 22.5	52.7 ± 25.3
Subgroup A	56.8 ± 31.3	34.0 ± 15.5	60.2 ± 18.5	57.2 ± 28.0
Subgroup B	52.3 ± 20.8	53.4 ± 28.9	46.0 ± 25.9	48.1 ± 24.6

Mean ± Standard Deviation. *, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively).

31. Significant decreases were observed in the combined Subgroups and were also seen in Subgroup A at both the mid- and the high doses. Despite decreases from the control of 50% or more, statistical significance was not achieved in Subgroup B. The CVs in this study were large, ranging from 50 to 120. The sperm morphology numbers show a very high background of abnormalities, approaching 45%. No changes in sperm morphology were observed with the test substance administration.

Summary

32. The two NP studies do not produce concordant data. The laboratory 1 study shows no effect, but lacks data from the low and mid-doses. The laboratory 6 data shows significant changes at the mid- and high dose, but these do not produce a dose response relationship as the lowest absolute numbers clearly occur at the mid-dose. Individual data have been extracted and tabularized (see page 5 of this draft).

Tamoxifen

Laboratory 3 – Tamoxifen

33. Sperm was collected from the left cauda epididymis

34. Sperm number methods: A 15 ml volume of 0.9% NaCl was added to the sperm/medium mixture for homogenization for at least 30 seconds. A 2 ml aliquot of the homogenized solution mixed with 20 µl of 10% formol was sonicated, and then stained with the addition of 200 µl of 1% eosin-Y. An appropriate

dilution of this suspension was placed in a Neubauer cell counter for spermatozoa count. The concentration (million sperm /g cauda weight) was reported.

35. Sperm morphology methods: A 250 µl aliquot of the sperm/medium mixture was diluted with 250 µl of 0.9% NaCl and 100 µl of 10% eosin-Y. After 2 hours of staining, two air-dried smears per male were prepared using 200 µl of the suspension for each smear. One slide per animal was examined by light microscopy for assessment of sperm morphology. Two hundred spermatozoa were assessed for each smear, where possible. The second slide for each animal was retained as a backup. The percentages of normal and abnormal heads, abnormalities of the flagellum and cytoplasmic droplets were reported.

36. Results: The laboratory 3 tamoxifen study sperm analyses are shown in Table 7. Despite tissue weight and histological parameters, sperm analysis of males treated up to 200 µg/kg/day revealed no alteration of sperm number or the frequency of abnormal sperm.

Table 7. Sperm count and morphology results for Tamoxifen in the laboratory 3 study

Tamoxifen Dose	Control	Low	Middle	High
	0	5 µg/kg/d	30 µg/kg/d	200 µg/kg/d
Sperm Count (10 ⁶ per g epididymis)				
Combined	787.3 ± 176.4	795.1 ± 100.2	759.3 ± 170.0	767.7 ± 86.0
Subgroup A	736.8 ± 116.2	784.4 ± 108.4	713.0 ± 115.3	712.6 ± 74.7
Subgroup B	837.8 ± 224.0	805.8 ± 102.7	805.5 ± 215.4	822.8 ± 59.0
Morphology				
% Abnormal				
Combined	10.6 ± 3.9	11.4 ± 3.9	11.5 ± 4.5	10.6 ± 2.8
Subgroup A	11.2 ± 3.2	13.4 ± 3.3	13.0 ± 5.3	10.8 ± 1.6
Subgroup B	10.1 ± 4.8	9.5 ± 3.8	10.1 ± 3.6	10.4 ± 3.8

Mean ± Standard Deviation

37. No statistical difference was observed for sperm number or morphology at any dose in either Subgroup. The CVs ranged from 11 to 27.

Laboratory 10 – Tamoxifen

38. After weight determination, the following parameters will be evaluated in the right epididymis of all animals sacrificed on schedule in a randomized sequence.

39. Numbers are not provided, only graphics of 1) daily sperm production, 2) number of teratosperms (which appears to be total % abnormal sperm), and 3) sperm motility. For the daily sperm production and sperm motility, there is a statement that statistical significance was not achieved. For teratosperms, the graphic indicates the low dose group had significantly fewer teratosperms. This suggests some variability in the background and/or technique.

40. In this study, the total number of animals was only 6, rather than 10, and the animals were not divided into Subgroups.

Summary

41. The results of the two studies are consistent. There were no findings for any effects by tamoxifen on sperm counts or sperm morphology in any treatment group.

CGS 18320B**Laboratory 8 - CGS 18320B**

42. Sperm count methods: The cauda epididymal sample was suspended in solution, examined in a counting chamber, and the number of sperm cells in a pre-defined number of erythrocyte fields counted. The results were calculated as millions per gram cauda epididymal tissue.

43. Sperm morphology methods: Immediately after sacrifice, the right epididymis was punctured at the cauda wall and drop of sperm fluid was sampled and treated with eosin Y 1% and incubated at room temperature. A 3 µl sample was placed on a slide and a cover slip applied. Approximately 200 classifiable sperm cells were evaluated under magnification for defects of the head, midportion, and tail.

44. Results: The laboratory 8 CGS 18320B study sperm analyses are shown in Table 8. There was no statistically significant difference between the vehicle control group and the CGS 18320B groups in either total cauda epididymal sperm numbers or sperm morphology.

Table 8. Sperm count and morphology results for CBS 18320B in the Laboratory 8 study

CGS 18320 B Dose	Control	Low	Middle	High
	0	0.3 mg/kg/d	3 mg/kg/d	30 mg/kg/d
Sperm Count (10 ⁶ per g cauda epididymis)				
Combined	636 ± 83	635 ± 98	643 ± 84	597 ± 95
Subgroup A	645 ± 61	615 ± 102	665 ± 77	617 ± 126
Subgroup B	627 ± 107	656 ± 100	621 ± 93	572 ± 41
Morphology				
% Normal				
Combined	98.4 ± 1.6	*91.7 ± 16.6	97.7 ± 1.1	97.8 ± 1.1
Subgroup A	97.5 ± 1.5	85.9 ± 23.1	97.9 ± 1.1	98.0 ± 1.2
Subgroup B	99.3 ± 1.1	97.4 ± 1.1	97.4 ± 1.2	97.6 ± 1.3

Mean ± Standard Deviation. *, **, Significantly different from control (p<0.05 and p<0.01, respectively).

45. No statistical difference was observed for sperm number or morphology at any dose in either Subgroup. The sperm count CVs were low and in a relatively narrow range from 10 to 20.

Laboratory 13 – CGS 18320B

46. At scheduled sacrifice, sperm from one epididymis was collected for cauda epididymal sperm count and for morphological evaluation of epididymal sperm.

47. Sperm count methods: Epididymal sperm count was done on cauda epididymal sample suspended in 0.9% NaCl. A sample was examined in a counting chamber under a light microscope, and the number of sperm cells in a pre-defined number of erythrocyte fields was counted. The number of sperm (in million per 1 gram of cauda epididymal tissue) was calculated.

48. Sperm morphology methods: Immediately after sacrifice of the animals, the left epididymis (for animal nos. 10, 20, 30 and 40 the right epididymis) was isolated, trimmed, and a small puncture was done in the cauda wall. A drop of sperm fluid was sampled and treated with eosin Y 1% and incubated at room temperature for 5-10 minutes. A 25 µl sample was placed on a slide and covered with a cover slip. Approximately 200 classifiable sperm cells were evaluated under magnification as to defects in head, midportion and tail. Sperm was classified as normal, abnormal, or tailless.

49. Results: The laboratory 13 CGS 18320B study sperm analyses are shown in Table 9. There was no statistically significant difference between the vehicle control group and the l-thyroxine substance groups in either total cauda epididymal sperm numbers or sperm morphology. Although a total group size of 10 animals per sex was used, the study did not compare the possible increase in statistical power by dividing the male and female groups into two Subgroups of five.

Table 9. Sperm count and morphology results for CBS 18320B in the Laboratory 13 study

CGS 18320 B Dose	Control	Low	Middle	High
	0	0.3 mg/kg/d	3 mg/kg/d	30 mg/kg/d
Sperm Count (10^6 per mg cauda epididymis)				
Combined	213.3 ± 60.6	243.7 ± 56.9	245.8 ± 54.9	190.4 ± 26.2
Morphology				
% Normal	93.8	92.5	93.6	92.7
% Abnormal				
Combined	4.2 ± 2.6	5.6 ± 1.8	5.6 ± 2.5	4.7 ± 1.2
% Tailless				
Combined	2.0 ± 1.5	1.9 ± 1.4	0.9 ± 0.6	2.6 ± 3.8

Mean ± Standard Deviation

50. The laboratory did not subdivide the experiment into two individual Subgroups. The sperm count CVs were modest and ranged from 14 to 28.

Summary

51. The results of the two studies are consistent. There were no findings for any effects by CGS 18320B on sperm counts or sperm morphology in any treatment group.

Methyl Testosterone

Laboratory 3 – Methyl Testosterone

52. Sperm was collected from the left cauda epididymis

53. Sperm number methods: A 15 ml volume of 0.9% NaCl was added to the sperm/medium mixture for homogenization for at least 30 seconds. A 2 ml aliquot of the homogenized solution mixed with 20 µl of 10% formol was sonicated, and then stained with the addition of 200 µl of 1% eosin-Y. An appropriate dilution of this suspension was placed in a Neubauer cell counter for spermatozoa count. The concentration (million sperm /g cauda weight) was reported.

54. Sperm morphology methods: A 250 µl aliquot of the sperm/medium mixture was diluted with 250 µl of 0.9% NaCl and 100 µl of 10% eosin-Y. After 2 hours of staining, two air-dried smears per male were prepared using 200 µl of the suspension for each smear. One slide per animal was examined by light microscopy for assessment of sperm morphology. Two hundred spermatozoa were assessed for each smear, where possible. The second slide for each animal was retained as a backup. The percentages of normal and abnormal heads, abnormalities of the flagellum and cytoplasmic droplets were reported.

55. Results: The laboratory 3 methyl testosterone study sperm analyses are shown in Tables 10 and 11. Sperm numbers were significantly decreased at the mid-dose. An examination of the individual data shows that 2 of 10 individual values were very low at the mid-dose and 3 of 10 individual values at the high dose. These values were clearly different and correlate with the individual histopathological findings in the epididymis. Morphologically abnormal spermatozoa were significantly increased at the mid-dose.

As with the sperm numbers, there was a close correlation between individuals which had an increased percentage of abnormal sperm morphology and animals showing histopathological findings in the epididymis as shown in the following table.

Table 10. Individual sperm analysis results and other data for Methyl Testosterone in the laboratory 3 study that influence interpretation

Correlation between individual sperm data and histopathology findings				
Dose Group and Animal number	Sperm Count (10 ⁶ /g of epididymis)	Total % of Abnormal sperm	Epididymal Histopathological changes	
			Oligospermia - diffuse	Intratubular spermatic debris - diffuse
Control Range of individuals	(645 to 1082)	(5.5 to 31.5)		
40 mg/kg/day				
KT3M3605	175	71.0	MARKED	MILD
KT3M3607	141	54.4	MILD	MODERATE
200 mg/kg/day				
KT4M3627	405	24.1	MILD	SLIGHT
KT4M3630	158	45.5	MARKED	--
KT4M3633	327	29.5	MILD	SLIGHT

Table 11. Sperm count & morphology results for Methyl Testosterone in the laboratory 3 study

Methyl Testosterone Dose	Control	Low	Middle	High
	0	10 mg/kg/d	40 mg/kg/d	200 mg/kg/d
Sperm Count (10 ⁶ per g epididymis)				
Combined	807.6 ± 123.1	766.1 ± 104.5	*649.0 ± 277.4	703.2 ± 331.7
Subgroup A	817.0 ± 46.9	785.5 ± 98.3	650.0 ± 289.2	740.2 ± 383.0
Subgroup B	798.1 ± 178.0	746.6 ± 118.1	648.0 ± 299.2	666.3 ± 312.2
Morphology				
% Abnormal				
Combined	12.0 ± 7.4	12.9 ± 6.0	*25.9 ± 20.3	21.2 ± 12.7
Subgroup A	11.5 ± 2.9	16.4 ± 5.6	26.0 ± 25.7	19.4 ± 10.3
Subgroup B	12.6 ± 10.7	9.4 ± 4.3	25.9 ± 16.4	22.9 ± 15.8

Mean ± Standard Deviation. *, **, Significantly different from control (p<0.05 and p<0.01, respectively).

56. Sperm numbers were not significantly decreased in the individual Subgroups, nor was sperm morphology. The parameters were only altered in the combined Subgroups. The significant changes in both parameters were at the mid-dose only, and absolute means rose in both Subgroups at the high dose versus the mid-dose. The CVs appeared to fall into two distinct groups. For the controls and low dose, the CVs ranged from 6-22. For the mid- and high doses, the CVs ranged from 42-52 indicating more individual variation within the groups.

Laboratory 12 – Methyl Testosterone

57. Sperm number methods: The right cauda epididymis was removed from all males and weighed. The distal end of the right cauda epididymis was cut twice with a scalpel, placed in a 20 ml vial containing 10 ml medium 199E, minced with a scissors and a sample of minced cauda was stained with IDENT, a DNA specific stain. Stained samples were placed into a slide chamber, and the number of sperm in the sample was counted using the CASA fluorescent mode.

58. Sperm morphology methods: Duplicate samples of minced right cauda epididymis were placed on labelled microscopic slides, which were dried, fixed and stained using a multicoloured single step stain

(0.8% trypan blue, 0.4% naphthol yellow, 0.2% eosin Y in 1% acetic acid). Two slides were prepared from each male and up to 200 sperm per slide were examined on each slide. Abnormal characteristics were divided into abnormal head, abnormal tail, multiple abnormality with subclassifications of detached head, abnormal shaped head, coiled/kinked tail, and abnormal sized tail.

59. Results: The laboratory 12 methyl testosterone study sperm analyses are shown in Table 12. Sperm numbers were significantly decreased at the high dose, and the percent abnormal sperm were significantly increased at the high dose. The right cauda epididymides weights were also significantly decreased compared with the control group at all doses. As there were no differences in the sperm parameters measured in the 10 or 40mg/kg/day groups compared with the control group, sperm parameters do not appear to be the most sensitive endpoint when compared to histopathology.

Table 12. Sperm count and morphology results for Methyl Testosterone in the Laboratory 12 study

Methyl Testosterone Dose	Control	Low	Middle	High
	0	10 mg/kg/d	40 mg/kg/d	200 mg/kg/d
Sperm Count (10^6 per g epididymis)				
Combined	466 ± 180	462 ± 262	506 ± 124	**102 ± 74
Subgroup A	566 ± 214	524 ± 379	564 ± 136	**103 ± 39
Subgroup B	366 ± 49	401 ± 42	448 ± 89	**100 ± 103
Morphology				
% Normal				
Combined	98.0	97.3	97.6	**63.1
Subgroup A	97.7	97.1	97.0	**60.7
Subgroup B	98.3	97.5	98.2	**65.5
% Abnormal				
Combined	2.0 ± 0.9	2.7 ± 1.0	2.4 ± 0.9	**36.9 ± 15.1
Subgroup A	2.3 ± 1.1	2.9 ± 0.5	3.0 ± 0.7	**39.3 ± 17.3
Subgroup B	1.7 ± 0.7	2.5 ± 1.3	1.8 ± 0.7	**34.5 ± 14.2

Mean ± Standard Deviation. *, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively).

60. The significant decreases in sperm counts and corresponding significant increases in abnormal sperm were observed in both the combined Subgroups and both individual Subgroups at the high dose. In parallel, there were sharp increases in abnormal sperm at the high dose that resulted in statistically significant changes. The CV range was particularly large, from 11 to 103, in this laboratory.

Summary

61. The results of the two methyl testosterone studies are not concordant. In the laboratory 3 study, there were decreases of 20 and 13% at the mid- and high doses, respectively, for sperm counts. In the laboratory 12 study, the numbers were +8% and -78%, respectively. Thus, one study shows no consistent evidence of a decrease in sperm counts or morphological changes, and the other study is very clear at showing changes in both parameters.

Flutamide

Laboratory 2 – Flutamide

62. Sperm number methods: Determination of spermatozoa density was performed in a suspension (0.9% NaCl) of minced cauda epididymis tissue by counting of spermatozoa in a hemocytometer and calculation of spermatozoa density per g epididymis. The samples used for counting had been headed (>70°C) shortly.

63. Sperm morphology methods: During necropsy a sperm sample was taken from the right cauda epididymis and suspended in a formalin citrate Eosin G fixation solution to stain permits for morphological examination. Morphological changes of the head, upper and middle tail were evaluated on 200 spermatozoa using alight microscope.

64. Results: The laboratory 2 flutamide study sperm analyses are shown in Table 13. There was a statistically significant decrease in sperm number at the high dose when the Subgroups were combined. There was also a rise in the percentage of abnormal sperm and sharp drop in the percentage of motile sperm at the high dose. This laboratory did not report the combined values and the separate Subgroups are reported here. This laboratory reported only mean values and did not record standard deviations in the final report.

Table 13. Sperm count and morphology results for Flutamide in the Laboratory 2 study

Flutamide Dose	Control	Low	Middle	High
	0	1 mg/kg/d	10 mg/kg/d	100 mg/kg/d
Sperm Count (10^6 per g cauda epididymis)				
	644.5 ± 185.6	703.0 ± 285.5	840.4 ± 217.5	*383.9 ± 203.2
Subgroup A	601.6 ± 169.6	705.4 ± 286.3	780.3 ± 157.5	497.5 ± 220.7
Subgroup B	687.3 ± 210.0	700.7 ± 318.4	900.6 ± 269.4	270.3 ± 109.0
Morphology				
% Normal				
Subgroup A	98.7	99.0	98.4	96.9
Subgroup B	98.7	99.0	98.6	92.1
Motility – 1 Min				
Subgroup A	80	81	78	47
Subgroup B	90	87	78	46

Mean ± Standard Deviation. *, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively).

65. The changes in the high dose groups were not statistically significant when analyzed as Subgroups of 5 animals. The sperm count CVs were from 20 to 45 in this laboratory.

Laboratory 11 – Flutamide

66. At termination, the right caudal epididymis was excised and weighed on the day of necropsy.

67. Sperm number methods: The sperm was collected from the right caudal epididymis. Using a Thoma hemocytometer under the microscope, sperm after dilution were counted and calculated as sperm per 1 gram caudal epididymis. In the same sample, sperm motility was evaluated as the number of motile sperm/number of sperm examine x 100.

68. Sperm morphology methods: For morphology, smear preparation of the sperm samples were made and sperm form anomalies index (number of subnormal sperm/number of sperm examined x 100) was calculated.

69. Results: The laboratory 11 flutamide study sperm analyses are shown in Table 14. Sperm numbers were significantly decreased at the high dose. Statistically significant decreases in abnormalities were observed at the mid- dose.

Table 14. Sperm count and morphology results for Flutamide in the Laboratory 11 study

Flutamide Dose	Control	Low	Middle	High
	0	1 mg/kg/d	10 mg/kg/d	100 mg/kg/d
Sperm Count (10^6 per g epididymis)				
Combined	585.9 ± 90.2	647.2 ± 99.4	575.6 ± 119.4	**300.3 ± 122.4
Subgroup A	545.2 ± 109.1	667.8 ± 138.7	527.2 ± 95.1	**274.6 ± 103.5
Subgroup B	626.5 ± 47.6	626.8 ± 44.0	624.0 ± 131.1	**326.0 ± 146.2
Morphology				
% Abnormal				
Combined	6.50 ± 8.01	3.87 ± 2.68	*2.73 ± 1.18	4.90 ± 1.96
Subgroup A	8.53 ± 1.50	4.87 ± 3.50	*3.00 ± 1.23	*4.33 ± 2.08
Subgroup B	4.47 ± 2.79	2.87 ± 0.84	2.47 ± 1.21	5.47 ± 1.86

Mean ± Standard Deviation. *, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively).

70. Sperm numbers were significantly decreased at the high dose in both the combined Subgroups and in both Subgroups A and B. Statistically significant decreases in abnormalities were observed in Subgroup A at the mid- and high dose, which resulted in a statistically significant decreases in abnormalities in the combined Subgroups at the mid-dose only. Absolute numbers of abnormalities the Subgroup A control was twice that of other groups and appeared to account for the statistical significance. The CVs were low in the vehicle and the low and mid-dose groups (a range of 7-21). At the high dose, the CVs jumped to a range of 38-45, indicating more individual variation within the groups.

Summary

71. The results of the two studies are consistent for the sperm counts. The laboratory 2 study observed significant decreases in the combined Subgroups at the high dose as did the laboratory 11 study. The degree of significance was larger in the laboratory 11 study and significance occurred in both individual Subgroups, whereas it did not in the laboratory 2 study. Neither study saw a dose related change in sperm morphology in the combined Subgroups.

p,p'-DDE

Laboratory 6 –*p,p'*-DDE

72. Sperm number methods: The sample was collected from the left cauda by slicing the weighed cauda and allowing the sliced cauda to sit in 10 mls of warmed (37 °C) Dulbecco's Phosphate Buffered Saline (DPBS) with calcium and magnesium, supplemented with 1.0% w/v bovine serum albumin for 15 minutes. One milliliter was added to a centrifuge tube containing one drop of 10% neutral buffered formalin, 100 µl of sample was then added to a DNA stain and distilled water, and 6 µl of this solution was placed on a microcell® slide. Counts were performed using the Hamilton Thorne Research IVOS (Integrated Visual Optical System). All settings are described in the final report. The total counts are the mean of three individual analysis runs.

73. Sperm morphology methods: The sample was collected from the left cauda by slicing the weighed cauda and allowing the sliced cauda to sit in 10 mls of warmed media for 15 minutes. Two slides were prepared with the suspension after it had sat for the 15 minutes. The slides were stained with Papanicolaou stain. Epididymal sperm morphology was performed. The stained slides were evaluated manually by phase contrast microscopy at a minimum power of 200x, and the slides were archived for any future re-

evaluations. Five hundred sperm were evaluated (Filler, R., (1993). Male Reproductive Toxicology, Methods in Toxicology, Volume 3A, Methods of Evaluation of Rat Epididymal Sperm Morphology, pg 334-343.) from each animal and the percent normal sperm calculated: $((500 \text{ sperm counted} - \text{total sperm with abnormalities}) \div 500) * 100$.

74. Results: The laboratory 6 *p,p'*-DDE study sperm analyses are shown in Table 15. The only statistically significant difference between the vehicle control and treatment groups was a decrease in the sperm numbers at the low dose.

Table 15. Sperm count and morphology results for DDE in the Laboratory 6 study

<i>p,p'</i> -DDE Dose	Control	Low	Middle	High
	0	12.5 mg/kg/d	50 mg/kg/d	200/150 mg/kg/d
Sperm Count (10^6 per mg epididymis)				
Combined	547.5 ± 199.9	*245.7 ± 222.7	676.1 ± 197.6	487.6 ± 304.6
Subgroup A	653.0 ± 115.1	303.8 ± 309.8	662.0 ± 236.0	378.7 ± 272.1
Subgroup B	442.1 ± 221.0	187.6 ± 84.8	690.2 ± 178.0	651.1 ± 366.1
Morphology				
% Normal				
Combined	93.5	91.9	93.0	92.0
Subgroup A	94.2	90.9	92.8	92.4
Subgroup B	92.8	92.8	93.1	91.4

Mean ± Standard Deviation. *, **, Significantly different from control ($p < 0.05$ and $p < 0.01$, respectively).

75. No statistical difference was observed for sperm number or morphology at any dose in either Subgroup. This was despite a greater than 50% decrease in the low dose values for Subgroup B from the respective vehicle control. The sperm count CVs ranged from 25 to 90.

Laboratory 7 – *p,p'*-DDE

76. Sperm number methods: The right cauda epididymis was removed from all males and weighed. The sperm suspension of cauda epididymis was prepared by mincing and homogenizing in the deionized water, and was stained with IDENT, a DNA specific-stain. The sperm (head) number per 1 gram cauda epididymis was calculated using the Hamilton Thorne Research IVOS.

77. Sperm morphology methods: The mass of sperm was taken from the right cauda epididymis and suspended in Hanks' solution containing 0.5 w/v% bovine serum albumin. Two slides per animal were prepared with the suspension and stained with eosin-anilin blue solution. The sperm morphology was evaluated using the light microscope. Two hundred sperms per slide were evaluated, and the percentage of abnormal sperm rate was calculated.

78. **Results:** The laboratory 7 *p,p'*-DDE study sperm analyses are shown in Table 16. There was no statistically significant difference between the vehicle control group and the *p,p'*-DDE substance groups in either total cauda epididymal sperm numbers or sperm morphology.

Table 16. Sperm count and morphology results for DDE in the laboratory 7 study

<i>p,p'</i> -DDE Dose	Control	Low	Middle	High
	0	12.5 mg/kg/d	50 mg/kg/d	100 mg/kg/d
Sperm Count (10^6 per g cauda epididymis)				
Combined	960.7 ± 244.8	1028.9 ± 197.7	1050.8 ± 244.4	1127.9 ± 203.3
Subgroup A	1165.2 ± 76.1	1006.8 ± 127.4	1021.0 ± 205.0	1119.4 ± 229.3
Subgroup B	756.1 ± 156.1	1051.0 ± 265.6	1080.7 ± 300.3	1136.3 ± 200.5
Morphology				
% Abnormal				
Combined	6.1 ± 2.3	5.8 ± 2.3	5.1 ± 1.8	5.7 ± 2.3
Subgroup A	5.3 ± 2.3	6.1 ± 2.4	5.4 ± 1.6	5.1 ± 2.4
Subgroup B	6.9 ± 2.2	5.4 ± 2.4	4.8 ± 2.2	6.2 ± 2.4

Mean ± Standard Deviation

79. No statistical difference was observed for sperm number or morphology at any dose in either Subgroup. The sperm counts were in a relatively narrow range from 17-28.

Summary

80. The results of the two studies are basically consistent. There were no dose related findings for any effects by DDE on sperm counts or sperm morphology. There appear to be sharp variations in group results in the laboratory 6 study (compare Subgroups A and B at the high dose), so the statistical significance in the low dose group is equivocal.

Propylthiouracil

Laboratory 1 – Propylthiouracil

81. **Methods:** Immediately after necropsy and organ weight determination the cauda epididymis was taken from all male animals. The following parameters were determined: Sperm morphology and Sperm head count (cauda epididymis). Sperm morphology and sperm head counts (cauda epididymis) were evaluated from the animals of the control and high dose group, only.

82. **Results:** The laboratory 1 propylthiouracil study sperm analyses are shown in Table 17. There was no statistically significant difference between the vehicle control group and the high dose propylthiouracil substance group in either total cauda epididymal sperm numbers or sperm morphology. Therefore, the two lower dose groups were not analyzed. The data for the two individual Subgroups was not presented in the final report for this study.

Table 17. Sperm count and morphology results for PTU in the Laboratory 1 study

Propylthiouracil Dose	Control	Low	Middle	High
	0	0.1 mg/kg/d	1.0 mg/kg/d	10 mg/kg/d
Sperm Count (10^6 per mg cauda epididymis)				
	434 ± 90.7	Not done	Not done	492 ± 105.3
Morphology				
% Normal	97.3	Not done	Not done	96.9
% Abnormal	2.8 ± 1.51	Not done	Not done	3.0 ± 1.32

Mean ± Standard Deviation

83. No data for individual Subgroups was found, and only the vehicle and high dose groups were analyzed. The CVs for the two analyses were both 21.

Laboratory 10 – Propylthiouracil

84. After weight determination, the following parameters will be evaluated in the right epididymis of all animals sacrificed on schedule in a randomized sequence

85. Specific values were not provided in the final report, only graphics of 1) daily sperm production, 2) number of teratosperms (which appears to be total % abnormal sperm), and 3) sperm motility. None of the graphics has a designation that would indicate that statistical significance was achieved by any test substance group relative to the controls.

86. In this study, the total number of animals was only 6, rather than 10, and the animals were not divided into Subgroups. Due to the graphical results, no CVs were available.

Summary

87. The results of the two studies appear to be consistent. There was no evidence for any effects by PTU on sperm counts or sperm morphology in any treatment group. To make an unequivocal conclusion, however, would require the quantitative results from the laboratory 10 study.

L-Thyroxine**Laboratory 9 – L-Thyroxine**

88. At scheduled necropsy, the right epididymis was removed and weighed for sperm analyses.

89. **Sperm number methods:** The right caudal epididymis was homogenized and diluted. Then, sperm heads per 1 g of epididymal tissue were counted.

90. **Sperm morphology methods:** Smear samples of sperm collected from right caudal epididymis or vas deferens were air-dried. Sperms (at least 200 per animal) were examined and classified as either normal or abnormal. Number of abnormal sperms and type of abnormalities were recorded, and the frequency of each type of abnormality was calculated.

91. **Results:** The laboratory 9 l-thyroxine study sperm analyses are shown in Table 18. Sperm numbers and morphology were performed in the vehicle control and high dose 1.0 mg l-thyroxine/kg body weight group. There was no statistically significant difference between the vehicle control group and the high dose l-thyroxine substance group in either total cauda epididymal sperm numbers or sperm morphology. Therefore, the two lower dose groups of 0.1 and 0.01 mg/kg were not analyzed.

Table 18. Sperm count and morphology results for l-Thyroxine in the laboratory 9 study

l-Thyroxine Dose	Control	Low	Middle	High
	0	0.01 mg/kg/d	0.1 mg/kg/d	1.0 mg/kg/d
Sperm Count (10^6 per g epididymis)				
Combined	640.5 ± 197.9	Not done	Not done	561.0 ± 119.7
Subgroup A	691.5 ± 132.8	Not done	Not done	582.5 ± 145.6
Subgroup B	589.4 ± 252.9	Not done	Not done	539.5 ± 99.4
Morphology				
% Abnormal				
Combined	3.4 ± 5.96	Not done	Not done	1.8 ± 1.48
Subgroup A	2.0 ± 1.22	Not done	Not done	0.9 ± 1.24
Subgroup B	4.7 ± 8.60	Not done	Not done	2.7 ± 1.15

Mean ± Standard Deviation

92. No statistical difference was observed for sperm number or morphology at any dose in either Subgroup. CVs ranged from 18 to 42 with higher values distributed between the vehicle groups and the high dose groups.

Laboratory 13 – L-Thyroxine

93. At scheduled sacrifice, sperm from one epididymis was collected for cauda epididymal sperm count and for morphological evaluation of epididymal sperm.

94. **Sperm count methods:** Epididymal sperm count was done on cauda epididymal sample suspended in 0.9% NaCl. A sample was examined in a counting chamber under a light microscope, and the number of sperm cells in a pre-defined number of erythrocyte fields was counted. The number of sperm (in million per 1 gram of cauda epididymal tissue) was calculated.

95. **Sperm morphology methods:** Immediately after sacrifice of the animals, the left epididymis (for animal nos. 10, 20, 30 and 40 the right epididymis) was isolated, trimmed, and a small puncture was done in the cauda wall. A drop of sperm fluid was sampled and treated with eosin Y 1% and incubated at room temperature for 5-10 minutes. A 25 µl sample was placed on a slide and covered with a cover slip. Approximately 200 classifiable sperm cells were evaluated under magnification as to defects in head, midportion and tail. Sperm was classified as normal, abnormal, or tailless.

96. **Results:** The laboratory 13 l-thyroxine study sperm analyses are shown in Table 19. There was no statistically significant difference between the vehicle control group and the l-thyroxine substance groups in either total cauda epididymal sperm numbers or sperm morphology. Although a total group size of 10 animals per sex was used, the study did not compare the possible increase in statistical power by dividing the male and female groups into two Subgroups of five.

Table 19. Sperm count and morphology results for l-Thyroxine in the Laboratory 13 study

l-Thyroxine Dose	Control	Low	Middle	High
	0	0.01 mg/kg/d	0.1 mg/kg/d	1.0 mg/kg/d
Sperm Count (10^6 per mg cauda epididymis)				
	120.7 ± 27.5	141.0 ± 44.9	153.8 ± 41.5	155.6 ± 30.1
Morphology				
% Normal	96.7	96.8	96.7	95.3
% Abnormal	2.7 ± 1.7	2.0 ± 1.3	2.9 ± 1.9	4.1 ± 1.6
% Tailless	0.6 ± 0.7	1.2 ± 1.0	0.4 ± 0.5	0.6 ± 0.5

Mean ± Standard Deviation

97. The laboratory did not subdivide the experiment into two individual Subgroups. The sperm count CVs were modest and ranged from 19 to 32.

Summary

98. The results of the two studies are consistent. There were no findings for any effects by l-thyroxine on sperm counts or sperm morphology in any treatment group.

ANNEX 9

**Vaginal Smear Data
Generated for the Enhanced TG 407 Validation Studies**

1. The purpose of this Annex is to record for the vaginal smear data the basic findings in a common and comparable format for the following compound classes:
 - Estrogens: ethinyl oestradiol, genistein, and nonylphenol.
 - Antiestrogens: tamoxifen
 - Aromatase inhibitors: CGS 18320B.
 - And other compounds where the data could be made available.

2. A number of laboratories did not include the vaginal smear data in their final report. Further, there had been no agreement on the format of these data. In some cases, vaginal histopathology at necropsy were reported rather than vaginal smears for several days.

3. Therefore, this information was urgently requested from the laboratories conducting studies with test substances that could specifically interact with estrogen pathways (ethinyl oestradiol, genistein, nonylphenol, tamoxifen, and CGS 18320B) in a standardized format by the Secretariat (May, 2003). The technical purpose was to provide a means for comparisons with histological data for the female reproductive tract tissues. In turn, this was to investigate the possible loss of synchronization in the estrous cycle in the female rats, particularly, with the putative weak oestrogens, genistein and nonylphenol.

4. Detailed data were also available for one flutamide, one DDE study, one PTU study, and two thyroxine studies, and these data have been included.

5. No vaginal smear data in the detailed form used here were reported and available in the five other cases (2 methyl testosterone, 1 flutamide, 1 DDE, and 1 PTU). Neither were these data specifically requested from these laboratories by the Secretariat.

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Ethinyl Estradiol – Laboratories 2 and 5**Table 1. Laboratory 2 – EE experiments**

Estrous cycle / smear data for Individual Female Group A (n=5)										
Animal Number	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33
Vehicle Controls										
Animal 21	P	M	D	P	E	E/M	E	D *		
Animal 22	P	P	E	D	P	E	E	M	D *	
Animal 23	P	E	D	D/P	P	E	D *			
Animal 24	E	M/D	E	E	D *					
Animal 25	D	P	E/M	D	P/E	E	M	D *		
EE Group One (low dose -- 10 µg/kg/d)										
Animal 26	D	D	P/E	P/E	D *					
Animal 27	D	P	M/D	D	P	E	D *			
Animal 28	D	D	P	M	E/M	D *				
Animal 29	P	E	D	D	P/E	D *				
Animal 30	D	D	D/P	D	P/E	D *				
EE Group Two (mid or intermediate dose -- 50 µg/kg/d)										
Animal 31	D	M/D	P	D	E	D *				
Animal 32	D	D	E	E	E/M	M	D *			
Animal 33	P	P	E	D	P/E	M	M	M	D *	
Animal 34	P	P	E/M	D	P/E	M	D *			
Animal 35	D	D	E	E	P/E	D *				
EE Group Three (mid or intermediate dose – 200 µg/kg/d)										
Animal 36	P	E	M/D	P	E	E/M	E	D *		
Animal 37	P	E	D	D/P	P/E	E	D *			
Animal 38	D	P	E	E/M	D *					
Animal 39	E	M	P	P	E	D *				
Animal 40	E	M	E	D	P/E	M	D *			

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

Table 1 continued. Laboratory 2 – EE experiments

Note: The group B had the same numbering sequence for the animals.

Estrous cycle / smear data for Individual Female Group B (n=5)										
Animal Number	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33
Vehicle Controls										
Animal 21	E	M	D	D	E	M	D *			
Animal 22	P	P	E	E	D	D *				
Animal 23	D	D	P	E	D *					
Animal 24	P	M	D	D/P	E	D *				
Animal 25	E	E	P	P/E	E	E/M	D *			
Test Substance Dose Group One (low dose)										
Animal 26	D	D	P	P	P	E/M	D *			
Animal 27	M	D	E	D	D *					
Animal 28	D	D	P	E	E	D *				
Animal 29	D	D	P	P/E	E	D *				
Animal 30	M/D	D	D	P	E	E/M	D *			
Test Substance Dose Group Two (mid or intermediate dose)										
Animal 31	P	P	E	M	D *					
Animal 32	M	D	E	E	E	D *				
Animal 33	P	M/D	D/P	D	E	M	M/D	P	E **	
Animal 34	D	D	D/P	E	E	E/M	E	M	E **	
Animal 35	D	D	P	P/E	E	D *				
Test Substance Dose Group Three (mid or intermediate dose)										
Animal 36	E	M/D	D	P	E	M/D	D *			
Animal 37	M	D	D	E/M	P	D *				
Animal 38	D	P	P	E	D *					
Animal 39	D	D	P	E	M	M/D	M	D *		
Animal 40	P	E	D	D	D *					

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

** necropsied as animal reached terminal day without diestrous.

Table 2. Laboratory 5 – EE experiments

Note the added days in this study.

Estrous cycle / smear data for Individual Female Group A (n=5)													
Animal Number	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34
Vehicle Controls													
41	P	E	M	D	P	E	M	D	P	E	M	D*	
42	P	E	M	D	P	E	M	D	P	E	E	M	D*
43	E	M	D	P	E	M	D	P	E	M	D*		
44	E	M	D	P	E	M	D	P	E	M	D*		
45	D	D	E	M	D	D	E	M	D*				
Test Substance Dose Group One (low dose, 10 µg/kg/day)													
51	E	M	D	P	E	M	D	P	E	M	D*		
52	P	E	M	D	P	E	M	D	E	E	M	D*	
53	D	D	E	M	D	D	E	M	D*				
54	E	M	D	P	E	M	D	P	E	M	D*		
55	D	E	M	D	D	E	E	M	D*				
Test Substance Dose Group One (mid dose, 50 µg/kg/day)													
61	M	D	D	E	M	D	D	E	E	M	D*		
62	M	D	D	P	E	M	D	D	P	P	M	D*	
63	D	P	E	M	D	D	P	E	M	D*			
64	P	E	M	D	D	P	E	M	D*				
65	M	D	P	E	M	D	D	D	E	M	D*		
Test Substance Dose Group One (high dose, 200 µg/kg/day)													
71	D	D	D	D	D	O	O	O	D*				
72	M	M	O	E	E	M	M	D	E	E	E	E	E*
73	D	O	D	D	O	E	E	D	D	D	D*		
74	O	D	D	O	D	O	D	D	D*				
75	E	E	O	E	E	E	E	O	E	E	E	E	E*

D = diestrous; E = estrous; M = metestrous; P = proestrous; O = others

“ * “ means the necropsy at each stage

Table 2 continued. Laboratory 5 – EE experiments

Note the added days in this study.

Estrous cycle / smear data for Individual Female Group B (n=5)													
Animal Number	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34
Vehicle Controls													
46	D	P	E	M	D	P	E	M	D*				
47	M	D	D	D	E	M	D	D	E	E	D*		
48	D	E	M	D	D	E	M	D	E	E	M	D*	
49	D	D	E	M	D	D	E	M	D*				
50	D	P	E	M	D	P	E	M	D*				
Test Substance Dose Group One (low dose, 10 µg/kg/day)													
56	D	P	E	M	D	D	E	E	M	D*			
57	D	P	E	M	D	D	E	M	D*				
58	M	D	P	E	M	D	P	E	M	D*			
59	M	D	E	M	D	D	E	M	D*				
60	P	E	M	D	P	E	M	D	D	E	M	D*	
Test Substance Dose Group One (mid dose, 50 µg/kg/day)													
66	M	D	D	D	E	M	D	D	D	P	E	D*	
67	D	D	D	D	D	D	E	E	E	M	D*		
68	P	E	M	D	D	E	E	M	D*				
69	P	E	M	D	D	D	E	E	M	D*			
70	E	M	D	D	E	M	D	O	O	M	D*		
Test Substance Dose Group One (high dose, 200 µg/kg/day)													
76	D	O	O	D	D	E	M	O	O	P	P	O	O*
77	D	D	D	D	O	M	O	D	O	O	O	O	O*
78	E	D	D	D	O	D	O	D	D*				
79	E	M	D	D	E	M	D	D	D	D	E	M	E*
80	D	D	D	D	O	D	D	D	O	O	O	O	O*

D = diestrous; E = estrous; M = metestrous; P = proestrous; O = others

“ * “ means the necropsy at each stage

Genistein – Laboratories 4 and 12**Table 3. Laboratory 4 – Genistein experiments**

Estrous cycle / smear data for Individual Female Group A (n=5)										
Animal Number	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33
Vehicle Controls										
1101	M	D	P	E	M	D*				
1102	E	M	D	P	E	M	D*			
1103	M	D	P	E	M	D*				
1104	M	D	P	E	M	D*				
1105	P	E	M	D	P	E	M	D*		
Genistein Dose Group One (low dose: 120 mg/kg)										
2101	E	M	D	P	E	M	D*			
2102	E	M	D	P	E	M	D*			
2103	P	E	M	D	P	E	M	D*		
2104	D	P	E	M	D*					
2105	P	E	M	D	P	E	M	D*		
Genistein Dose Group Two (mid or intermediate dose: 400 mg/kg)										
3101	P	E	M	D	P	E	M	D*		
3102	P	E	M	D	P	E	M	D*		
3103	D	P	E	M	D*					
3104	E	M	D	D	D*					
3105	M	D	P	E	M	D*				
Genistein Dose Group Three (high dose: 1000 mg/kg)										
4101	D	P	E	M	D*					
4102	D	P	E	M	D*					
4103	M	D	P	E	M	D*				
4104	M	D	P	E	M	D*				
4105	P	E	M	D	P	E	M	D*		

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

Table 3 continued. Laboratory 4 – Genistein experiments

Estrous cycle / smear data for Individual Female Group B (n=5)										
Animal Number	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33
Vehicle Controls										
1106	D	D	D	D	P	E	M	D*		
1107	P	E	M	D	P	E	M	D*		
1108	M	D	P	E	M	D*				
1109	P	E	M	D	P	E	M	D*		
1110	D	P	E	M	D*					
Genistein Dose Group One (low dose: 120 mg/kg)										
2106	D	P	E	M	D*					
2107	E	M	D	P	E	M	D*			
2108	P	E	M	D	P	E	M	D*		
2109	P	E	M	D	P	E	M	D*		
2110	M	D	P	E	M	D				
Genistein Dose Group Two (mid or intermediate dose: 400 mg/kg)										
3106	D	P	E	M	D*					
3107	P	E	M	D	P	E	M	D*		
3108	D	P	E	M	D*					
3109	M	D	P	E	M	D*				
3110	E	M	D	D	P	E	M	D*		
Genistein Dose Group Three (high dose: 1000 mg/kg)										
4106	D	P	E	M	D*					
4107	M	D	P	E	M	D*				
4108	E	M	D	P	E	E	M	D*		
4109	D	P	E	E	M	D*				
4110	P	E	M	D	P	E	M	D*		

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

Table 4. Laboratory 12 – Genistein experiments

Estrous cycle / smear data for Individual Female Group A (n=5)										
Animal Number	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	
Vehicle Controls										
41	M	D	P	O	M	D*				
42	M	O	M	D	P	O	M	D*		
43	D	D	P	O	M	D*				
44	D	P	O	M	D*					
45	O	M	D	P	O	M	P	P	O*	
Test Substance Dose Group One (120 mg/kg/day)										
51	P	O	M	D	P	O	M	D*		
52	M	D	P	O	M	D*				
53	O	M	O	M	D*					
54	O	M	D	P	O	M	M	P	O*	
55	D	P	O	O	M	P	P	O	D*	
Test Substance Dose Group Two (400 mg/kg/day)										
61	O	D	D	P	O	M	M	P	O*	
62	O	M	M	P	O	M	M	P	O*	
63	D	D	M	D	O	O	M	M	O*	
64	M	D	P	O	P	D*				
65	O	O	M	D	P	O	O	O	M*	
Test Substance Dose Group Three (1000 mg/kg/day)										
71	O	O	M	O	O	O	M	P	O*	
72	M	M	P	O	M	M	O	O	M*	
73	M	O	M	O	O	O	O	O	O*	
74	O	M	M	P	O	O	M	O	O*	
75	O	O	O	O	O	O	O	O	O*	

D = diestrous; E = estrous; M = metestrous; P = proestrous; O = others

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

Table 4 continued. Laboratory 12 – Genistein experiments

Estrous cycle / smear data for Individual Female Group A (n=5)										
Animal Number	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	
Vehicle Controls										
46	P	O	D	D	P	O	M	D*		
47	O	D	M	P	O	D*				
48	D	P	O	D	D*					
49	D	D	P	O	D*					
50	O	D	M	P	O	D*				
Test Substance Dose Group One (120 mg/kg/day)										
56	O	M	D	D	O	M	D*			
57	O	M	M	D	O	M	D*			
58	O	M	M	M	O	M	D*			
59	M	P	O	M	O	O	O	M	D*	
60	D	P	O	M	P	P	O	M	M*	
Test Substance Dose Group Two (400 mg/kg/day)										
66	O	M	M	P	O	M	D*			
67	D	P	O	M	M	P	O	D*		
68	O	M	M	P	O	O	M	D*		
69	O	M	O	O	O	M	M	P	O*	
70	M	D	P	O	M	D				
Test Substance Dose Group Three (1000 mg/kg/day)										
76	M	M	M	O	O	M	P	O	M*	
77	P	O	M	D	P	P	O	D*		
78	O	O	O	M	M	O	O	M	M*	
79	P	O	O	O	M	D*				
80	M	M	M	O	M	D*				

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

Nonylphenol – Laboratories 1 and 5**Table 5. Laboratory 1 – Nonylphenol data**

Estrous cycle / smear data for Individual Female Group A (n=5)										
Animal Number	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33
Vehicle Controls (0 mg/kg body weight)										
41	P	E	M	D	P	E	M	D*		
42	P	E	M	D	P	E	M	D*		
43	D	P	E	M	D*					
44	E	M	M	P	E	M	D*			
45	P	E	M	D	P	E	M	D*		
Test Substance Dose Group One (low dose: 20 mg/kg body weight)										
51	D	P	E	E	M	D*				
52	E	M	M	D	D*					
53	P	E	M	D	D*					
54	D	P	E	M	M	D*				
55	D	P	E	M	D*					
Test Substance Dose Group Two (mid dose: 80 mg/kg bodyweight)										
61	P	P	E	M	D*					
62	E	M	D	P	E	M	D*			
63	P	P	P	D	E	M	D*			
64	E	M	M	D	E	M	D*			
65	P	E	M	D	D*					
Test Substance Dose Group Three (high dose: 200 / 150 mg/kg body weight ¹⁾)										
71	M	M	M	E	E	M	M	E	M*	
72	P	E	M	D	D*					
73	P	E	M	M	D*					
74 ²⁾	-	-	-	-	-	-	-	-	-	-
75	M	M	D	P	E	M	P	P	P*	

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

¹⁾ Dose reduction on day 15²⁾ Animal 74 was found dead on day 14

Table 5 continued. Laboratory 1 – Nonylphenol data

Estrous cycle / smear data for Individual Female Group B (n=5)										
Animal Number	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33
Vehicle Controls (0 mg/kg body weight)										
46	E	M	D	P	E	M	D*			
47	M	D	P	E	M	D*				
48	P	E	M	D	P	E	M	M	M*	
49	P	E	M	D	P	E	M	D*		
50	D	P	E	E	M	M	D*			
Test Substance Dose Group One (low dose: 20 mg/kg body weight)										
56	D	P	E	M	D*					
57	D	P	E	M	D*					
58	E	M	D	P	E	M	D*			
59	P	E	M	D	P	P	E	M	M*	
60	E	M	D	D	D*					
Test Substance Dose Group Two (mid dose: 80 mg/kg bodyweight)										
66	D	D	E	M	D*					
67	M	M	D	E	M	M	D*			
68	D	D	D	P	D*					
69	P	E	M	D	P	E	M	M	M*	
70	M	M	E	E	D*					
Test Substance Dose Group Three (high dose: 200 / 150 mg/kg body weight ¹⁾)										
76	D	P	E	E	M	M	D*			
77	D	P	P	E	E	M	D*			
78	D	D	P	E	M	D*				
79	P	E	M	P	E	M*				
80	D	P	E	M	D*					

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

¹⁾ Reduction of the dose on day 15

Table 6. Laboratory 6 - Nonylphenol data

Estrous cycle / smear data for Individual Female Group A (n=5)										
Animal Number	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33
Vehicle Controls										
IGJ269F	P	E	D	D	P	E	M	D*		
IGJ253F	E	D	D	P	E	M	D*			
IGJ277F	D	P	E	D	D*					
IGJ258F	D	D	P	E	M	D*				
IGJ283F	E	E	D	D	P	E	E	D*		
Test Substance Dose Group One (low dose: 20 mg/kg body weight)										
IGJ274F	D	P	E	D	D*					
IGJ278F	E	D	D	P	E	D*				
IGJ282F	E	E	D	D	P	E	E	D*		
IGJ276F	D	D	P	E	D*					
IGJ267F	D	P	E	M	D*					
Test Substance Dose Group Two (mid or intermediate dose: 80 mg/kg body weight)										
IGJ293F	E	D	D	P	E	D*				
IGJ275F	D	E	D	D	P	E	D*			
IGJ284F	D	P	E	D	D*					
IGJ300F	P	E	D	D	D*					
IGJ259F	D	D	D	P	E	D*				
Test Substance Dose Group Three (high dose: 300/250 mg/kg body weight)										
IGJ280F	D	D	D	P	E	D*				
IGJ298F	E	D	D	P	P	E	D*			
IGJ294F	P	P	P	P	P	D*				
IGJ270F (Dead)										
IGJ271F	P	E	D	D	D*					

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

Table 6 continued. Laboratory 6 - Nonylphenol data

Estrous cycle / smear data for Individual Female Group B (n=5)										
Animal Number	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33
Vehicle Controls										
IGJ286F	E	D	D	P	P	E	M	D*		
IGJ292F	D	P	E	D	D*					
IGJ273F	E	E	D	P	E	D*				
IGJ251F	P	E	D	D	P	E	M	D*		
IGJ297F	P	E	D	P	P	E	M	D*		
Test Substance Dose Group One (low dose: 20 mg/kg body weight)										
IGJ260F	P	E	D	D	P	E	D*			
IGJ299F	P	E	M	D	D*					
IGJ295F	P	E	D	D	P	E	M	D*		
IGJ266F	D	P	E	E	D*					
IGJ265F	D	E	D	D	P	E	D*			
Test Substance Dose Group Two (mid or intermediate dose: 80 mg/kg body weight)										
IGJ281F	P	E	D	D	P	E	D*			
IGJ285F	D	P	E	D	D*					
IGJ289F	D	D	P	E	D*					
IGJ272F	P	E	D	D	P	E	D*			
IGJ264F	D	P	E	D	D*					
Test Substance Dose Group Three (high dose: 300/250 mg/kg body weight)										
IGJ296F	D	E	D	D	D*					
IGJ288F	D	P	E	D	D*					
IGJ254F	P	E	D	D	D*					
IGJ279F	D	P	E	D	D*					
IGJ290F	D	D	P	E	D*					

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

Tamoxifen – Laboratories 3 and 10**Table 7. Laboratory 3 – Tamoxifen data**

Estrous cycle / smear data for Individual Female Group A (n=5)										
Animal Number	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33
Vehicle Controls										
KT1F5005	M	D	D	NC	NC	D *				
KT1F5006	D	E	M	NC	NC	E	NC	D *		
KT1F5008	M	D	D	NC	NC	M	D *			
KT1F5011	E	M	D	NC	NC	M	D *			
KT1F5012	M	D	P	NC	NC	D *				
Test Substance Dose Group One (low dose: 5 µg/kg body weight)										
KT2F5023	P	E	M	NC	NC	E	NC	D *		
KT2F5025	P	E	M	NC	NC	E	NC	M	D * ≠	
KT2F5026	E	M	D	NC	NC	M	D *			
KT2F5029	E	M	D	NC	NC	M	D *			
KT2F5032	M	M	D	NC	NC	D *				
Test Substance Dose Group Two (mid or intermediate dose: 30 µg/kg body weight)										
KT3F5044	D	D	D	NC	NC	D	D *			
KT3F5046	P	M	D	NC	NC	D *				
KT3F5048	D	D	D	NC	NC	D	D *			
KT3F5049	D	D	D	NC	NC	D *				
KT3F5052	E	D	D	NC	NC	D	D *			
Test Substance Dose Group Three (mid or intermediate dose: 200 µg/kg body weight)										
KT4F5063	D	D	D	NC	NC	NC	NC	NC	NC	NC *
KT4F5064	D	D	D	NC	NC	NC	NC	NC	NC	NC *
KT4F5066	D	D	D	NC	NC	NC	NC	NC *		
KT4F5067	D	D	D	NC	NC	NC	NC	NC	NC	NC *
KT4F5072	D	D	D	NC	NC	NC	NC	NC	NC	NC *

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Histopathologic diagnoses of vagina confirmed diestrous morphology, except where specified (≠)

NC = Not Checked.

Table 7 continued. Laboratory 3 – Tamoxifen data

Estrous cycle / smear data for Individual Female Group B (n=5)										
Animal Number	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33
Vehicle Controls										
KT1F5003	D	E	M	NC	NC	E	NC	D *		
KT1F5004	D	D	E	NC	NC	P	NC	NC	D *	
KT1F5007	E	M	D	NC	NC	M	D *			
KT1F5009	D	E	M	NC	NC	E	NC	D *		
KT1F5010	M	D	D	NC	NC	D *				
Test Substance Dose Group One (low dose: 5 µg/kg body weight)										
KT2F5024	E	M	D	NC	NC	M	D *			
KT2F5027	P	E	M	NC	NC	E	NC	D *		
KT2F5028	P	E	M	NC	NC	E	NC	D *		
KT2F5030	D	D	E	NC	NC	D * ≠				
KT2F5031	D	E	M	NC	NC	P	NC	NC	D *	
Test Substance Dose Group Two (mid or intermediate dose: 30 µg/kg body weight)										
KT3F5043	D	D	D	NC	NC	D *				
KT3F5045	P	M	D	NC	NC	D *				
KT3F5047	D	P	M	NC	NC	P	M	D *		
KT3F5050	D	D	D	NC	NC	M	D *			
KT3F5051	M	D	D	NC	NC	D *				
Test Substance Dose Group Three (mid or intermediate dose: 200 µg/kg body weight)										
KT4F5065	D	D	D	NC	NC	NC	NC	NC	NC	NC *
KT4F5068	D	D	D	NC	NC	NC	NC	NC *		
KT4F5069	D	D	D	NC	NC	NC	NC	NC	NC	NC *
KT4F5070	D	D	D	NC	NC	NC	NC	NC	NC	NC *
KT4F5071	D	D	D	NC	NC	NC	NC	NC *		

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Histopathologic diagnoses of vagina confirmed diestrous morphology, except where specified (≠)

NC = Not Checked.

Table 8. Laboratory 10 – Tamoxifen Data

Estrous cycle / smear data for Individual Female (only one group with n=6)										
Animal Number	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33
Vehicle Controls										
F1	P	P	D	D	D *					
F2	P	P	E	M	D *					
F3	E	P	E	D	P	E	M	D *		
F4	E	P	E	M	D *					
F5	P	E	E	E	M	M	D *			
F6	D	P	E	D	D *					
Test Substance Dose Group One (low dose: 5 µg/kg body weight)										
F13	P	P	E	E	E	E	E	D *		
F14	P	P	E	M	P	P	M	M	D *	
F15	P	P	P	E	E	E	M	D *		
F16	M	P	P	E	E	M	D	D *		
F17	P	D	P	E	E	M	M	M	D *	
F18	D	P	E	E	M	M	D *			
Test Substance Dose Group Two (mid or intermediate dose: 30 µg/kg body weight)										
F19	P	P	P	D	D *					
F20	D	D	D	D	D *					
F21	P	P	D	D	D *					
F22	P	E	P	P	M	D *				
F23	E	D	P	E	M	M	D *			
F24	M	P	P	P	M	D *				
Test Substance Dose Group Three (high dose: 200 µg/kg body weight)										
F25	P	P	D	D	D *					
F26	D	D	P	P	M	D *				
F27	D	D	D	D	D *					
F28	D	M	D	D	D *					
F29	D	D	D	P	P	D *				
F30	P	P	P	E	M	M	D	D	D *	

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

CGS 18320B – Laboratories 8 and 13.

Laboratory 8 did not retain the vaginal smear data, but did include detailed vaginal histopathology. This table summarizes the vaginal histopathology findings.

Table 9. Laboratory 8 – CGS 18320B

Estrous cycle / smear data for Individual Female Group A (n=5)										
Vehicle Controls										
Animal Number	451	452	453	454	455	456	457	458	459	460
Observations	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
CGS 18320B Dose Group One (low dose: 0.3 mg/kg body weight)										
Animal Number	461	462	463	464	465	466	467	468	469	470
Observations	NAF	Atrophy ++	Atrophy ++	NAF	NAF	Atrophy +	Atrophy +	Atrophy +	Atrophy +	Atrophy ++
CGS 18320B Dose Group Two (mid or intermediate dose: 3 mg/kg body weight)										
Animal Number	471	472	473	474	475	476	477	478	479	480
Observations	Atrophy ++	Atrophy ++	Atrophy ++	Atrophy ++	Atrophy ++	Atrophy ++	Atrophy ++	Atrophy ++	Atrophy ++	Atrophy ++
CGS 18320B Dose Group Three (high dose: 30 mg/kg body weight)										
Animal Number	481	482	483	484	485	486	487	488	489	490
Observations	Atrophy ++	Atrophy ++	Atrophy ++	Atrophy ++	Atrophy ++	Atrophy ++	Atrophy ++	Atrophy ++	Atrophy ++	Atrophy ++

NAF – No abnormal findings or observations for the animal in this tissue.

Grading for this laboratory: + slight, ++ moderate, +++ severe

Table 10. Laboratory 13 – CGS 18320B

Estrous cycle / smear data for Individual Female (n=10)							
Animal Number	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32
Vehicle Controls							
41	E	M	D	D*			
42	D	P	M	P	E	D*	
43	D	D	D	D*			
44	D	D	P	P	E	D*	
45	E	M	D	D*			
46	E	M	D	D*			
47	P	M	D	D*			
48	D	D	P	E	M	D*	
49	D	D	D	E	M	D*	
50	D	D	E	E	M	D*	
CGS 18320B Dose Group One (low: 0.3 mg/kg body weight)							
51	D	D	D	D*			
52	D	D	D	D*			
53	D	D	D	D*			
54	D	D	D	D*			
55	D	D	D	D*			
56	D	D	D	D*			
57	D	D	D	D*			
58	D	D	D	D*			
59	D	D	D	D*			
60	D	D	D	D*			

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

Table 10 continued. Laboratory 13 – CGS 18320B experiments

Estrous cycle / smear data for Individual Females (n=10)							
Animal Number	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32
CGS 18320B Dose Group Two (mid or intermediate dose: 3 mg/kg body weight)							
61	D	D	D	D*			
62	D	D	D	D*			
63	D	D	D	D*			
64	D	D	D	D*			
65	D	D	D	D*			
66	D	D	D	D*			
67	D	D	D	D*			
68	D	D	D	D*			
69	D	D	D	D*			
70	D	D	D	D*			
CGS 18320B Dose Group Three (high dose: 30 mg/kg body weight)							
71	D	D	D	D*			
72	D	D	D	D*			
73	D	D	D	D*			
74	D	D	D	D*			
75	D	D	D	D*			
76	D	D	D	D*			
77	D	D	D	D*			
78	D	D	D	D*			
79	D	D	D	D*			
80	D	D	D	D*			

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

Flutamide – Laboratory 2**Table 11. Laboratory 2 – Flutamide experiments**

Estrous cycle / smear data for Individual Female Group A (n=5)															
Animal Number	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32
Vehicle Controls															
21	D	D	P	E	D	P	E	M	D	D	E	E	D*		
22	P	E	D	D	P	E	D	D	P	E	D*				
23	D	D	P	E	D	D	P	E	D	P	P	E	D*		
24	P	E	M	D	D	P	E	D	D	P	E	E	M	D*	
25	E	D	D	P	E	D	D	P	P	D	D*				
Test Substance Dose Group One (low dose: 1 mg/kg bodyweight)															
26	D	P	E	D	D	P	E	D	D	P	E	D*			
27	D	D	P	E	M	No data	P	E	D	D	E	E	D*		
28	E	E	D	D	P	E	D	P	E	E	D*				
29	D	P	M	P	P	D	No data	P	E	D	D*				
30	P	P	E	D	D	P	P	P	E	M	M	P	E	D*	
Test Substance Dose Group Two (mid or intermediate dose: 10 mg/kg body weight)															
31	P	P	E	D	D	P	E	D	D	P	E	D*			
32	D	D	P	E	D	P	No data	P	E	D	D*				
33	P	P	E	M	M	No data	E	D	P	P	E	D*			
34	D	D	E	M	D	D	P	E	D	D	P	E	D*		
35	E	M	M	D	E	D	No data	P	M	D	P	M	M	D*	
Test Substance Dose Group Three (high dose: 100 mg/kg body weight)															
36	E	E	D	D	E	E	E	E	E	E	D*				
37	E	D	D	D	E	E	D	D	E	M	M	D*			
38	D	P	E	D	D	No data	E	M	D	D	P	D*			
39	D	P	E	D	P	E	E	D	D	D	E	D*			
40	D	P	E	E	D	P	E	E	D	P	E	E	D*		

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

Table 11 continued. Laboratory 2 – Flutamide experiments

Estrous cycle / smear data for Individual Female Group B (n=5)															
Animal Number	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32
Vehicle Controls															
21	P	P	E	M	D	P	E	M	D	No data	E	D*			
22	E	E	D	D	P	E	M	D	D	E	D*				
23	D	P	E	M	M	D	P	E	D	D	P	E	E	D*	
24	E	M	D	P	P	E	D	D	D	P	P	D*			
25	D	D	E	E	M	D	E	E	E	M	M	M	D*		
Test Substance Dose Group One (low dose: 1 mg/kg bodyweight)															
26	D	P	E	M	D	P	E	D	D	P	E	D*			
27	D	P	E	E	D	D	No data	E	D	D	P	E	D*		
28	D	P	E	M	D	P	E	M	M	M	E	D*			
29	E	D	P	E	E	D	No data	P	E	D	D*				
30	M	P	D	E	D	D	P	E	D	D	P	M	D*		
Test Substance Dose Group Two (mid or intermediate dose: 10 mg/kg body weight)															
31	P	E	D	D	P	E	D	D	P	E	D*				
32	E	D	D	P	E	D	No data	P	E	D	P	E	E	D*	
33	D	D	D	P	E	M	D	D	P	E	D*				
34	D	D	P	E	M	D	D	P	E	D*					
35	P	E	M	D	No data	E	D	P	E	E	E	E	D*		
Test Substance Dose Group Three (high dose: 100 mg/kg body weight)															
36	P	E	M	M	M	E	E	No data	M	E	M	D*			
37	E	M	D	P	E	D	P	E	M	M	M	M	E	D*	
38	D	D	D	P	E	D	D	D	P	E	D*				
39	E	D	D	D	P	E	E	D	D	D	D*				
40	E	E	M	D	E	E	D	D	D	E	D	P	E	E	D*

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

DDE – Laboratory 6**Table 12. Laboratory 6 – *p,p'*-DDE**

Estrous cycle / smear data for Individual Females (groups combined)								
Vehicle Controls								
Animal #	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31
IGI755F	D	D	P	E	D*			
IGI751F	E	D	D	P	E	D*		
IGI723F	M	D	P	E	D*			
IGI739F	D	E	E	D	D*			
IGI759F	D	D	P	E	D*			
IGI767F	E	M	D	P	E	E	D*	
IGI745F	D	D	P	E	D*			
IGI737F	P	E	D	D	D*			
IGI730F	D	P	E	E	D*			
IGI758F	P	E	D	D	P	E	D*	
Test Substance Dose Group One (low dose: 12.5 mg/kg body weight)								
Animal #	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31
IGI735F	D	E	M	D	D*			
IGI742F	D	D	P	E	D*			
IGI736F	D	D	P	E	M	D*		
IGI720F	E	M	D	P	E	D*		
IGI762F	P	E	D	D	P	E	D*	
IGI722F	D	P	E	E	D			
IGI746F	D	D	P	E	M	D*		
IGI725F	D	D	P	E	D			
IGI729F	E	D	D	P	E	E	D*	
IGI760F	D	P	E	D	D*			

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Table 12 continued. Laboratory 6 – p,p'-DDE

Estrous cycle / smear data for Individual Females (groups combined)								
Test Substance Dose Group Two (mid or intermediate dose: 50 mg/kg body weight)								
Animal #	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31
IGI748F	P	E	D	D	P	E	D*	
IGI740F	D	D	P	E	E	D*		
IGI731F	D	D	D	D	D*			
IGI765F	P	E	M	P	E	E	D*	
IGI743F	D	P	E	D	D*			
IGI721F	D	P	E	D	D*			
IGI724F	D	P	E	D	D*			
IGI741F	M	D	P	E	M	D*		
IGI754F	P	E	E	D	D*			
IGI733F	D	D	D	D	D*			
Test Substance Dose Group Three (high dose: 200/150 mg/kg body weight)								
Animal #	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31
IGI744F	D	P	E	M	D*			
IGI757F	D	P	E	D	D*			
IGI764F X								
IGI761F	D	D	P	E	M	D*		
IGI756F	D	P	P	E	D*			
IGI747F X								
IGI718F X								
IGI749F	D	P	E	D	D*			
IGI719F	E	E	M	D	P	E	M	D*
IGI763F	P	E	D	D	P	E	E	D*

NOTE: X - ANIMAL DEAD

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Propylthiouracil – Laboratory 1

Table 13. Laboratory 1 – PTU experiments

Estrous cycle / smear data for Individual Female Group A (n=5)										
Animal Number	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33
Vehicle Controls										
41	P	E	M	D	P	E	M	D*		
42	E	M	D	P	E	M	D*			
43	P	E	M	D	P	E	M	D*		
44	P	E	M	D	P	E	M	D*		
45	D	P	E	D	P	M	D*			
Test Substance Dose Group One (low dose: 0.1 mg/kg bodyweight)										
51	E	D	P	E	M	D*				
52	M	D	P	E	M	D*				
53	D	D	D	D	D*					
54	P	E	E	D	P	E	M	D*		
55	D	D	P	D	D*					
Test Substance Dose Group Two (mid or intermediate dose: 1.0 mg/kg body weight)										
61	P	E	M	D	P	E	D*			
62	E	M	D	P	E	M	D*			
63	P	P	E	M	D*					
64	D	P	E	M	D*					
65	M	D	P	E	M	D*				
Test Substance Dose Group Three (high dose: 10.0 mg/kg body weight)										
71	M	D	P	E	M	D*				
72	P	E	E	D	P	E	M	D*		
73	E	M	D	P	E	M	D*			
74	P	E	D	D	P	E	D*			
75	M	D	P	E	M	D*				

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

Table 13 continued. Laboratory 1 – PTU experiments

Estrous cycle / smear data for Individual Female Group B (n=5)										
Animal Number	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33
Vehicle Controls										
46	D	P	E	M	D*					
47	P	E	M	D	P	E	M	D*		
48	E	M	D	P	E	M	D*			
49	D	P	E	M	D*					
50	D	P	E	M	D*					
Test Substance Dose Group One (low dose: 0.1 mg/kg bodyweight)										
56	E	M	D	P	E	M	D*			
57	D	P	E	M	D*					
58	M	D	P	E	M	D*				
59	P	M	D	D	D*					
60	D	D	D	D	D*					
Test Substance Dose Group Two (mid or intermediate dose: 1.0 mg/kg body weight)										
66	P	E	M	D	P	E	M	D*		
67	P	E	M	D	P	E	M	D*		
68	D	P	E	M	D*					
69	D	P	E	M	D*					
70	P	E	M	D	P	E	M	D*		
Test Substance Dose Group Three (high dose: 10.0 mg/kg body weight)										
76	E	M	D	P	M	D*				
77	E	M	D	P	E	M	D*			
78	P	E	M	D	P	E	M	D*		
79	D	P	E	M	D*					
80	M	P	E	M	M	D*				

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

Thyroxine – Laboratories 9 and 13**Table 14. Laboratory 9 – l-Thyroxine experiments**

Estrous cycle / smear data for Individual Female Group A (n=5)										
Animal Number	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33
Vehicle Controls										
50101		E	E	D	D	P	E	D*		
50102		D	D	P	E	D*				
50103		P	M	D	D	D*				
50104		D	P	E	D	D*				
50105		D	P	E	D	D*				
Test Substance Dose Group One (low dose: 0.01 mg/kg body weight)										
50201		D	P	E	M	D*				
50202		D	D	E	D	D*				
50203		D	E	M	D	D*				
50204		M	D	D	P	E	D*			
50205		P	E	D	D	P	E	D*		
Test Substance Dose Group Two (mid or intermediate dose: 0.1 mg/kg body weight)										
50301		P	E	D	D	D*				
50302		P	E	D	D	D*				
50303		P	E	M	D	D*				
50304		P	E	D	D	D*				
50305		P	E	M	D	P	E	D*		
Test Substance Dose Group Three (high dose: 1 mg/kg body weight)										
50401		M	D	P	E	D*				
50402		E	E	M	D	P	E	D*		
50403		E	E	D	D	E	E	D*		
50404		P	E	D	D	E	E	D*		
50405		P	E	D	D	D*				

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

Table 14. Laboratory 9 – l-Thyroxine experiments

Estrous cycle / smear data for Individual Female Group B (n=5)										
Animal Number	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33
Vehicle Controls										
50106		E	D	D	D	P	E	D*		
50107		P	E	D	D	P	E	D*		
50108		D	D	P	E	D*				
50109		E	M	D	D	E	D*			
50110		P	E	M	D	P	E	D*		
Test Substance Dose Group One (low dose: 0.01 mg/kg body weight)										
50206		E	M	D	D	E	D*			
50207		D	P	E	E	M	D*			
50208		E	D	D	E	E	D*			
50209		D	D	D	D	D*				
50210		D	P	E	M	D*				
Test Substance Dose Group Two (mid or intermediate dose: 0.1 mg/kg body weight)										
50306		E	E	M	D	P	E	D*		
50307		P	E	M	D	P	E	D*		
50308		P	E	D	D	E	E	D*		
50309		E	E	D	P	E	E	D*		
50310		E	E	D	D	P	E	D*		
Test Substance Dose Group Three (high dose: 1 mg/kg body weight)										
50406		D	D	E	D	D*				
50407		M	D	P	E	D*				
50408		D	P	E	D	D*				
50409		P	E	D	D	E	E	D*		
50410		D	P	E	D	D*				

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

Table 15. Laboratory 13 – l-Thyroxine experiments

Estrous cycle / smear data for Individual Female (n=10)							
Animal Number	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32
Vehicle Controls							
41	P	M	D	D*			
42	D	D	D	E	D*		
43	M	D	D	E	M	D*	
44	P	M	D	P	E	M	D*
45	D	M	P	M	M	D*	
46	D	D	E	M	D*		
47	D	D	D	E	M	D*	
48	D	D	E	M	D*		
49	D	D	E	M	D*		
50	P	E	D	P	E	M	D*
l-Thyroxine Dose Group one (low dose: 0.0.1 mg/kg body weight)							
51	D	D	E	M	D*		
52	D	D	E	M	D*		
53	D	P	E	M	D*		
54	D	D	P	D*			
55	D	D	E	M	D*		
56	D	D	D	P	E	M	D*
57	D	P	M	D*			
58	P	D	D	D*			
59	P	D	D	E	M	D*	
60	E	M	D	P	E	M	D*

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

Table 15 continued. Laboratory 13 – l-Thyroxine experiments

Estrous cycle / smear data for Individual Female (n=10)							
Animal Number	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32
l-Thyroxine Dose Group Two (mid or intermediate dose: 0.1 mg/kg body weight)							
61	P	M	D	D*			
62	D	P	E	M	D*		
63	D	D	D	E	D*		
64	D	D	E	M	D*		
65	M	P	D	D*			
66	D	D	D	E	M	D*	
67	M	D	D	E	M	D*	
68	D	D	E	M	D*		
69	D	D	E	M	D*		
70	D	D	E	D*			
l-Thyroxine Dose Group Three (high dose: 1 mg/kg body weight)							
71	D	D	E	M	D*		
72	D	D	E	M	D*		
73	D	D	E	M	D*		
74	D	E	M	D*			
75	D	D	P	E	M	D*	
76	D	P	E	M	D*		
77	E	M	D	D*			
78	D	D	D	E	M	D*	
79	D	D	E	M	D*		
80	P	P	D	P	E	M	D*

D = diestrous; E = estrous; M = metestrous; P = proestrous

* = animal necropsied

Example: D * would indicate necropsied on a given day during diestrous according to the smears

ANNEX 10

Laboratory Measurements and Summary Results Generated for Phase-1 Feasibility Studies with the Updated TG 407

1. The purposes of this Annex are to describe the updated measurements made in various laboratories during Phase-1 feasibility studies for the TG 407. All studies were conducted for 28-days. However, these were with the exception of laboratory 2's study with flutamide not complete TG 407 studies, but only pilots to study the enhancements. A number of studies were conducted with only one sex which was believed to be the target sex for test substance. Many studies omitted the functional and motor batteries, and even some standard endpoints, particularly the more expensive and resource intensive histopathological measurements.
2. The measurements conducted to study possible enhancements are listed in Table 1 (tissue weights), Table 2 (Histopathology), and Table 3 (Hormonal, sperm, and estrous cycle).
3. Summary results of these measurements are described in the following set of tables. These are described as to whether the measurement achieved statistical significance and whether this was a significant increase (↑) or a significant decrease (↓). If a measurement was made and no significant change occurred, then no change is indicated by (↔). If the measurement was not made, or was not reported to the Secretariat, the table cell has been left blank. The summary results are presented in Table 4 (Male rats: Tissue weights, spermatology, and histopathology) and Table 5 (Female rats: Tissue weights, estrous cycle, and histopathology).
4. A particular area of interest was the feasibility of conducting and gaining valuable information from a battery of serum hormonal analyses. The summary results of these hormonal analyses are provided in Table 6, and the individual means recorded are provided in Table 7. The laboratories experienced considerable variation with a number of the hormonal analyses with CVs sometimes exceeding 100. The analyses were also expensive, as few laboratories had the on-site capability to perform the radioimmunoassays and samples had to be sent to external clinical laboratories. Further, the results were often not significant even with potent compounds and were at times erratic with sample sizes of n=5. As a result, the laboratories concluded that luteinising hormone, follicle stimulating hormone, testosterone, oestradiol, prolactin, and corticosterone would not be included in the enhancements studies in Phase-2. However, the three thyroid hormones, T₃, T₄, and thyroid stimulating hormone were to be retained.
5. As noted in the main body of the report, several of these studies have been subsequently published in the scientific literature as well as other related 407 studies. A reference list is included for the benefit of the reader.

407 Publications

- Andrews, P, Freyberger A, Hartmann E, Eiben R, Loof I, Schmidt U, Temerowski M, Becka M. (2001). Feasibility and potential gains of enhancing the subacute rat study protocol (OECD test guideline no. 407) by additional parameters selected to determine endocrine modulation. A pre-validation study to determine endocrine-mediated effects of the antiandrogenic drug flutamide. *Arch Toxicol* 75:65-73.
- Andrews, P, Freyberger A, Hartmann E, Eiben R, Loof I, Schmidt U, Temerowski M, Folkerts A, Stahl B, Kayser M. (2002). Sensitive detection of the endocrine effects of the estrogen analogue ethinylestradiol using a modified enhanced subacute rat study protocol (OECD test guideline no. 407). *Arch Toxicol* 76:194-202.
- Aso S, Anai M, Noda S, Imatanaka N, Yamasaki K, Maekawa A. (2000). Twenty-eight-day repeated-dose toxicity studies for detection of weak endocrine disrupting effects of nonylphenol and atrazine in female rats. *J. Toxicol. Pathol.* 13:13-20.
- Okazaki K, Okazaki S, Nishimura S, Nakamura H, Kitamura Y, Hatayama K, Nakamura A, Tsuda T, Katsumata T, Nishikawa A, Hirose M. (2001). A repeated dose (28 days) oral toxicity study of methoxychlor in rats, based on the 'Enhanced OECD test guideline 407' for screening for endocrine-disrupting chemicals. *Arch Toxicol* 75:513-521.
- Okazaki K, Imazawa T, Nakamura H, Fukukawa F, Nishikawa A, Hirose M. (2002). A repeated 28-day oral dose toxicity study of 17 α -methyltestosterone in rats, based on the 'Enhanced OECD test guideline 407' for screening for endocrine-disrupting chemicals. *Arch Toxicol* 75:635-642.
- Okazaki K, Okazaki S, Nakamura H, Kitamura Y, Hatayama K, Wakabayashi S, Tsuda T, Katsumata T, Nishikawa A, Hirose M. (2002). A repeated 28-day oral dose toxicity study of genistein in rats, based on the 'Enhanced OECD test guideline 407' for screening for endocrine-disrupting chemicals. *Arch Toxicol* 76:553-559.
- Toyoda K, Shibutani M, Tamura T, Koujitani T, Uneyama C, Hirose M. (2000). Repeated dose (28 days) oral toxicity study of flutamide in rats, based on the draft protocol for the 'Enhanced OECD test guideline 407' for screening for endocrine-disrupting chemicals. *Arch Toxicol* 74:127-132.
- Yamasaki, K, Sawaki M, Noda S, Imatanaka N, Takatsuki M. (2002). Subacute oral toxicity study of ethinylestradiol and bisphenol A, based on the draft protocol for the 'Enhanced OECD test guideline no. 407.' *Arch Toxicol* 76:65-74.
- Yamasaki, K, Tago Y, Nagai K, Sawaki M, Noda S, Takatsuki M. (2002). Comparison of toxicity studies based on the draft protocol for 'Enhanced OECD test guideline no. 407' and the research protocol of 'Pubertal Development and Thyroid Function in Immature Male Rats' with 6-*n*-propyl-2-thiouracil. *Arch Toxicol* 76:495-501.

**Table 1. Updated TG 407 Phase 1 Feasibility:
Enhanced tissue weight measurements performed**

Laboratory	Testes Separately (Right and Left) ¹	Seminal Vesicles with Coagulating Glands	Prostate	Thyroid	Ovaries
2	▲	▲	▲	▲	▲
5	▲	▲	▲	▲	▲
7	▲	▲	▲	▲	▲
8	▲	▲	▲		▲
9		▲		▲	▲
10	▲	▲	▲		▲
11	▲	▲	▲	▲	▲

¹ In the standard 407 protocol, both testes were previously weighed together.

**Table 2. Updated TG 407 Phase 1 Feasibility:
Histopathological analyses performed**

Laboratory	Pituitary	Vagina	Ovaries	One Testes	One Epididymidis	Seminal Vesicles and Coagulating Glands	Mammary glands
2	▲	▲	▲	▲	▲	▲	
5		▲	▲	▲	▲	▲	
7		▲	▲	▲	▲	▲	
8			▲	▲		▲	▲
9	▲	▲	▲	▲	▲	▲	
10			▲	▲		▲	
11			▲	▲	▲	▲	▲

**Table 3. Updated TG 407 Phase 1 Feasibility:
Hormone, sperm and estrous cycle enhancements performed**

Laboratory	Hormonal									Other			
	Lutenizing Hormone (LH)	Follicle Stimulating Hormone (FSH)	Testosterone	17 β -Estradiol	Prolactin	Corticosterone	Thyroid Stimulating Hormone (TSH)	T3	T4	Sperm number	Sperm motility	Sperm morphology	Estrous cycling
2	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
5	▲	▲	▲	▲	▲	▲				▲	▲		▲
7	▲	▲	▲ ²	▲ ²	▲	▲				▲	▲	▲	▲
8	▲ ²	▲ ²	▲	▲ ²	▲ ²					▲	▲	▲	▲
9							▲	▲	▲				
10										▲	▲	▲	▲
11	▲	▲	▲	▲	▲					▲ ¹	▲ ¹	▲ ¹	

¹ Performed with Subgroup A, but not Subgroup B

² Did not performed on both sexes or with both Subgroups

Table 4: Summary of Findings in Male Rats in Phase-1 of the Updated TG 407 Validation

Male Rats				Absolute Tissue Weights						Spermatology			Histopathology						
Chemical	Lab	Doses (mg/kg/d)	MTD	Pituitary	Thyroid	Prostate	Testes	Epididymis	ASG	Count	Motility	Morphology	Pituitary	Thyroid	Testis	Epididymis	prostate	ASG	Mammary Gland
Flutamide	2 Study A ^a	1, 10, 100	yes	↑	↔	↓	↔	↓	↓	↔	↔	↔	↑	↔	↑	↑	↑	↑	
	2 Study B ^a	1, 10, 100	yes	↔	↔	↓	↔	↓	↓	↔	↓	↔	↑	↔	↑	↑	↑	↑	
	8	0.25, 1, 4	no	↔		↔		↓?	↓?	↔	↔	↔			↔	↔			↑
	10	0.5, 3, 18	no			↓	↔	↓	↓	↓	↔	↔			↔	↔			
	11 Study A ^a	0.15, 0.6, 2.5, 4	no	↔	↔	↓DL	↔	↓?	↔	↔	↔	↔			↔	↔	↑	↑	↔
11 Study B ^a	0.15, .6, 2.5, 4	no	↔	↓	↓	↔	↔	↓						↔	↔	↑	↑	↑	
EE	5	3, 12, 48 (µg)	no	↑	↔	↔	↔			↔	↔				↔	↔			
Methyl testosterone	8	5, 20, 80	no				↓	↓	↔	↔	↔	↔			↑	↑			
Methoxychlor	7	25, 100, 400	yes	↑	↑	↓	↓			↓	↔				↑	↑	↑	↑	
Tamoxifen	7	12.5, 50, 200	yes	↔	↔	↔	↑			↔	↔				↔	↔			
Propyl thiouracil	9	0.01, 0.1, 1	yes	↑	↑								↑	↑	↔				

Summary of findings: ↑, increase; ↓, decrease; ↔, no change; equiv – equivocal; cell blank, not measured/no data submitted to Secretariat

MTD – maximum tolerated dose; ASG – male accessory sex glands as a unit, DL – dorsolateral prostate

^a Laboratories 2 and 11 performed the studies as two subgroups of 5 termed Study A and Study B, respectively.

Table 5: Summary of Findings in Female Rats in Phase-1 of the Updated TG 407 Validation

Female Rats				Absolute Tissue Weights				Estrus (Vaginal smears)	Histopathology					
Chemical	Lab	Doses (mg/kg/d)	MTD	Pituitary	Thyroid	Ovary	Uterus		Pituitary	Thyroid	Uterus	Vagina	Ovary	Mammary Gland
Flutamide	2 Study A ^a	1, 10, 100	yes	↔	↔	↔	↔	↔	↔	↔	↔	↔	↑	
	2 Study B ^a	1, 10, 100	yes	↓	↔	↔	↔	↔	↔	↔	↔	↔	↑	
	8	0.25, 1, 4	no	↔		↔		↔						↑
	10	0.5, 3, 18	no			↔		↔						
	11 Study A ^a	0.15, 0.6, 2.5, 4	no			↔					↔	↔	↑	
11 Study A ^a	0.15, 0.6, 2.5, 4	no			↔					↔	↔	↔		
EE	5	3, 12, 48 (µg)	Equiv.	↔	↑	↔	↔	↔			↑	↑	↔	
Methyl testosterone	8	5, 20, 80	no	↓		↓		↑			↑	↑	↑	↑
Methoxychlor	7	25, 100, 400	yes	↓	↔	↓	↑	↑			↑	↑	↑	
Tamoxifen	7	12.5, 50, 200	yes	↔	↔	↓	↓	↑			↑	↑	↑	
Propyl thiouracil	9	0.01, 0.1, 1	yes	↑	↑				↑	↑				

Summary of findings: ↑, increase/effect seen; ↓, decrease; ↔, no change; equiv - equivocal; cell blank, not measured/no data submitted to Secretariat
 MTD – maximum tolerated dose;

^a Laboratories 2 and 11 performed the studies as two subgroups of 5 termed Study A and Study B, respectively.

Table 6: Summary of Hormonal Studies in Male and Female Rats in Phase-1 of the Updated TG 407 Validation

Chemical	Lab	Sex	MTD	LH	FSH	Prolactin	Estradiol	Testosterone	Corticosterone	T3	T4	TSH	
Flutamide	2 Study A ^a	male	yes	↑	↑	↔	↑	↑	↔	↔	↓	↔	
	2 Study B ^a	male	yes	↑	↑	↔	↑	↑	↔	↔	↔	↔	
	2 Study A ^a	female	yes	↔	↔	↔	↔	↔	↔	↔	↔	↔	
	2 Study B ^a	female	yes	↔	↔	↔	↔	↔	↔	↔	↔	↔	
	8	male	no	↔	↔	↔	↑	↑					
		female	no	↔	↔								
	11 Study A ^a	male	no	↔	↔	↔	↔	↑					
11 Study B ^a	male	no	↔	↔	↔	↔	↔						
EE	5	male	yes	↔	↔	↑	↔	↔	↔				
		female	yes	↔	↔	↔	↔		↔				
Methyl Testosterone	8	male	no					↔					
		female	no	↔	↑	↔	↔	↔					
Methoxychlor	7	male	yes	↔	↑	↑	↔	↔	↔				
		female	yes	↔	↑	↔	↔		↔				
Tamoxifen	7	male	yes	↑	↔	↑		↓	↔				
		female	yes	↔	↑	↔	↔		↔				
Propyl Thiouracil	9	male	yes							↓	↓	↑	
		female	yes							↔	↓	↑	

Summary of findings: ↑, increase; ↓, decrease; ↔, no change; blank, not measured/no data

MTD – maximum tolerated dose; LH – leutinising hormone; FSH – follicle stimulating hormone; TSH – thyroid stimulating hormone

^a Laboratories 2 and 11 performed the studies as two subgroups of 5 termed Study A and Study B, respectively.

Table 7: Compilation of Mean Hormonal Values in Phase-1 of the Updated TG 407 Validation

Chemical	Lab	Sex	Dose	LH	FSH	Prolactin	Estradiol	Testost.	Corticoster.	T3	T4	TSH
				ng/mL	ng/mL	ng/mL	pg/mL	ng/mL	ng/mL	nmol/L	nmol/L	mmol/L
Flutamide	2 Study A ^a	male	C	0.37	1.03	20.97	93.1	0.58	159.6	1.89	52.1	1.46
			Low	0.32	1.59	22.40	95.5	1.82	165.2	1.86	51.3	2.32
			Mid	1.06	4.80*	25.67	96.2	6.23*	66.6	1.87	56.7	3.25
			High	2.52*	8.14*	25.31	111.2*	9.62*	162.0	1.66	38.4*	3.98
	2 Study B ^a	male	C	0.60	1.54	16.95	99.4	1.27	183.9	1.85	46.2	3.01
			Low	0.73	2.26	19.72	104.1	2.44	116.3	1.84	55.6	2.40
			Mid	1.40*	5.13	29.06	100.8	4.95	268.9	1.89	50.7	2.25
			High	2.71*	9.19*	21.24	115.2*	11.53*	136.6	1.66	38.2	1.93
	2 Study A ^a	fem.	C	1.77	1.28	110.98	120.2	0.03	94.24	1.86	37.2	1.46
			Low	1.92	1.09	127.99	123.0	0.03	83.19	1.84	28.8	1.60
			Mid	1.57	0.63	103.81	115.5	0.03	66.21	1.89	32.5	1.23
			High	2.33	1.52	89.08	114.9	0.03	91.56	1.86	29.3	2.32
	2 Study B ^a	fem.	C	1.88	2.14	122.76	118.7	0.03	66.85	2.04	34.7	1.85
			Low	1.87	2.38	85.84	128.7	0.03	91.87	2.02	40.7	2.61
			Mid	1.68	1.78	71.18	127.9	0.03	68.60	2.04	36.4	1.88
			High	1.72	1.71	71.23	133.2	0.03	83.60	1.96	34.9	1.82
Flutamide	8	male	C	3.0	17.5	22.9	4.7	2.2				
			Low	2.9	15.9	17.7	4.8	2.3				
			Mid	3.1	17.7	28.3	5.2	3.0				
			High	3.3	19.1	28.9	5.7*	3.9*				
		fem.	C	2.8	11.1							
			Low	2.7	10.6							
			Mid	2.8	10.3							
			High	2.9	12.6							
Flutamide	11 Study A ^a	male	C	1.7	8.92	12.9	< 2.50	1.5				
			1	1.6	10.73	24.6	< 2.50	2.7				
			2	< 1.6	10.42	14.7	< 2.50	2.4				
			3	< 1.9	11.85	14.3	< 2.50	3.6*				
			4	2.1	12.00	18.5	< 2.50	4.8*				
	11 Study B ^a	male	C	< 1.4	10.2	7.29	< 2.50	1.9				
			1	< 1.2	10.2	< 7.76	< 2.50	1.9				
			2	1.6	11.4	8.16	< 2.50	2.3				
			3	1.8	12.8	< 14.60	< 2.50	1.9				
			4	1.9	13.2	12.34	< 2.50	3.8				

* - Statistically significant; < one or more samples were below the limit of detection (LOD), and the LOD value was used to generate the mean. C - control

^a Laboratories 2 and 11 performed the studies as two subgroups of 5 termed Study A and Study B, respectively.

Table 7 continued: Compilation of Mean Hormonal Values in Phase-1 of the Updated TG 407 Validation

Chemical	Lab	Sex	Dose	LH	FSH	Prolactin	Estradiol	Testosterone	Corticoster.	T3	T4	TSH	
				ng/mL	ng/mL	ng/mL	pg/mL	ng/mL	ng/mL	nmol/L	nmol/L	mmol/L	
Ethinyl estradiol	5	male	C	13.6	313	62		2.82	137				
			Low	15.7	305	67		3.49	65				
			Mid	13.5	282	83	11	4.17	80				
			High	12.9	310	162*	13	2.59	49				
		fem.	C	13.1	210	37	17		413				
			Low	10.9*	230	34	17		243				
			Mid	13.4	251	44	18		235				
			High	13.0	300	55	15		306				
Methyl testosterone	8	male	C					1.6					
			Low					3.2					
			Mid					2.6					
			High					1.1					
		fem.	C	2.6	9.8	12.1	11.4	< 0.1					
			Low	2.7	10.2	99.1	11.5	< 0.1					
			Mid	2.6	10.0	12.2	8.3	< 0.1					
			High	2.5	11.7*	24.8	8.7	< 0.1					
Methoxy-chlor	7	male	C	7.7	179	36	12	2.43	88				
			Low	9.8	191	44	12	2.26	42				
			Mid	10.3	256*	104*	9	0.72	50				
			High	9.9	324*	114*	12	0.85	64				
		fem.	C	6.1	249	39	26		176				
			Low	6.7	317	47	16		163				
			Mid	5.9	349	218	14		62				
			High	6.3	394*	122	9		141				
Tamoxifen	7	male	C	8.9	236	41		2.87	74				
			Low	9.9	214	74*		2.04	75				
			Mid	11.7*	225	98*		1.37*	107				
			High	12.1*	243	83*		1.38	114				
		fem.	C	10.3	162	42	12		117				
			Low	9.9	234	56	21		124				
			Mid	9.3	291*	151	14		133				
			High	9.2	248*	193	9		123				
Propyl thiouracil	9	male	C							82.7	5.88	6.54	
			Low							66.5	5.26	6.06	
			Mid							67.2	4.20*	8.06	
			High							41.7*	< LOD	34.4*	
		fem.	C								80.6 (7.2)	3.40 (1.03)	5.08 (1.63)
			Low								65.1 (9.4)	3.72 (.73)	5.54 (1.93)
			Mid								60.2 (10.9)	2.52 (.66)	5.92 (2.25)
			High								71.9 (17.8)	.96 (1.01)*	25.5 (12.2)*

* - Statistically significant; < one or more samples were below the limit of detection (LOD), and the LOD value was used to generate the mean. C - control