Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

13-Jul-2012

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

REPORT ON THE RETROSPECTIVE ANALYSIS OF 2-GENERATION REPROTOXICITY DATA

Series on Testing and Assessment

No. 176

This document is only available in PDF format.

JT03324751

ENV/JM/MONO(2012)21
Unclassified
English - Or. English

OECD Environment, Health and Safety Publications
Series on Testing and Assessment

No. 176

REPORT ON THE RETROSPECTIVE ANALYSIS OF 2-GENERATION REPROTOXICITY DATA

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS
A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris 2012

Also published in the Series on Testing and Assessment:

No. 1, *Guidance Document for the Development of OECD Guidelines for Testing of Chemicals (1993; reformatted 1995, most recently revised 2009)*

No. 2, *Detailed Review Paper on Biodegradability Testing (1995)*

No. 3, *Guidance Document for Aquatic Effects Assessment (1995)*

No. 4, *Report of the OECD Workshop on Environmental Hazard/Risk Assessment (1995)*

No. 5, *Report of the SETAC/OECD Workshop on Avian Toxicity Testing (1996)*

No. 6, *Report of the Final Ring-test of the Daphnia magna Reproduction Test (1997)*

No. 7, *Guidance Document on Direct Phototransformation of Chemicals in Water (1997)*

No. 8, *Report of the OECD Workshop on Sharing Information about New Industrial Chemicals Assessment (1997)*

No. 9, *Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides during Agricultural Application (1997)*

No. 10, *Report of the OECD Workshop on Statistical Analysis of Aquatic Toxicity Data (1998)*

No. 11, *Detailed Review Paper on Aquatic Testing Methods for Pesticides and industrial Chemicals (1998)*

No. 12, *Detailed Review Document on Classification Systems for Germ Cell Mutagenicity in OECD Member Countries (1998)*

No. 13, *Detailed Review Document on Classification Systems for Sensitising Substances in OECD Member Countries 1998)*

No. 14, *Detailed Review Document on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries (1998)*

No. 15, *Detailed Review Document on Classification Systems for Reproductive Toxicity in OECD Member Countries (1998)*

No. 16, *Detailed Review Document on Classification Systems for Skin Irritation/Corrosion in OECD Member Countries (1998)*

No. 17, *Environmental Exposure Assessment Strategies for Existing Industrial Chemicals in OECD Member Countries (1999)*

No. 18, *Report of the OECD Workshop on Improving the Use of Monitoring Data in the Exposure Assessment of Industrial Chemicals (2000)*

No. 19, *Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals used in Safety Evaluation (1999)*

No. 20, *Revised Draft Guidance Document for Neurotoxicity Testing (2004)*

No. 21, *Detailed Review Paper: Appraisal of Test Methods for Sex Hormone Disrupting Chemicals (2000)*

No. 22, *Guidance Document for the Performance of Out-door Monolith Lysimeter Studies (2000)*

No. 23, *Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (2000)*

No. 24, *Guidance Document on Acute Oral Toxicity Testing (2001)*

No. 25, *Detailed Review Document on Hazard Classification Systems for Specifics Target Organ Systemic Toxicity Repeated Exposure in OECD Member Countries (2001)*

No. 26, *Revised Analysis of Responses Received from Member Countries to the Questionnaire on Regulatory Acute Toxicity Data Needs (2001)*

No 27, *Guidance Document on the Use of the Harmonised System for the Classification of Chemicals which are Hazardous for the Aquatic Environment (2001)*

No 28, *Guidance Document for the Conduct of Skin Absorption Studies (2004)*

No 29, *Guidance Document on Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media (2001)*

No 30, *Detailed Review Document on Hazard Classification Systems for Mixtures (2001)*

No 31, *Detailed Review Paper on Non-Genotoxic Carcinogens Detection: The Performance of In-Vitro Cell Transformation Assays (2007)*

No. 32, *Guidance Notes for Analysis and Evaluation of Repeat-Dose Toxicity Studies (2000)*

No. 33, *Harmonised Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures (2001)*

No. 34, *Guidance Document on the Development, Validation and Regulatory Acceptance of New and Updated Internationally Acceptable Test Methods in Hazard Assessment (2005)*

No. 35, *Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies (2002)*

No. 36, *Report of the OECD/UNEP Workshop on the Use of Multimedia Models for Estimating Overall Environmental Persistence and Long Range Transport in the Context of PBTS/POPS Assessment (2002)*

No. 37, *Detailed Review Document on Classification Systems for Substances which Pose an Aspiration Hazard (2002)*

No. 38, *Detailed Background Review of the Uterotrophic Assay Summary of the Available Literature in Support of the Project of the OECD Task Force on Endocrine Disrupters Testing and Assessment (EDTA) to Standardise and Validate the Uterotrophic Assay (2003)*

No. 39, *Guidance Document on Acute Inhalation Toxicity Testing (2009)*

No. 40, *Detailed Review Document on Classification in OECD Member Countries of Substances and Mixtures which Cause Respiratory Tract Irritation and Corrosion (2003)*

No. 41, *Detailed Review Document on Classification in OECD Member Countries of Substances and Mixtures which in Contact with Water Release Toxic Gases (2003)*

No. 42, *Guidance Document on Reporting Summary Information on Environmental, Occupational and Consumer Exposure (2003)*

No. 43, *Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment (2008)*

No. 44, *Description of Selected Key Generic Terms Used in Chemical Hazard/Risk Assessment (2003)*

No. 45, *Guidance Document on the Use of Multimedia Models for Estimating Overall Environmental Persistence and Long-range Transport (2004)*

No. 46, *Detailed Review Paper on Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances (2004)*

No. 47, *Detailed Review Paper on Fish Screening Assays for the Detection of Endocrine Active Substances (2004)*

No. 48, *New Chemical Assessment Comparisons and Implications for Work Sharing (2004)*

No. 49, *Report from the Expert Group on (Quantitative) Structure-Activity Relationships [(Q)SARs] on the Principles for the Validation of (Q)SARs (2004)*

No. 50, *Report of the OECD/IPCS Workshop on Toxicogenomics (2005)*

No. 51, *Approaches to Exposure Assessment in OECD Member Countries: Report from the Policy Dialogue on Exposure Assessment in June 2005 (2006)*

No. 52, *Comparison of Emission Estimation Methods used in Pollutant Release and Transfer Registers (PRTRs) and Emission Scenario Documents (ESDs): Case Study of Pulp and Paper and Textile Sectors (2006)*

No. 53, *Guidance Document on Simulated Freshwater Lentic Field Tests (Outdoor Microcosms and Mesocosms) (2006)*

No. 54, *Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application (2006)*

No. 55, *Detailed Review Paper on Aquatic Arthropods in Life Cycle Toxicity Tests with an Emphasis on Developmental, Reproductive and Endocrine Disruptive Effects (2006)*

No. 56, *Guidance Document on the Breakdown of Organic Matter in Litter Bags (2006)*

No. 57, *Detailed Review Paper on Thyroid Hormone Disruption Assays (2006)*

No. 58, *Report on the Regulatory Uses and Applications in OECD Member Countries of (Quantitative) Structure-Activity Relationship [(Q)SAR] Models in the Assessment of New and Existing Chemicals (2006)*

No. 59, *Report of the Validation of the Updated Test Guideline 407: Repeat Dose 28-Day Oral Toxicity Study in Laboratory Rats (2006)*

No. 60, *Report of the Initial Work towards the Validation of the 21-Day Fish Screening Assay for the Detection of Endocrine Active Substances (Phase 1A) (2006)*

No. 61, *Report of the Validation of the 21-Day Fish Screening Assay for the Detection of Endocrine Active Substances (Phase 1B) (2006)*

No. 62, *Final OECD Report of the Initial Work towards the Validation of the Rat Hershberger Assay: Phase-1, Androgenic Response to Testosterone Propionate, and Anti-Androgenic Effects of Flutamide (2006)*

No. 63, *Guidance Document on the Definition of Residue (2006)*

No. 64, *Guidance Document on Overview of Residue Chemistry Studies (2006)*

No. 65, *OECD Report of the Initial Work towards the Validation of the Rodent Uterotrophic Assay - Phase 1 (2006)*

No. 66, *OECD Report of the Validation of the Rodent Uterotrophic Bioassay: Phase 2. Testing of Potent and Weak Oestrogen Agonists by Multiple Laboratories (2006)*

No. 67, *Additional Data Supporting the Test Guideline on the Uterotrophic Bioassay in rodents (2007)*

No. 68, *Summary Report of the Uterotrophic Bioassay Peer Review Panel, including Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow up of this Report (2006)*

No. 69, *Guidance Document on the Validation of (Quantitative) Structure-Activity Relationship [(Q)SAR] Models (2007)*

No. 70, *Report on the Preparation of GHS Implementation by the OECD Countries (2007)*

No. 71, *Guidance Document on the Uterotrophic Bioassay - Procedure to Test for Antioestrogenicity (2007)*

No. 72, *Guidance Document on Pesticide Residue Analytical Methods (2007)*

No. 73, *Report of the Validation of the Rat Hershberger Assay: Phase 3: Coded Testing of Androgen Agonists, Androgen Antagonists and Negative Reference Chemicals by Multiple Laboratories. Surgical Castrate Model Protocol (2007)*

No. 74, *Detailed Review Paper for Avian Two-generation Toxicity Testing (2007)*

No. 75, *Guidance Document on the Honey Bee (Apis Mellifera L.) Brood test Under Semi-field Conditions (2007)*

No. 76, *Final Report of the Validation of the Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances: Phase 1 - Optimisation of the Test Protocol (2007)*

No. 77, *Final Report of the Validation of the Amphibian Metamorphosis Assay: Phase 2 - Multi-chemical Interlaboratory Study (2007)*

No. 78, *Final Report of the Validation of the 21-day Fish Screening Assay for the Detection of Endocrine Active Substances. Phase 2: Testing Negative Substances (2007)*

No. 79, *Validation Report of the Full Life-cycle Test with the Harpacticoid Copepods Nitocra Spinipes and Amphiascus Tenuiremis and the Calanoid Copepod Acartia Tonsa - Phase 1 (2007)*

No. 80, *Guidance on Grouping of Chemicals (2007)*

No. 81, *Summary Report of the Validation Peer Review for the Updated Test Guideline 407, and Agreement of the Working Group of National Coordinators of the Test Guidelines Programme on the Follow-up of this Report (2007)*

No. 82, *Guidance Document on Amphibian Thyroid Histology (2007)*

No. 83, *Summary Report of the Peer Review Panel on the Stably Transfected Transcriptional Activation Assay for Detecting Estrogenic Activity of Chemicals, and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report (2007)*

No. 84, *Report on the Workshop on the Application of the GHS Classification Criteria to HPV Chemicals, 5-6 July, Bern Switzerland (2007)*

No. 85, *Report of the Validation Peer Review for the Hershberger Bioassay, and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report (2007)*

No. 86, *Report of the OECD Validation of the Rodent Hershberger Bioassay: Phase 2: Testing of Androgen Agonists, Androgen Antagonists and a 5 α-Reductase Inhibitor in Dose Response Studies by Multiple Laboratories (2008)*

No. 87, *Report of the Ring Test and Statistical Analysis of Performance of the Guidance on Transformation/Dissolution of*

Metals and Metal Compounds in Aqueous Media (Transformation/ Dissolution Protocol) (2008)

No. 88, *Workshop on Integrated Approaches to Testing and Assessment (2008)*

No. 89, *Retrospective Performance Assessment of the Test Guideline 426 on Developmental Neurotoxicity (2008)*

No.90, *Background Review Document on the Rodent Hershberger Bioassay (2008)*

No. 91, *Report of the Validation of the Amphibian Metamorphosis Assay (Phase 3) (2008)*

No. 92, *Report of the Validation Peer Review for the Amphibian Metamorphosis Assay and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report (2008)*

No. 93, *Report of the Validation of an Enhancement of OECD TG 211: Daphnia Magna Reproduction Test (2008)*

No. 94, *Report of the Validation Peer Review for the 21-Day Fish Endocrine Screening Assay and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report (2008)*

No. 95, *Detailed Review Paper on Fish Life-Cycle Tests (2008)*

No.96, *Guidance Document on Magnitude of Pesticide Residues in Processed Commodities (2008)*

No.97, *Detailed Review Paper on the use of Metabolising Systems for In Vitro Testing of Endocrine Disruptors (2008)*

No. 98, *Considerations Regarding Applicability of the Guidance on Transformation/Dissolution of Metals Compounds in Aqueous Media (Transformation/Dissolution Protocol) (2008)*

No. 99, *Comparison between OECD Test Guidelines and ISO Standards in the Areas of Ecotoxicology and Health Effects (2008)*

No. 100, *Report of the Second Survey on Available Omics Tools (2009)*

No. 101, *Report of the Workshop on Structural Alerts for the OECD (Q)SAR Application Toolbox, 15-16 May 2008, Utrecht, the Netherlands (2009)*

No. 102, *Guidance Document for using the OECD (Q)SAR Application Toolbox to Develop Chemical Categories According to the OECD Guidance on Grouping of Chemicals (2009)*

No. 103, *Detailed Review Paper on Transgenic Rodent Mutation Assays (2009)*

No. 104, *Performance Assessment: Comparsion of 403 and CxT Protocols via Simulation and for Selected Real Data Sets (2009)*

No. 105, *Report on Biostatistical Performance Assessment of the draft TG 436: Acute Toxic Class Testing Method for Acute Inhalation Toxicity (2009)*

No. 106, *Guidance Document for Histologic Evaluation of Endocrine and Reproductive Test in Rodents (2009)*

No. 107, *Preservative Treated Wood to the Environment for Wood held in Storage after Treatment and for Wooden Commodities that are not Covered and are not in contact with Ground. (2009)*

No. 108, *Report of the Validation of the Hershberger Bioassay (weanling model) (2009)*

No. 109, *Literature review on the 21-Day Fish Assay and the Fish Short-Term Reproduction Assay (2009)*

No. 110, *Report of the Validation Peer Review for the Weanling Hershberger Bioassay and Agreement of the Working Group of National Coordinators of the Test Guidelines Programme on the Follow-up of this Report (2009)*

No. 111, *Report of the Expert Consultation to Evaluate an Estrogen Receptor Binding Affinity Model for Hazard Identification (2009)*

No. 112, *The 2007 OECD List of High Production Volume Chemicals (2009)*

No. 113, *Report of the Focus Session on Current and Forthcoming Approaches for Chemical Safety and Animal Welfare (2010)*

No. 114, *Performance Assessment of Different Cytotoxic and Cytostatic Measures for the In Vitro Micronucleus Test (MNVIT): Summary of results in the collaborative trial (2010)*

No. 115, *Guidance Document on the Weanling Hershberger Bioassay in Rats: A Short-term Screening Assay for (Anti) Androgenic Properties (2009)*

No. 116, *Guidance Document 116 on the Conduct and Design of Chronic Toxicity and Carcinogenicity Studies, Supporting Test Guidelines 451, 452 and 453 – 2nd Edition (2011)*

No. 117, *Guidance Document 117 on the Current Implementation of Internal Triggers in Test Guideline 443 for an Extended One Generation Reproductive Toxicity Study, in the United States and Canada (2011)*

No. 118, *Workshop Report on OECD Countries' Activities Regarding Testing, Assessment and Management of Endocrine Disrupters Part I and Part II (2010)*

No. 119, *Classification and Labelling of chemicals according to the UN Globally Harmonized System: Outcome of the Analysis of Classification of Selected Chemicals Listed in Annex III of the Rotterdam Convention (2010)*

No. 120, *Part 1: Report of the Expert Consultation on Scientific and Regulatory Evaluation of Organic Chemistry Mechanism-based Structural Alerts for the Identification of DNA Binding Chemicals (2010)*

No. 120, *Part 2: Report of the Expert Consultation on Scientific and Regulatory Evaluation of Organic Chemistry Mechanism-based Structural Alerts for the Identification of DNA Binding Chemicals (2010)*

No. 121, *Detailed Review Paper (DRP) on Molluscs Life-cycle Toxicity Testing (2010)*

No. 122, *Guidance Document on the Determination of the Toxicity of a Test Chemical to the Dung Beetle Aphodius Constans (2010)*

No. 123, *Guidance Document on the Diagnosis of Endocrine-related Histopathology in Fish Gonads (2010)*

No. 124, *Guidance for the Derivation of an Acute Reference Dose (2010)*

No. 125, *Guidance Document on Histopathology for Inhalation Toxicity Studies, Supporting TG 412 (Subacute Inhalation Toxicity: 28-Day) and TG 413 (Subchronic Inhalation Toxicity: 90-Day) (2010)*

No. 126, *Short Guidance on the Threshold approach for Acute Fish Toxicity (2010)*

No. 127, *Peer Review Report of the Validation of the 21-day Androgenised Female Stickleback Screening Assay (2010)*

No. 128, *Validation Report of the 21-Day Androgenised Female Stickleback Screening Assay (2010)*

No. 129, *Guidance Document on using Cytotoxicity Tests to Estimate Starting Doses for Acute Oral Systemic Toxicity Tests(2010)*

No. 131, *Report of the Test Method Validation of Avian Acute Oral Toxicity Test (OECD Test Guideline 223) (2010)*

No. 132, *Report of the Multi-Laboratory Validation of the H295R Steroidogenesis Assay to Identify Modulators (2010)*

No.133, *Peer Review Report for the H295R Cell-Based Assay for Steroidogenesis (2010)*

No.134, *Report of the Validation of a Soil Bioaccumulation Test with Terrestrial Oligochaetes by an International ring test (2010)*

No.135, *Detailed Review Paper on Environmental Endocrine Disruptor Screening: The use of Estrogen and Androgen Receptor Binding and Transactivation Assays in Fish (2010)*

No. 136, *Validation Report of the Chironomid Full Life-Cycle Toxicity Test (2010)*

No. 137, *Explanatory Background Document to the OECD Test Guideline on In Vitro Skin Irritation Testing (2010)*

No. 138, *Report of the Workshop on Using Mechanistic Information in Forming Chemical Categories (2011)*

No. 139, *Report of the Expert Consultation on Scientific and Regulatory Evaluation of Organic Chemistry Mechanism Based Structural Alerts for the Identification of Protein-binding Chemicals (2011)*

No. 140, *Report of the WHO/OECD/ILSI (Hesi) Workshop on Risk Assessment of Combined Exposures to Multiple Chemicals (2011)*

No. 141, *Report of the Phase 1 of the Validation of the Fish Sexual Development Test for the Detection of Endocrine Active Substances (2011)*

No. 142, *Report of the Phase 2 of the Validation of the Fish Sexual Development Test for the Detection of Endocrine Active Substances (2011)*

No. 143, *Peer Review Report for the Validation of the Fish Sexual Development Test and Agreement of the Working Group of National*

Co-ordinators of the Test Guideline Programme on the Follow-up of the Peer Review (2011)

No. 144, *Validation Report for the Acute Chironomid Assay (2011)*

No. 145, *Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay: Retrospective Performance Assessment (2011)*

No. 146, *Syrian Hamster Embryonic (SHE) Cell PH 6.7 Cell Transformation Assay Prevalidation Study Report (2012)*

No. 147, *Syrian Hamster Embryonic (SHE) Cell PH 7.0 Cell Transformation Assay Prevalidation Study Report (2012)*

No. 148, *Guidance Document on the Androgenised Female Stickleback Screen (2011)*

No. 149, *Validation Report of the Balb/c 3T3 Cell Transformation Assay (2012)*

No. 152, *Case Study: Assessment of an Extended Chemical Category, the Short-chain Methacrylates, Targeted on Bioaccumulation (2011)*

No. 153, *Guidance Document for the Derivation of an Acute Reference Concentration (Arfc) (2011)*

No. 154, *Validation Report: Part 1 – Validation of Efficacy Methods for Antimicrobials used on Hard Surfaces (2011)*

No. 154, *Validation Report: Part 2 – Validation of Efficacy Methods for Antimicrobials used on Hard Surfaces (2011)*

No. 155, *Peer Review for the Validation of the Modified Skin Irritation Test Method using LabyCyte EPI-MODEL24; Additional Studies; and Agreement of the Working Group of National Coordinators on the Follow-up to the Peer Review* (2011)

No. 156, *Guidance Notes on Dermal Absorption* (2011)

No. 157, *Validation Report Phase 1 for the Zebrafish Embryo Toxicity Test (2011)*

No. 158, *Report of Progress on the Interlaboratory Validation of the OECD Harpacticoid Copepod Development and Reproduction Test (2011)*

No. 159, *Validation Report for the Skin Irritation Test Method using Labcyte Epi-Model24 (2011)*

No. 160, *Guidance Document on the Bovine Corneal Opacity and Permeability (BCOP) and Isolated Chicken Eye (ICE) Test Methods: Collection of Tissues for Histological Evaluation and Collection of Data on Non-Severe Irritants (2011)*

No. 161, *Peer Review Report for the Validation of the Stably Transfected Transcriptional Activation Assay for the Detection of Androgenic and Anti-Androgenic Activity of Chemicals (2011)*

No. 165, *Guidance Document on Crop Field Trials (2011)*

No. 166, *SIDS Initial Assessment Profiles agreed in the course of the OECD HPV Chemicals Programme from 1993 to 2013 (2012)*

No. 167, *Crosswalk of Harmonized U.S. - Canada Industrial Function and Consumer and Commercial Product Categories with EU Chemical Product and Article Categories (2012)*

No. 168, *The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins Binding to Proteins (2012)*

No. 169, *Use of the AOP to Develop Chemical Categories and Integrated Assessment and Testing Approaches (2012)*

No. 170, *Draft Guidance Document for Demonstrating Efficacy of Pool and Spa Disinfectants and Field Testing (2012)*

ABOUT THE OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 34 industrialised countries in North and South America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in eleven different series: **Testing and Assessment; Good Laboratory Practice and Compliance Monitoring; Pesticides; Biocides; Risk Management; Harmonisation of Regulatory Oversight in Biotechnology; Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; and Safety of Manufactured Nanomaterials.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (www.oecd.org/ehs/).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organisations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. UNDP is an observer. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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

FOREWORD

This document presents the report by the Netherlands on the 2010 retrospective analysis of two-generation reprotoxicity data, which was performed to assist in finalising the development of the Test Guideline for an Extended One-Generation Reproductive Toxicity Study (TG 443). The document also presents the conclusions regarding whether the information available from the production of a second generation would change the conclusion on the hazard characterization for risk assessment and/or the Globally Harmonized System (GHS) hazard classification of chemicals. These conclusions were developed at the combined meeting of the Working Group of the National Coordinators of the Test Guidelines Programme (WNT) and expert group on reproductive toxicity (Arlington, Virginia, US) on 19-21 October 2010.

The Dutch report of the 2010 retrospective analysis of two-generation reprotoxicity data is included in Appendix 1. The analysis procedure used in the retrospective analysis is included in Appendix 2, and the boundaries of the retrospective analysis are included in Appendix 3. The US report on the 2009 retrospective analysis of 350 multi-generation reproduction toxicity studies is included in Appendix 4.

The Joint Meeting of the Chemicals Committee and the working Party on Chemicals, Pesticides and Biotechnology (Joint Meeting) agreed to the declassification of this document on November 2010.

This document is published under the responsibility of the Joint Meeting.

TABLE OF CONTENTS

Introduction 20
Development of the Third Retrospective Data Analysis 20
Conclusions on whether the information available from the production of a second generation would change the conclusion on the hazard characterization for risk assessment and/or the GHS hazard classification of chemicals 21
Appendix 1: 2010 Retrospective Analysis of Two-Generation Reprotoxicity Data 22
Part I: Combined retrospective analysis of rat multi-generation reproductive toxicity studies: on the impact of parameters related to F1 mating and F2 offspring 22
Part II: On the impact of P1 mating and F2 offspring effects for EU Classification & Labelling 42
Annex I: Effects tables for studies indicated in the text, where the P1/F2 effects that have not been seen at lower dose levels or in other generations might have influenced Classification and Labelling. These effects are given in bold. The Dose Level (DL) which was determined to be the study reproductive LOAEL is indicated with a * 55
Annex II: EU Technical Committee on Classification & Labelling meeting minutes excerpts on the classification for reproductive toxicity of the substances: 60
Appendix 3: Boundaries of the Retrospective Data Analysis 86
Appendix 4: 2009 Retrospective Analysis of Two-Generation Reprotoxicity Data 88

INTRODUCTION

1. The project for developing a new Test Guideline (TG) on reproductive toxicity was included in the work plan of the Test Guideline Programme in 2007. The United States, Germany and the Netherlands led the project and submitted a first draft Test Guideline for an Extended One-Generation Reproductive Toxicity Study (EOGRTS) in 2008 for discussion by an Expert Group on reproductive toxicity. The aim of the project was to develop an improved, more efficient and less animal consuming OECD Test Guideline for testing toxicity to reproduction. It was expected to help harmonise the approach used for testing across various classes of chemicals and to improve integrated risk assessment using all information for the relevant reproductive endpoints, e.g. fertility and developmental toxicity. The project was based on a first retrospective analysis.

2. In order to assess the potential as well as the limitations of the proposed Test Guideline for an EOGRTS, a second retrospective analysis of 350 multi-generation reproduction toxicity studies from the United States and Canada was performed and presented to the Expert Group at its October 2009 meeting. This analysis aimed at assessing the contribution of the effects observed only in the second generation (F2) to hazard identification and characterisation, as well as to decisions on classification. However, experts could not reach agreement on a Test Guideline study design on the basis of that retrospective analysis report.

3. In February 2010, the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticide and Biotechnology (JM) agreed that, before taking a final decision on the draft EOGRTS Test Guideline, a third retrospective analysis on the 2-generation reproductive toxicity studies should be performed and should involve all interested countries.

Development of the Third Retrospective Data Analysis

4. The work on the third retrospective analysis started after the February 2010 Joint Meeting, led jointly by the Netherlands and the United States, on the basis of a wider 2-generation reproductive toxicity study database: the Netherlands included additional data from the RIVM data base, from Germany and from Notox B.V. (Netherlands). The 3rd Retrospective analysis aimed at combining the previous analyses and including newly identified examples using a single combined database, aiming at comprehensiveness in terms of the substances included. Approximately 150 additional 2-generation studies were added to the existing 350 studies (from US and Canada), leading to a total of about 500 studies in the Database.

5. Since the last Joint Meeting and prior to the November Joint Meeting, there were three meetings of the Expert Group and monthly conference calls. The first meeting was held in the Netherlands on 22 June 2010, to discuss the framework of the retrospective analysis. The second one was held in the Netherlands on 14 September 2010, to discuss the draft retrospective analysis report that was provided by the Netherlands at the end of August. The third one was a combined meeting of the Expert Group and National Coordinators for the Test Guidelines Programme; it was held in Arlington, Virginia, USA, on 19-21 October 2010.

6. The report of the retrospective analysis is as submitted by the Netherlands. Comments from the Arlington meeting were considered in order to develop the following conclusions on whether the information available from the production of a second generation would change the conclusions of the hazard characterization for risk assessment and/or the GHS hazard classification of chemicals.

Conclusions on whether the information available from the production of a second generation would change the conclusion on the hazard characterization for risk assessment and/or the GHS hazard classification of chemicals

Most of the National Coordinators and experts attending the Arlington meeting agreed that the extensive retrospective analyses indicated that the production of a 2nd generation would rarely affect hazard characterization either for risk assessment or for GHS hazard classification of chemicals,

A few participants at Arlington meeting disagreed with this conclusion. These participants considered that, based on examples provided and due to remaining scientific uncertainties with the analysis, it cannot be excluded that the 2nd generation would affect the hazard characterization, either for risk assessment or for GHS hazard classification of chemicals in a substantial number of studies.

APPENDIX 1: 2010 RETROSPECTIVE ANALYSIS OF TWO-GENERATION REPROTOXICITY DATA

Part I: Combined retrospective analysis of rat multi-generation reproductive toxicity studies: on the impact of parameters related to F1 mating and F2 offspring

A.H. Piersma [1,4], E. Rorije[2], E. Schenk[1], A. Muller[3], B.C. Hakkert[2]
Laboratory for Health Protection Research[1], Substance Expertise Center[2], Center for Substances and Integrated Risk Assessment[3], National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands, Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, The Netherlands[4]

Abstract

1.1 The two-generation reproductive toxicity study has been extensively used to assess the adverse effects of substances on reproduction. It involves exposure of the adult parent generation before and throughout mating and pregnancy, continued in its offspring through adulthood and mating, including the second generation offspring until weaning. Several studies have addressed the question whether the second generation mating and offspring could be omitted without influencing the interpretation of the study for hazard and risk assessment. This study aimed at a comprehensive combined retrospective analysis of all available two-generation studies that had been deemed relevant in the risk assessment context in a standardized approach. Using the USEPA ToxRefDB format, 498 rat multi-generation studies representing 438 different tested substances were collected. Risk assessment reports were analyzed to assess how studies had been interpreted, both in terms of Lowest Effect Levels (LEL) observed among generations as well as in terms of Lowest Observed Adverse Effect Levels (LOAEL) according to the interpretation given in the risk assessment reports. We found 33 studies in which the LEL of first offspring mating and second offspring parameters had been considered lower than the LEL of the parental and first offspring generation including its adult non-mating parameters. In 19 of these cases, in the risk assessment report the LOAEL had been considered identical between generations or other nonreproductive toxicity effects had determined overall substance LOAEL. An additional 10 studies showed inconsistencies of findings in a second mating or in the third generation. Another 4 studies revealed a lower LOAEL only in the F3 generation. Analysis of the remaining studies on the basis of LOAEL differences among generations showed 5 additional studies with differences between generations. However, detailed assessment of study reports revealed that these were no real differences in sensitivities between the generations in view of additional end point parameters. Therefore, this study concludes that in retrospect in 498 multi-generation studies, there had not been a single example where the second generation mating and offspring provided critical information for the interpretation of the study in terms of the determination of the reproductive LOAEL. These findings are in agreement with earlier, smaller studies and provide further justification for replacing the OECD 2-generation reproduction study with the proposed OECD extended one-generation reproductive toxicity study protocol in regulatory risk assessment testing strategies.

Introduction

1.2 In the early 1980ies, the two-generation reproduction toxicity study (OECD Test Guideline 416) was introduced as the globally agreed standard test protocol for assessing potential adverse effects of

industrial substances and pesticides on fertility and reproduction. This study protocol has been employed extensively for more than 25 years now, and hundreds of such studies have been performed worldwide. It was officially updated in 2001 to include additional parameters. The protocol requires premating exposure of male and female animals, usually rats (P0), followed by mating and pregnancy to generate an F1 offspring (Figure 1). This offspring generation is carried through to adulthood (P1) and mated to generate an F2 generation. The F2 animals are terminated at weaning. Exposure is continued throughout the study. The principle of the study design is in the exposure of the F1 animals throughout their entire reproductive cycle, starting with exposure of the gametes in the P0 which will give rise to the F1, continuing through fertilization, embryo-fetogenesis and postnatal development of the F1 and reproduction of the P1 generating the F2. This comprehensive exposure design covering the entire reproductive cycle in the F1/P1 generation allows adverse effects on reproductive function at any time in the reproductive cycle to be detected irrespective of the sensitive period of their causation in the reproductive cycle.

1.3 In principle this comprehensive study design including the entire reproductive cycle for reproductive toxicity assessment is generally considered as scientifically sound. However, extensive experience with the protocol over more than three decades has caused researchers to consider whether in actual practice parameters related to the mating of the P1 and the generation of the F2 do have an impact on overall conclusions on the reproductive toxicity of substances tested. In case each of the individual parameters measured in P1 mating and F2 offspring animals would be equally or less sensitive than those measured in P0 and F1 animals, the P1 mating (and subsequent generation of the F2) would not impact on the overall lowest observed adverse effect level (LOAEL) of the study. Removal of the P1 mating and beyond would significantly reduce cost, time and animal use in the study. Cooper et al. (2006) suggested that for 350 pesticide substances, with two possible exceptions, adverse effects would have been detected already in the first offspring generation. They proposed a novel protocol, the Extended One-Generation Reproduction Toxicity Study (EOGRTS), in which the F1 generation would be followed until adulthood by default, whereas the subsequent mating of the P1 and the generation of an F2 would be triggered on the basis of information collected within the study and/or on the basis of existing data on possible fertility effects. Furthermore, additional parameters and increases in the number of observations were suggested to increase the sensitivity and statistical power of the study. This proposal was followed by several studies assessing the impact of the second generation on the study conclusions of the two-generation study in existing risk assessment reports in retrospect. Janer et al., (2007) studied a database of 176 multi-generation study risk assessment reports and observed that in all cases the second generation affected neither the overall NOAEL nor the nature of the critical effect. Therefore, it was concluded that the second generation had no impact on the ensuing risk assessment or on classification and labeling. This study specifically included all available two-generation studies for substances classified and labelled as reproductive toxicants under European and Californian law. Several smaller studies followed (Myers et al., 2008, Beekhuijzen et al., 2009), and more recently Martin et al (2009) using a US EPA ToxRefDB dataset of 329 studies supported the hypothesis that the F2 generation would rarely impact either the qualitative or quantitative evaluations of these studies, with only in one case (fenarimol) effects observed in the second generation affecting the chronic reference dose.

1.4 Meanwhile, the EOGRTS protocol was formally forwarded by the USA, Germany and the Netherlands for adoption as a globally agreed OECD test guideline. In October 2008 and October 2009 OECD expert meetings in Paris followed. The debate on the necessity of the F2 generation focused on the principal argument of the essential need for exposing and assessing effects during the entire reproductive cycle within one generation, versus the practical argument that in retrospect the P1 mating and F2 generation parameters hardly ever if at all impacted on the study interpretation. It was decided that a systematic combined retrospective analysis of the internationally agreed interpretations (in the form of risk assessment summaries) of all available two-generation studies would be necessary before concluding about the impact of the second generation mating and offspring parameters, and its consequences for the design of an OECD EOGRTS protocol. This manuscript describes this combined retrospective analysis performed

at RIVM with input from the OECD expert group on the subject. It combines the ToxRefDB and RIVM databases with smaller datasets from Beekhuijzen (NOTOX) and EU New Substance data as provided by the German authority (BfR).

Materials and Methods

1.5 A combined database of multi-generation study results was generated on the basis of the ToxRefDB layout and its multi-generation study content (Martin et al., 2009), which already included additional study reports provided by the Canadian authority. The German (EU New Substances), RIVM (Janer et al., 2007) and NOTOX (Beekhuijzen et al., 2009) databases as well as a couple of individual studies indicated by the OECD expert group were added into the same format by RIVM, amounting to a database containing the results of 498 studies covering 438 substances in total.

1.6 The database contains for each study all parameters affected, stratified according to external dose level and generation, as described in publicly available consensus summary risk assessment reports and supporting publicly available scientific publications. The lowest effect level (LEL) is defined as the lowest dose administered at which an effect had been noted in the risk assessment summary. These LEL are subject to further interpretation as to biological relevance and statistical significance. The lowest observed adverse effect level (LOAEL) is defined as the lowest dose at which an effect was found that had been interpreted in the summary report as relevant for feeding into risk assessment. The latter had been decided on the basis of statistical significance and/or biological relevance dependent on expert judgment using a weight of evidence approach as customary in regulatory risk assessment. The current retrospective analysis primarily collected data (LEL) and their interpretation (LOAEL) from existing risk assessment reports and on purpose did not produce new interpretations and risk assessments, in line with the retrospective character of the exercise. Studies selected were further scrutinized in the original reports whenever possible to put the initial findings into context for consideration of the specificity of the P1/F2 findings.

1.7 The reports included JMPR (115), OECD SIDS (21), EU RAR or DAR (33), Canada PMRA (12), Californian EPA (4), US NIH (1), EU C&L (confidential, 9) and US EPA (315) risk assessment summaries. This distribution of risk assessment report (RAR) sources is reminiscent of the primary databases from which they were derived. For selected studies, alternative RARs were consulted for further information. If several multigeneration studies were available for the same substance, usually only the most relevant study on the basis of the RAR was entered into the database. For a considerable number of studies there were multiple (independent) summaries of the same (unpublished) study. Of the 438 substances in the database 27 substances were classified in Europe for fertility (10 x R60 and 17 x R62) and 44 substances were classified for developmental toxicity (23 x R61 and 19 x R63) under EU legislation. Additionally 2 substances classified as “may cause harm to breastfed babies”, R64, are present in the 438 substance database. Since fertility and developmental classification are combined for several substances, the total number of substances classified as reproductive toxicants (R60-63) in the database is 49 out of a total of 438 substances. Within each generation effects were distinguished as either premating (F) effects or postmating (P) effects, to allow the assessment of the impact of mating on study outcome (see Figure 1).

1.8 After completion of the database, studies were selected in which specific effects had been observed in the P1/F2 (and beyond if an F3 was generated) at a dose level below the Lowest Effect Level observed in the F0/P0/F1. This is equivalent to comparing the LELs for the life stages on the left side versus the right side of the vertical dashed line indicated in Figure 1. In addition, for completeness the same analysis was also performed using the LOAELs in order to additionally include those studies for which the interpretation had resulted in a lower LOAEL in the P1/F2 when compared to F0/P0/F1 effects.

Subsequently all substances that were selected based on the quantitative comparison of the LELs and/or LOAELs were evaluated in detail.

Results

LEL comparison

1.9 Comparison of the LELs in the P1/F2 with LELs in the F0/P0/F1 (see Figure 1) revealed 33 studies in the database for which the P1/F2 generation showed the lowest LEL (Table 1), as reported in the risk assessment summary ("source" in tables 2 to 5). Of the 33 substances in Table 1, 27 have an entry in Annex VI, and only 4 are classified for fertility (R60, R62). An additional two are classified for developmental toxicity (R61). Most of the remaining 23 classified substances carried a classification as toxic (Xn, T, T+) and/or harmful to the environment (N).

1.10 For 19 of the 33 studies selected on the basis of the LEL comparison, the study summaries revealed that the effects seen in P1/F2 had not been interpreted as occurring at a lower dose than in the F0/P0/F1. LOAELs for these studies had been considered to be similar in both generations or other nonreproductive toxicity effects had determined overall substance LOAEL. Details of these studies and the relevant interpretation in the risk assessment reports are given in Table 2.

1.11 In an additional 10 studies of the 33, apparent P1/F2 specificity had not been reproduced in a parallel mating (e.g. F2b versus F2a) or in the third generation F3, suggesting that the P1/F2 findings could have occurred by chance. Relevant effects and the interpretation in the risk assessment reports for these studies are summarized in Table 3.

1.12 Furthermore, 4 studies showed the Lowest Effect Levels in the P2/F3 generation only, which would not have been detected if the study would have been terminated at the second generation offspring (Table 4).

LOAEL comparison

1.13 A similar comparison between F0/P0/F1 and P1/F2 generations was performed using the LOAEL values (again as had been reported in the risk assessment summaries). This additional analysis was to identify those studies in which, although the LELs between generations were similar, the interpretation of the study nevertheless had resulted in a lower LOAEL for the P1/F2 as compared to the F0/P0/F1 generation. The results of this LOAEL analysis revealed 5 additional studies which had been interpreted by USEPA as having a unique sensitivity in the P1/F2. Detailed assessment of study summaries revealed additional findings showing that the P1/F2 was in fact not more sensitive than the F0/P0/F1. Some cases indicated discrepancies among risk assessment reports as to LOAEL determination.

Discussion

Aim and approach

1.14 This study aimed at a combined retrospective analysis of the impact of the P1/F2 generation on the overall interpretation of the study. The study aimed at consistency and comprehensiveness, being based on one single standardized database including all existing regulatory rat multi-generation reproduction studies available worldwide that had been considered relevant in the risk assessment. Earlier studies (Janer et al. 2007, Myers et al. 2008, Beekhuijzen et al. 2009, Martin et al. 2009) used smaller databases and were not carried out in identical ways, complicating combined interpretation. The retrospective nature of the current analysis is exemplified by the primary use of existing internationally agreed study interpretations from existing risk assessment reports. This is a critical feature of the present study, as it guarantees that the

historical regulatory interpretation of studies is taken into account. It allows us to analyze how the study had actually impacted on risk assessment and classification & labeling. It could be argued that both the study designs and expert judgment have changed over the years, causing older regulatory assessments to be of less relevance nowadays. Whereas this suggestion is very difficult to address objectively, our current analysis has not given us reason to believe that major changes have taken place that could have affected the current study outcome.

Outcome for risk assessment

1.15 The present retrospective analysis of the combined database of 498 multi-generation studies indicated that in 33 studies LEL were observed in subsequent generations at a lower dose level than in the P0/F1 generations. Therefore, in over 90% of the studies collected the overall sensitivity of the P1/F2 generation was not higher than that of the P0/F1 generation, as judged by simply comparing overall LEL values among and within generations. In addition however, the remaining 33 studies contained additional findings in their study summaries on the basis of which the generation specificity of the P1/F2 effect had been considered questionable. In 19 cases (Table 2), the risk assessment reports revealed that the effect in question had been considered not relevant for the determination of the LOAEL. This could be on the basis of the limited incidence or magnitude of the effect, an absence of dose-response, similar but less pronounced trends in the P0/F1 generation, or because of dominating substance characteristics such as cholinesterase inhibition or genotoxicity. There were 10 studies (Table 3) in which the P1/F2 effect had been considered not relevant because it was not reproduced in a parallel or in a subsequent generation. As already discussed in Janer at al. (2007), effects may occur in one generation but not in another by chance. Multiple testing compromises the type-I error rate, which is especially evident in protocols with many parameters, such as the two-generation reproduction study. A similar situation of possible chance findings is relevant for multi-generation studies, in which we observed 4 studies (Table 4) with effects found only in the P2/F3 generation. In addition, these latter cases have been dismissed as not relevant in view of analyzing the two-generation protocol, which would not have seen these effects. This study therefore indicates that in all 498 studies the P1/F2 generation had not had an impact on the overall conclusion of the two-generation study from the study for risk assessment (RA) purposes.

Comparison of this study with Martin et al. (2009)

1.16 In a similar analysis for specific reproductive P1/F2 effects, Martin et al, 2009 identified 16 substances using a 316 substance/329 multi-generation studies subset of the current database. Twelve of these substances appeared in the current selection as well (marked with * in Table 1). The remaining 4 substances did not show a unique P1/F2 effect in the current analysis because the multi-generation studies for these substances had been recorded as having the most sensitive LEL to be a reproductive non-mating effect in the F1. For Clethodim and Dicyclohexylphthalate, the critical effects were prostate and seminal vesicle weight changes in the adult F1. For Thiamethoxam, testicular atrophy was observed in F1 adults. Tributylchlorostannane affected testis and epidydimis weights in adult F1. These effects do not require mating of the F1 generation and breeding of the F2, and are therefore considered to be part of the F0/P0/F1 effects in the current analysis (left of the dotted line in Figure 1).

Outcome for Classification & Labelling

1.17 For classification & labelling (C&L) (EU-CLP 2008), the discussion is more complex. The primary assumption of EU-CLP is that it is the observation of a reproductive toxic effect considered not secondary to general toxicity that drives C&L. Consequently, the presence of a LOAEL for specific reproductive toxic effects is critical for both risk assessment and C&L. The LOAEL is based on biological relevance and statistical significance of findings as assessed by expert judgment. The determination of the LOAEL includes careful consideration of the incidence, magnitude and severity (IMS) of findings. There

is however discussion among experts about whether differences in IMS may lead to a different classification. For example, in this view a limited but biologically significant pup body weight decrease might lead to a lower classification level than biologically significant fetal death occurring at the same dose level. In that case, similar LELs found in different generations would not be sufficient to judge the impact of the P1/F2 generation on C&L as the nature of the effects observed at the LEL should also be taken into account.

1.18 It is noteworthy that of the 33 substances with observed differences in LEL between generations in this study, only 4 carry a classification for fertility (and development) and two carry a classification for development only. Given that 26 of the 33 have an EU Annex VI entry it is likely that these have been assessed for C&L. This suggests that also for C&L, the generation specificity of effects at the LELs has not been critical in C&L for these substances. Of around 120 substance entities that have a classification for fertility, for only around 26 of them is a two-generation study available (Janer et al., 2007). This indicates that classification for fertility is not solely based on information from the two-generation study. Rather, information about effects on reproductive organs and/or fertility from other types of studies may more often have generated a classification than two-generation study results.

1.19 In order to provide further factual input for the discussion on the impact of IMS on C&L, we are currently studying the approximately 50 substances which have a multigeneration study and in addition carry an EU classification for fertility and/or development. Substances with a classification for development will be included, given that F2 findings are mostly induced prenatally and therefore will lead to such a classification. This study aims at revealing how IMS in these studies have differed between generations. These findings will be reported separately.

Summary

1.20 In summary, the present study combines and elaborates existing databases, provides a new comprehensive retrospective analysis, and confirms earlier conclusions about the limited contribution of the second generation offspring in the two-generation reproductive toxicity study to the interpretation for risk assessment and C&L. It supports the replacement of the two-generation reproduction toxicity study with the EOGRTS on the basis of existing experience with multi-generation studies. EOGRTS is further supported by arguments of increased parameter numbers and statistical power, enhanced economy and reduced animal use.

References

Beekhuijzen M, Zmarowski A, Emmen H, Frieling W. To mate or not to mate: a retrospective analysis of two-generation studies for evaluation of criteria to trigger additional mating in the extended one-generation design. Reprod Toxicol. 2009 Sep;28(2):203-8.

Cooper RL, Lamb JC, Barlow SM, Bentley K, Brady AM, Doerrer NG, Eisenbrandt DL, Fenner-Crisp PA, Hines RN, Irvine LF, Kimmel CA, Koeter H, Li AA, Makris SL, Sheets LP, Speijers G, Whitby KE. A tiered approach to life stages testing for agricultural chemical safety assessment. Crit Rev Toxicol. 2006 Jan;36(1):69-98.

Dang ZC, E Rorije, Th Hogen Esch, A Muller, BC Hakkert, AH Piersma (2009) Retrospective analysis of relative parameter sensitivity in multi-generation reproductive toxicity studies. Reprod.Tox.28:196-202.

EU CLP - Regulation (EC) No 1272/2008 Regulation on classification, labelling and packaging of substances and mixtures, Annex VI: Harmonised classification and labelling for certain hazardous substances. 20-9-2010, updated with ATP 1 to the Regulation http://ecb.jrc.ec.europa.eu/esis/index.php?PGM=cla

EU REACH - Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency. Official Journal of the European Union, L 396 Volume 49, 30 December 2006. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:396:SOM:EN:HTML

Janer G, Hakkert BC, Slob W, Vermeire T, Piersma AH (2007). A retrospective analysis of the two-generation study: What is the added value of the second generation? Reprod. Tox. 24:79-102.

Mangelsdorf I, Buschmann J, Orthen B. Some aspects relating to the evaluation of the effects of chemicals on male fertility. Regul Toxicol Pharmacol. 2003 Jun;37(3):356-69

Martin MT et al. (2009) Profiling the Reproductive Toxicity of Chemicals from Multigeneration Studies in the Toxicity Reference Database. Tox. Sci.110(1):181-190.

Myers DP, Willoughby CR, Bottomley AM, Buschmann J. An analysis of the results from Two-Generation Reproduction Toxicity Studies to assess the value of mating animals of the second (F1) generation for the detection of adverse treatment-related effects on reproductive performance. Reprod Toxicol. 2008 Sep;26(1):47-50.

OECD TG 415 One-Generation Reproduction Toxicity Study, OECD 1983. http://www.oecd.org/dataoecd/18/12/1948458.pdf

OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents, OECD, 1995. http://www.oecd.org/dataoecd/50/41/37477972.pdf

OECD TG 421. Reproduction/Developmental Toxicity Screening Test, OECD 1995. http://www.oecd.org/dataoecd/18/14/1948474.pdf

OECD TG 414. Prenatal Developmental Toxicity Study, OECD 2001. http://www.oecd.org/dataoecd/18/15/1948482.pdf

OECD TG 416 Proposal for updating guideline 416, Two-Generation Reproduction Toxicity Study, OECD 2001. http://www.toxi.ucl.ac.be/RRIOS/OCDE%20TG%20416.pdf

OECD Draft Proposal for an Extended One-Generation Reproductive Toxicity Study. October 2009. http://www.oecd.org/dataoecd/55/24/43965303.pdf

van der Jagt, K, S Munn, J Tørsløv & J de Bruijn. Alternative approaches can reduce the use of animals under REACH; 2004. http://ecb.jrc.ec.europa.eu/documents/REACH/PUBLICATIONS/Reducing the use of test animals under REACH IHCP report.pdf.

References to multigeneration studies for the 38 substances, discussed in tables 1 to 5.

ABC (American Biogenics Corporation). 1986. Two-generation reproduction study in albino rats with methyl bromide - results of both generations (Study No. 4500-1525) (Unpublished final report).

Ambrose, A.M., Larson, P.S., Borzelleca, J.F., Blackwell Smith, R. & Hennigar, G.R. (1971) Toxicologic studies on 2,4-dichlorophenyl-p-nitrophenyl ether (TOK). Toxicol. Appl. Pharmacol. 19:263-275.

Barton, S.; Hastings, M. (1996) Bensulide: Two Generation Reproduction Study in Rats: Lab Project Number: 11430: 491205. Unpublished study prepared by Inveresk Research International. 237 p.

Becker, H.; Et Al. (1986) T54 Desmedipham: Multiple Generation Re- Production Study With Desmedipham Technical In Rats: Laboratory Project Id: Pf-82.810. Unpublished Study Prepared By Research & Consulting Co., Ag. 1057 p.

Beyer BK (1991b) Multigeneration rat reproduction study. Study No. MRD-88-331. Unpublished report dated 26th November 1991 from Exxon Biomedical Sciences Inc., East Millstone, NJ, USA. Submitted to WHO by Amvac Chemical Corp., City of Commerce, CA, USA.

Borzelleca, J.F., Larson, P.S., Crawford, E.M., Hennigar, G.R., Jr, Kuchar, E.J. & Klein, H.H. (1971) Toxicological and metabolic studies on pentachloronitrobenzene. Toxicol. Appl. Pharmacol., 18, 522-534.

Bottomley, A.; Bowman, A.; Offer, J.; et al. (1986) 2,4-DB--Effect on Two Generations of the Rats: Laboratory Project ID: UNC/138- R. Unpublished study prepared by Huntingdon Research Centre Ltd. 549 p.

Cozens, D.D.; Allen, P.A.; Clark, R.; et al. (1980) Effect of CGA 48-988 on Reproductive Function of Multiple Generations in the Rat: CBG 181/80254. (Unpublished study received Apr 15, 1981 under 100-607; prepared by Huntingdon Research Centre, England)

Cozens, D.; Barton, S.; Offer, J.; Et Al. (1985) Effect Of Ethofen-Prox (Mti-500) On Multiple Generations Of The Rat: Hrc Rept. No. Mtc 67/85706. Unpublished Study Prepared By Huntingdon Research Centre Ltd. 461 P.

Eiben, R. (1984) MEB 6447 (Triadimefon): Two-generation Study with Rats: Supplementary Study: Report No. 12712. Unpublished study prepared by Bayer AG, Institute of Toxicology. 168 p.

Eigenberg, D.; Elcock, L. (1996) A Two-Generation Reproduction Study in Rats Using Technical Grade Cyfluthrin Administered Via the Diet: Lab Project Number: 93-672-UZ: 107408: 93-672-UZ1. Unpublished study prepared by Bayer Corp. 973 p.

Eigenberg, D. (1997) A Supplementary Two-Generation Dietary Reproduction Study in Rats Using Technical Grade Cyfluthrin: Lab Project Number: 94-672-CK: 107474: 94-672-CK1. Unpublished study prepared by Bayer Corp. 771 p.

Eisenlord, G.; Loquvam, G.S.; Leung, S. (1969) Results of Reproduction Study of Rats Fed Diets Containing SD 15418 over Three Generations: Report No. 47. (Unpublished study received on un-known date under 9G0844; prepared by Hine Laboratories, Inc.

EPA 1979. U.S. Environmental Protection Agency. Toxicology One-Line Summary: Triadimefon. Environmental Fate and Effects Division, Washington, DC, 1993.8-14. AND U.S. National Library of Medicine. Hazardous Substances Databank. Bethesda, MD, 1995.8-17. Therein a reference to: Mobay Chemical Corporation. 1979. MRID No. 00032541. Available from EPA. Write to FOI, EPA, Washington, DC 20460. (IRIS database).

Eschbach, B.; Aerni, R.; Hopley, J.; Et Al. (1991) Propetamphos: Two Generation Reproduction Study In Rats: Final Report: Lab Project Number: 442 R: 1309: Bs2238. Unpublished Study Prepared By Sandoz Agro Ltd. 943 P.

Hazelden, K.; Wilson, J. (1988) Tcmtb Two Generation Reproduction Study In Rats: Report No. 5168: Iri Project No. 435334. Unpublished Study Prepared By Inveresk Research International. 156 P.

Hellwig, J. (1992a) Report Reproduction Study with Reg. No. 83 258 (Vinclozolin) in Rats Continuous Dietary Administration over 2 Generations (2 Litters in the First and 2 Litters in the Second Generation): Lab Project Number: 92/11251: 71R0375/88053.

Hellwig, J. (1992b) Reproduction Study with Reg. No. 205259 (Epoxiconazole) in Rats: Continuous Dietary Administration over 2 Generations: Lab Project Number: 92/10689: 70R0959/88098. Unpublished study prepared by BASF Aktiengesellschaft. 1203 p.

Hellwig, J. (1993) Reproduction Toxicity Study with Mepiquat Chloride in Rats: Continuous Dietary Administration over 2 Generation: Lab Project Number: 93/10983. Unpublished study prepared by BASF Aktiengesellschaft Dept. of Toxicology. 1702 p.

Higgins, C. (1990) LS860263: Reproductive Performance Study in Rats Treated Continuously Through Two Successive Generations: Final Report: Lab Project Number: 90/0754: RHA/175/860263: 90/RHA175/0754. Unpublished study prepared by Life Science Research Ltd.

Hodge, M.C., Banham, P.B., Glaister, J.R., Richards, D., Taylor, K. & Weight, T.M. (1977) PP557: 3-Generation reproduction study in rats. Unpublished report from Imperial Chemical Idustries Ltd, Alderley Edge, England, Report no. CTL/P/361. Submitted to WHO by FMC Corporation, Princeton, New Jersey, USA.

Hoffman, D.G., Adams, E.R., Markham, J.K., Owen, N.V., Gibson, W.R. & Morton, D.M. (1977) A multi-generation reproduction study with EL-222 in the rat. Unpublished report No. R-715, R-1345, R-956, R-966 dated November 1977 from Lilly Research Laboratories, USA. Submitted to WHO by DowElanco Europe, Wantage, Oxon, United Kingdom.

Holzum, B. 1989. W VII/117 (c.n. fuberidazole) - Two generation study in rats …text blacked out… Report No. 17590. Date 1989-01-10. GLP. Unpublished.

Horstmann, G. (1998) HOE 115008--Substance Technical: Two-Generation Feeding--Reproduction Toxicity Study in Rats: Lab Project Number: 96.0699: C001514: 98.0762. Unpublished study prepared by Hoechst Marion Roussel Deustchland GmbH. 1646 p. {OPPTS 870.38)

Malley, L. (1990) Reproductive And Fertility Effects With In 43898: Multigeneration Reproduction Study In Rats: Lab Project Number: 8478-001: 693-89. Unpublished Study Prepared By E. I. Du Pont De Nemours And Co., Inc. 1401 P.

Masters, R. (1993) Technical Dicamba: A Study of the Effect on Reproductive Function of Two Generations in the Rat: Lab Project Number: SNC 140/921437. Unpublished study prepared by Huntingdon Research Centre Ltd. 392 p.

Milburn, G. (1997) ZA1296: Multigeneration Study in the Rat: Lab Project Number: CTL/P/5147: RR0691. Unpublished study prepared by Zeneca Central Toxicology Laboratory. 4607 p.

Morseth, S. (1988) Two-Generation Reproduction Study In Rats: Fat 80'023: Hla Study No. 2386-100. Unpublished Study Prepared By Hazleton Laboratories America, Inc. 986 p.

Moxon, M. (1994) ICIA5504: Multigeneration Study in the Rat: Lab Project Numbers: CTL/P/4213: 5504/83: RR0604. Unpublished study prepared by Zeneca Central Toxicology Lab. 2127 p.

Muller, Wolfgang (1993) Two Generation Oral (Dietary Administration) Reproduction Toxicity Study in the Rat (With One Litter in the P and Two Litters in the F1 Generation): Topsin-M: Final Report-Volume 1: Lab Project Number: 996-683-004. Unpublished study

Nagymagtenyi L, Schulz H and Desi I (1997) Behavioural and functional neurotoxicological changes caused by cadmium in a three-generational study in rats. Hum. Exp. Toxicol. 16, 691-699.

Nair RS, Johannsen FR, Schroeder RE, Auletta CS (1989) Evaluation of chronic and reproductive effects of p-Nitrochlorobenzene (PNCB) in rats. The Toxicologist 9(1):212 (Abstr.), and Monsanto Co (1984a) A Two Generation Reproduction Study of p-Nitro-chloro-benzene in rats. Project No. 80-2513 (BD-80-472), Final Report, Contractor: Bio/dynamics Inc., report submitted to: Monsanto Co., Date: April 19, 1984, EPA-OTS0557087 and EPAOTS0526382

Neeper-Bradley TL and Tyl RW. Bushy Run Centre (1989) Two-generation reproduction study of p-cresol (CAS No.106-44-5) administered by gavage to Sprague-Dawley (CD) rats. Project report 52-512. November 13, 1989. Unpublished data submitted by Bushy Run Research Center to The American Chemistry Council Cresols Panel, Washington, DC, EPA/OTS0529224

Palmer, A., Lovell., M. & Newman, A. (1972) Effect of thiophanate-methyl on reproductive function of multiple generations in the rat. Unpublished report from Huntingdon Research Centre, Huntingdon, Cambs, United Kingdom. Submitted to WHO by Nippon Soda Co. Ltd, Tokyo, Japan.

Schardein, J. (1991) PCNB: Two-Generation Reproduction Study in Rats: Lab Project Number: 399-086. Unpublished study prepared by International Research and Development Corp. 633 p.

Schilling, K.; Gembardt, C.; van Ravenzwaay, B. (2001) BAS 510 F Two Generation Toxicity Study in Wistar Rats Continuous Dietary Administration: Final Report: Lab Project Number: 70R0179/97136: 2001/1000117. Unpublished study prepared by BASF Aktiengesellschaft.

Suter, P., Vogel, W., Armstrong, J.M. & Terrier, C.H. (1989) Two generation reproduction study in the rat. Unpublished report No. 071561 from RCC Itingen, Switzerland, 27 June 1989. Aventis document A41694. Submitted to WHO by Aventis CropScience, Frankfurt am Main, Germany.

Tesh, J.; Willoughby, C.; Secker, R. Et Al. (1986) Nf-114: Effects Upon Reproductive Performance And Teratogenic Responses Of Rats Treated Continuously Throughout Three Successive Generations: Laboratory Project Id 85/Nis008/184. Unpublished Study.

WIL Research Laboratories. (2001) Inhalation two-generation reproductive toxicity study of 1-bromopropane in rats. Study No. WIL-380001. Study Director, D. Stump. Ashland (OH): Study sponsored by Brominated Solvents Committee (BSOC).

Wazeter, F.; Goldenthal, E. (1973) Teratology Study In Rabbits: Rh- 787. Unpublished Study Prepared by International Research And Development Corp. 27 p.

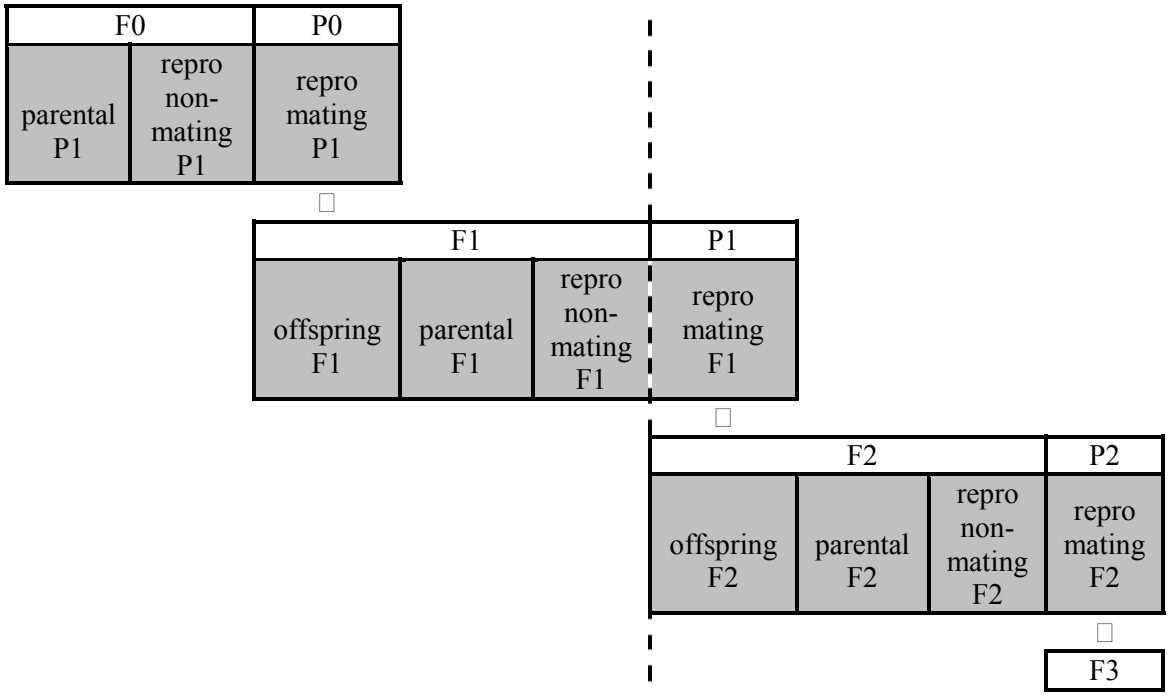


Figure 1. Schematic representation of the different stages in multigeneration reproduction toxicity study protocols. Within generations, four groups of parameters are distinguished in the database (see shaded boxes): "offspring" parameters observed until weaning at postnatal day 21, "parental" general toxicity parameters observed from weaning onward and including adulthood, "repro"ductive "nonmating" parameters observed in adult animals, and "repro"ductive parameters related to "mating". In the text, life phases before mating are indicated as (F) (white boxes), and after mating as (P), whereas the index (0,1,2,3) indicates the generations such that the first offspring generation emanating from the study is designated as the F1. The generation indications in the grey boxes are the original EPA ToxRefDB nomenclature. The analysis of the impact of the second generation is primarily done by comparing parameters occurring to the left of the dashed line (F0/P0/F1 effects, white boxes) with those observed to the right of that line (P1/F2 effects, and if present also P2/F3 effects).

Table 1: Studies showing an LEL that is lower in P1/F2 or P2/F3 than in F0/P0/F1

	Substance NAME	CAS number	EU C&L as Reproductive Toxicant, R60-63	EU Annex VI	table nr.
1	1-bromopropane	106-94-5	Repr.Cat.2 R60, Cat.3 R63	YES	2
2	1-chloro-4-nitrobenzene	100-00-5		YES	2
3*	2,4-DB	94-82-6		YES	2
4*	Azoxystrobin	131860-33-8		YES	2
5*	Bromuconazole	116255-48-2		NO	2
6	Cadmium chloride	10108-64-27440-43-9	Repr.Cat.2, R60-61	YES	4
7*	Carbaryl	63-25-2		YES	2
8*	Chlorethoxyfos	54593-83-8		NO	2
9	Cyfluthrin	68359-37-5		YES	2
10*	Desmedipham	13684-56-5		YES	2
11*	Epoxiconazole	106325-08-0		NO	2
12	Ethofenprox	80844-07-1		NO	2
13*	Fenarimol	60168-88-9	Repr.Cat.3, R62-63, R64	YES	3
14	Flusilazole	85509-19-9	Repr.Cat.2, R61	YES	3
15	Fuberidazole	3878-19-1		YES	3
16*	Mepiquat chloride	24307-26-4		YES	2
17	Mesotrione	104206-82-8		YES	3
18	Metalaxyl	57837-19-1		YES	3
19	Methyl bromide	74-83-9		YES	2
20	Mevinphos	7786-34-7		YES	2
21	Nitrofen	1836-75-5	Repr.Cat.2, R61	YES	4
22	p-Cresol	106-44-5		YES	3
23	Permetrhin	52645-53-1		YES	4
24*	Propetamphos	31218-83-4		YES	2
25	Quintozene	82-68-8		YES	3
26	Sodium cyanurate	2624-17-1		NO	2
27*	TCMTB	21564-17-0		YES	3

28	Thiophanate methyl	23564-05-8		YES	3
29	Triadimefon	43121-43-3		YES	3
30	Triazophos	24017-47-8		YES	2
31 *	Triclosan	3380-34-5		YES	2
32	Triflumizole	68694-11-1		NO	4
33	Vinclozolin	50471-44-8	Repr.Cat.2, R60-61	YES	2

* studies also identified by Martin et al (2009)

Table 2: Studies showing lower P1/F2 LEL as compared to F0/P0/F1 in the database with additional considerations on the specificity of this effect

Name	P1/F2 effects	Additional considerations	Source(s)	Study reference
1-Bromo-propane (C&L: R60)	P1: low dose (1): ns reduction fertility P1: dose level 2: reduction nr. of implantation sites; ns reduction in fertility indices.	LOAEL is set at dose level 2 based on: P0 reduced prostate wt, F1 reduced litter wt, and incr estrous cycle length. Reduction in fertility indices of P1 at low dose and dose level 2 were non significant.	CERHR, NIH Publication No. 04-4479	WIL, 2001
1-Chloro-4-nitrobenzene	P1; low dose: lower mating index, not statistically significant.	LOAEL was set at mid dose based on statistically significant decrease in litter survival of the F1 generation.	OECD SIDS	Nair, 1989
2,4-DB	F2 ; mid dose: increase in pelvic dilatation, not statistically significant.	LOAEL is set at high dose based on multiple effects (incl. reproductive effects) in all generations.	EPA ToxRefDB & JMPR	Bottomley, 1986
Azoxystrobin	F2 females (not males); mid dose: Relative liver weight incr and bw decr.	LOAEL based on same effects at high dose in all generations plus reduced food consumption. No fertility effects recorded in this study.	EPA ToxRefDB	Moxon, 1994
Bromuconazole	F2 females (not males); mid dose: relative liver weight effect.	LOAEL was based on a.o. (F1) pup body wt effects at high dose	EPA ToxRefDB	Higgins, 1990
Carbaryl	F2; mid and high dose: statistically non-significant decrease in survival and lactation but statistically significant for trends.	F1 shows same trend for survival, effect is evident at mid dose both in F1 and F2. JMPR also mentions P0 and P1 liver weight effects and red bw during lactation at mid dose. JMPR sets LOAEL at middose on all these effects. EPA does not mention these effects at middose, and sets LOAEL at middose based only on survival and lactation index trend in F2.	EPA ToxRefDB & JMPR	Tyl, 2001
Chlorethoxyfos	F2; dose level 3 of 4: Decreased pup body weight	LOAEL is based on tremors in the F1 and F2 at dose level 4, the body weight effect is deemed not relevant for LOAEL.	EPA ToxRefDB	Malley, 1990
Cyfluthrin	F2; low dose: Decreased litter weight	LOAEL is based on litter wt effects, neurotox and decreased food consumprion in F1 and F2 at middose. A supplementary study (1997) does not show these specific F2 effects.	EPA ToxRefDB	Eigenberg 1996, 1997.
Desmedipham	F2; low dose: Decr. relative liver and	LOAEL was based on spleen wt and anemia in the P1 at mid dose.	EPA ToxRefDB	Becker, 1986

	kidney weight			
Epoxyconazole	F2; low and mid dose: Decr viability index at PND4	LOAEL was based on multiple serious effects including decreased viability index at the high dose	EPA ToxRefDB	Hellwig, 1992b
Ethofenprox	F2; low and mid dose: Incr. thyroid gland wt	LOAEL was set at mid dose based on liver wt effects in F1 and F2. Thyroid gland wt effect is first seen in P0 and F1at high dose, this effect only seen as toxicologically relevant at high dose	EPA ToxRefDB, & JMPR	Cozens, 1985
Mepiquat chloride	F2; low dose: Delayed eye opening on day 15.	LOAEL was based on multiple effects at mid dose (including delayed eye opening)	EPA ToxRefDB	Hellwig, 1993
Methyl bromide	F2 reduced bw at mid and high dose at day 28 only. Offspring viability not affected.	No effects on litter size, sex ratio, survival through lactation, or grossly observable abnormalities. OECD: ”For risk assessment it is more relevant that the substance is genotoxic”	OECD SIDS	ABC, 1986
Mevinphos	P1; low dose: ns red. mating and fertility indices at low and mid dose.	LOAEL at high dose based on: red. abs testes, epididymis, and incr rel ovarian w; absent corpora lutea, red mating and fertility indices, cholinesterase inhibition (more severe), and red bw gain in the F1. Cholinesterase inhibition at all doses in all generations	JMPR & EPA ToxRefDB	Beyer, 1991
Propetamphos	F2; low dose: Decr. litter size and no milk in stomach	LOAEL is based on cholinesterase effect mid dose all generations	EPA ToxRefDB	Eschbach, 1991
Sodium cyanurate	F2; high dose: Kidney chronic inflammation of calculi	No LOAEL was determined in absence of any other effect in the study	EPA ToxRefDB	Wazeter, 1973
Triazophos	F2; mid dose: inc loss of pups	Pup loss in F1 and F2 at high dose. F2 pup loss at mid dose was not considered due to treatment because 5 of the 12 lost pups in this group were in the same litter. JMPR sets LOAEL at high dose	JMPR	Suter, 1989
Triclosan	F2; low and mid dose: Incr pup bw. P1; mid dose: incr bw	LOAEL is based on decr bw in all generations at high dose. Pup viability was decr in both F1 and F2 at high dose	EPA ToxRefDB	Morseth, 1988
Vinclozolin (C&L: R60-61)	F2 red epididymal weight at the low dose.	General tox and dev tox in all generations at next higher dose. Lowest recorded effect dose of all 4 studies. JMPR summary considers this a marginal effect.	EPA ToxRefDB & JMPR	Hellwig, 1992

Table 3: Studies in which apparent P1/F2 specificity was not confirmed in parallel matings, or in the subsequent generation, and/or substance shows other forms of toxicity

	P1/F2 effect	Additional considerations	Source(s)	Study reference
Fenarimol (C&L: R62-63, R64)	F2b (not F2a); Reduction in liveborn litter size	JMPR concluded that fertility effects were mediated by aromatase inhibition, and are not relevant for humans, therefore these are not used for determining ADI	EPA ToxRefDB & JMPR	Hoffman, 1977
Flusilazole (C&L: R61)	F2b (not F2a): Hydronephrosis F2a (not F2b): decr pup survival	Absence of dose-response and incidence at historical control levels, therefore considered not toxicologically significant in the JMPR summary. No effects at the same dose level in a more recent study (Mullin)	JMPR	Pastoor 1986, Mullin 1990
Fuberidazole	F2b; mid and high does: Viability index decr in F2b at mid and high dose, No dose response in the F2a.	EU RAC discussion of this substance (sep.2010) concluded that the lack of consistence in the (reproductive effects is the basis for not classifying this substance for fertility or developmental toxicity.	EU DAR	Holzum, 1989
Mesotrione	F2; dose level 2 and 3 (of 4): decr survival at day 22 and 29	F1 and F3 survival was not affected at mid doses. F1, F2 and F3 pup survival is affected at high dose (dose 4). Low dose general tox effects (tyrosine) in P2 and F3 was used for LOAEL setting	EPA ToxRefDB	Milburn, 1997
Metalaxyl	F2b; high dose: liver effect	Liver effect considered not biologically relevant (JMPR and EPA), no F2a effects, no other effects reported, NOAEL is set at high dose (JMPR and EPA)	EPA ToxRefDB & JMPR	Cozens, 1980
p-cresol	F2: increase in stillbirths	Both in F1 and F2 non-dose-related increases in stillbirths, considered not relevant, overall NOAEL was set at high dose.	OECD SIDS	Neeper-Bradley, 1989
Quintozene	F2b (not F2a); red bw of adults (three-gen.study)	Effects considered not dose-related, F3 generation without effects. More recent study shows similar body weight effects throughout generations	JMPR & EPA ToxRefDB	Borzelleca, 1971 Schardein, 1991
TCMTB	F2b (not F2a): Decreased pup weight in F2b on lactation day 21	EPA: questionable significance because pup wt was ns reduced at days 7 or 14 in the F2b pups, and no consistent pup weight effects were seen in the F0 and F1a generations.	EPA ToxRefDB	Hazelden, 1988

Thiophanate methyl	F2b (not F2a); low and mid dose: red viable litter size and weight gain, no other effects at these two doses	NOAEL set at mid dose based on slightly reduced litter size and bw effects at high dose. A more recent study (1993) was used to establish EPA OPPT LOAEL, and is summarized in the ToxRefDB	US EPA IRIS & ToxRefDB	Palmer, 1972 Muller, 1993
Triadimefon	F2; mid dose: decreased bodyweight gain, no bw effect at high dose	No effects seen in F3 (EPA). No effects in any generation at same dose level in an additional study (ToxRefDB and JMPR)	EPA ExToxNet PIP, ToxRefDB & JMPR	EPA, 1979 Eiben, 1984

Table 4: Multi-generation studies in which effects were observed in P2/F3 at a dose level below doses with observed effects in P0/F1 or P1/F2. These effects would not have been observed in a 2-generation study.

	P2/F3 effect	**Additional considerations**	**Source(s)**	**Study reference**
Cadmium (C&L: R62-63)	F3: red open field exploration (only effect in study)	no effects in F2 or F1	EU RAR	Nagymajtenyi , 1997
Nitrofen (C&L: R61)	P2: Body weight slightly decr at mid dose in breeding the F3a, and at low dose in breeding the F3b.	This decrease was due to lower initial body weights of rats used for breeding.	JMPR	Ambrose, 1971
Permethrin	F3: Liver hypertrophy at all doses.	No liver hypertrophy effects in F2, F1 or P0 at any dose, tremors at high dose in P1, F1 and F2 determined LOAEL.	EPA ToxRefDB & JMPR.	Hodge 1977
Triflumizole	P2; low and mid dose: increased gestation length	F1 offspring and F2 offspring effects at highest dose only	EPA ToxRefDB	Tesh, 1986

Table 5: Studies selected on the basis of LOAEL at a lower dose level in the P1/F2 or P2/F3 when compared to the LOAEL from the P0/F1 generations1,2

	P1/F2 effect	Additional considerations	Source(s)	Study reference
Bensulide, CAS 741-58-2	F2; high dose: decreased viability index and pup survival index, ns (LOAEL)	P0; high dose: decreased brain weight, decreased fertility index (LEL?). Mid and high dose: cholinesterase inhibition in all generations (LEL)	EPA ToxRefDB	Barton, 1996
Dicamba CAS 1918-00-9	F2; mid dose: body and organ weight effects (LOAEL)	P0 and P1 also show kidney wt effects, but these are deemed not toxicologically significant. No fertility effects.	EPA ToxRefDB	Masters, 1993
Iodosulfuron-methyl-sodium CAS 144550-36-7	F2; high dose: litter size effects at high dose (LOAEL)	EPA: Reduced P0 bw and F1 survival at high dose (LEL). EU-DAR: reduced bw in P0 and P1, and reduced pup survival in F1 and F2 at high dose determined overall LOAEL	EPA ToxRefDB & EU DAR	Horstmann, 1998
Cyanazine CAS 21725-46-2	F3: high dose: relative brain weight effect (LOAEL)	9% bw decrease in the P0 at same dose (LEL but not LOAEL). The LOAEL effect (brain weight) is only seen in third generation	EPA ToxRefDB	Eisenlord, 1969
Boscalid / Nicobifen CAS 188425-85-6	F2; mid and high dose: reduced body weight (LOAEL)	P0 and F1 only show liver hypertrophy (LEL) at these doses, low dose is unaffected. Incidental high litter size in F2 accompanied lower pup body weight	EPA ToxRefDB, & EU DAR	Schilling, 2001

1. none of these 5 substances carry an EU classification for fertility or developmental toxicity

2. except for Boscalid, these substances all appear in EU Annex VI. Boscalid is currently under evaluation for classification in the EU.

Part II: On the impact of P1 mating and F2 offspring effects for EU Classification & Labelling

Emiel Rorije, André Muller, Erna Schenk, Betty Hakkert, Aldert Piersma (RIVM, Bilthoven, the Netherlands)
Report for the OECD Working Group on the Extended One Generation Reproductive Toxicity study protocol
October 13, 2010

Summary

2.1 This report describes the analysis of the 50 substances present in the multigeneration database which carry a classification for reproductive toxicity or a label for lactation to assess the impact of P1 mating and F2 offspring effects for EU Classification & Labelling. The first stage of this assessment showed that for 24 substances end points were reported to be affected in the P1/F2 generation that had not been observed in earlier stages of the study, or that occurred at lower doses than in earlier phases of the study. Of these 24 substances, for 15 substances it was concluded that in view of all data in the study the observed F2 effects were very unlikely to have been essential for arriving at the classification given. The 9 remaining substances showed P1/F2 effects that might have been reason for classification. For 7 of these substances, the historic record of the minutes of the Technical Committee on Classification & Labelling (TCC&L) were studied. Our analysis of these minutes indicates that in the view of the TTC&L the P1/F2 parameters did not play a crucial role in the classification of these substances. For the two remaining substances either the P0 mating findings would have sufficed for the classification given, or the effects were not reproduced in a second mating of the same generation. For these two substances the TCC&L records will be analysed at a later stage. In summary, for a subset of the 50 substances out of 498 in the multigeneration database that carry a classification for reproductive toxicity or a label for lactation, specific effects in the P1/F2 generation were observed for a number of substances. However, in none of these cases the P1/F2 generation of the multigeneration study appears to have provided crucial information that determined the classification & labelling.

Introduction

2.2 In the previously provided Part 1 of the analysis, taking all studies in the database into account, studies were identified that showed effects at lower doses in the P1/F2 generations as compared to earlier stages of the protocol. This was done by comparing the lowest effect levels in the different generation groups, irrespective of the type of effects. The assumption was that both for Risk Assessment (NOAEL or LOAEL determination) as well as for Classification & Labelling (C&L) the most relevant effects are those occurring at the lowest effect level (LEL) or, taking into account expert interpretation, at the lowest observed adverse effect level (LOAEL).

2.3 It has been argued in the OECD expert group on the EOGRTS that specifically for C&L purposes specific (reproductive) effects occurring both at as well as above the LEL or LOAEL level, can influence the decision on classification for reproductive effects (fertility as well as developmental effects). In order to assess whether and how often in retrospect the above situation has actually occurred, this present paper reports on the 50 substances in the database of multi-generation studies that carry a classification for reproductive toxicity or a label for lactation. The studies pertaining to these substances were analyzed for the occurrence of unique effects in the P1/F2 generations, which were not observed in the F0/P0/F1 generations, not only at the study LEL or LOAEL, but at any dose level in the studies.

Results

2.4 In total 49 substances in the database carry a classification according to EU regulations (Annex VI) as Reproductive Toxicants (R60,61,62 and/or 63) (**Table 1**). Additionally 1 substance which is classified as "possibly harmful to breast feeding babies" (R64) has also been included in this analysis, making a total of 50 substances.

2.5 All 66 studies in the combined multi-generation database for these 50 substances were analyzed for the occurrence of unique P1/F2 effects, irrespective of dose level (**Table 2**). Subsequently the type and severity of the effect in the P1/F2 has been compared with the effects in the P0/F1 generations wherever possible. This analysis resulted in 24 substances / 26 studies with an observed unique P1/F2 effect..

2.6 For 15 of these 24 substances, detailed observation of the study data revealed that the unique P1/F2 effect was very unlikely to have been essential for arriving at the classification given. These 15 cases are reviewed in a dedicated chapter below.

2.7 The remaining 9 substances / 10 studies showed a P1/F2 effect that potentially might have resulted in Classification and Labelling for reproductive effects. These 9 cases are reviewed in another dedicated chapter below. For these 9 substances, documentation of meeting minutes from the EU Classification and Labelling working group has been investigated in detail to establish whether the P1/F2 effects observed in these 10 studies had played a role in the decision to classify these substances for reproductive toxicity.

2.8 For 1-bromopropane, cadmium chloride, DEHP, BBP and Benomyl full study summaries of all effects are given in **Annex I** as examples of the information summarized in the database. The minutes of the EU Technical Committee on C&L (TCC&L) that contain the reasoning at the basis for conclusions in the C&L working group is given for 7 substances in **Annex II** to this report, with our assessment of their implications for the role of the P1/F2 generation of the two-generation reproduction study.

2.9 The results strongly suggest that for all 50 substances that carry a classification for reproductive toxicity or a label for lactation which do have a two-generation reproduction toxicity study, the classification would have been the same in the absence of the P1/F2 generation data in these two-generation studies.

Table 1. 49 substances classified for reproductive toxicity (R60-63) in the combined retrospective database of multi-generation studies. Lindane, is labelled only as R64 (lactation label) without a classification for reproductive toxicity.

Name	Reproductive C&L (Cat.2)	Name	Reproductive C&L (Cat.3)
Glufosinate ammonium	Cat.2 R60 Cat.3 R63	2,4-dinitrotoluene	Cat.3 R62
1-Bromopropane	Cat.2 R60, Cat.3 R63	2-hydroxyethyl picramic acid	Cat.3 R62
2-methoxyethanol	Cat.2 R60-61	Acrylamide	Cat.3 R62
Benomyl	Cat.2 R60-61	Bisphenol A	Cat.3 R62
Boric acid	Cat.2 R60-61	Molinate	Cat.3 R62
Cadmium chloride	Cat.2 R60-61	Nitrobenzene	Cat.3 R62
Carbendazim	Cat.2 R60-61	Octamethylcyclotetrasiloxane	Cat.3 R62
DEHP	Cat.2 R60-61	Carbon disulphide	Cat.3 R62-63
Potassium dichromate	Cat.2 R60-61	Nonylphenol	Cat.3 R62-63
Vinclozolin	Cat.2 R60-61	Piperazine	Cat.3 R62-63
DIHP	Cat.2 R61	Tepraloxydim	Cat.3 R62-63

Dinocap	Cat.2 R61	Fenarimol	Cat.3 R62-63, R64
Ethylene thiourea	Cat.2 R61	Amitrole	Cat.3 R63
Fluazifop butyl	Cat.2 R61	Chlorotoluron	Cat.3 R63
Flumioxazin	Cat.2 R61	Cyproconazole	Cat.3 R63
Flusilazole	Cat.2 R61	Fenpropimorph	Cat.3 R63
Isoxaflutole	Cat.2 R61	Fentin hydroxide	Cat.3 R63
Nickel sulphate	Cat.2 R61	Mancozeb	Cat.3 R63
Nitrofen	Cat.2 R61	Maneb	Cat.3 R63
PFOA	Cat.2 R61	Metconazole	Cat.3 R63
Tridemorph	Cat.2 R61	Myclobutanil	Cat.3 R63
Azafenidin	Cat.2 R61 Cat.3 R62	Tebuconazole	Cat.3 R63
BBP	Cat.2 R61 Cat.3 R62	Toluene	Cat.3 R63
DBP	Cat.2 R61 Cat.3 R62		
Dinoseb	Cat.2 R61 Cat.3 R62	Lindane	R64
Linuron	Cat.2 R61 Cat.3 R62		

Table 2. 24 classified substances (from a total of 50), with 26 different studies in which a unique P1/F2 effect is observed at any dose level. A unique effect is defined as an effect that has not been seen in earlier stages within the study irrespective of dose level. If the same effect was observed in the P0/F1 at a higher dose level only, this is also scored as a unique P1/F2 effect. 4 studies given in this table do not show any unique P1/F2 effects (NO/NO score), the substance involved does however have another study which does show a unique P1/F2 effect.

	Study	New P1/F2 effect(s) at LEL?	New P1/F2 effect(s) above LEL?	C&L
1-Bromopropane*	WIL, 2001	YES	YES	Cat.2 R60, Cat.3 R63
Amitrole	Richard, 1995	YES	NO	Cat.3 R63
	Gaines, 1973	NO	YES	
BBP	Aso, 2005	NO	YES	Cat.2 R61 Cat.3 R62
	Tyl, 2004	YES	YES	
Benomyl	Mebus, 1991	YES	YES	Cat.2 R60-61
Cadmium chloride*	Nagymajtenyi, 1997	YES	YES	Cat.2 R60-61
DBP	Wine, 1997	YES	YES	Cat.2 R61 Cat.3 R62
DEHP	Schilling, 2001	NO	YES	Cat.2 R60-61
DIHP	McKee, 2006	NO	YES	Cat.2 R61
Fenarimol*	Hoffman, 1977	YES	YES	Cat.3 R62-63, R64
	Markham, 1978	NO	NO	
Fenpropimorph	Merkle, 1982	YES	NO	Cat.3 R63
Fentin hydroxide	Young, 1986	YES	YES	Cat.3 R63
Fluazifop butyl	Willoughby, 1981	NO	YES	Cat.2 R61
Flusilazole*	Pastoors, 1986	YES	YES	Cat.2 R61
	Mullin, 1990	NO	NO	
Lindane	King, 1991	NO	YES	R64
Molinate	Gilles, 1989	NO	NO	Cat.3 R62
	Moxon, 1997	NO	YES	
Myclobutanil	Brown, 1985	NO	YES	Cat.3 R63
Nickel sulphate	Ambrose, 1976	YES	NO	Cat.2 R61
Nitrofen*	Ambrose, 1971	YES	NO	Cat.2 R61
Nonylphenol	NTP, 1997	NO	YES	Cat.3 R62-63
Octamethylcyclo-tetrasiloxane	Dow, 2001	YES	YES	Cat.3 R62
PFOA	Luebker, 2005	NO	YES	Cat.2 R61
Piperazine	Wood, 1994	NO	YES	Cat.3 R62-63
Tepraloxydim	Hellwig, 1997	YES	NO	Cat.3 R62-63
Vinclozolin*	Hellwig, 1992	YES	NO	Cat.2 R60-61
	Hellwig, 1994	NO	NO	

*1-bromopropane, cadmium chloride, fenarimol, nitrofen, flusilazole and viclozolin were identified already in part 1 of the analysis as studies where effects in the P1/F2 determined the study LEL. For a discussion of the specific effects in these studies see also part 1 of this analysis.

15 substances with unique P1/F2 effects which are unlikely to have influenced EU C&L

2.10 The reasons why the observed P1/F2 effects are not likely to have influenced the classification and labeling conclusion for the remaining 15 substances are given below.

Amitrole

2.11 The Gaines, 1973 study shows a reduced number of litters in the F2a cohort at dose level 2 of 4. Only thyroid hyperplasia and reduced food consumption are noted at this dose level in the F0. At the next dose level (conc. 5x higher), clear effects in the F1 are observed (reduced number of pups born and reduced survival). There is a newer study (Richard, 1995) which uses the concentration from dose level 2 in the Gaines study as the high dose. In this study already very clear P0 and F1 effects are noted at this concentration; fertility effects both in the P0 and P1 (red nr of implantation sites), the F1 (significantly decreased mean litter size at day 1 post partum, decreased F1 pup body weights during lactation), but also P1 effects not observed in the P0 (significantly decreased mating indices, decreased fertility indices, increased length of gestation). These "new" P1 effects are however merely a different way of expressing the effects seen already in the P0 and F1 generations.

DBP

2.12 Wine, 1997 (EURAR) study for DBP shows F1 litter size and epididymal weight effects at low dose, and F2 pup body weight effects. At middose F2 as well as F1 pup bodyweight effect is noted, and additionally F1 litter size effects and epididymis and testes atrophy are observed. At the high dose again F2 pup body weight effects are seen, but now also a P1 pregnancy and fertility effect is noted which is not seen in the P0. At high dose the F1 shows litter size effects together with F1 pup bw, ovary, testes, epididymis, liver, prostate and sperm effects. The effects seen in the F1 are more than sufficient for classification.

DIHP

2.13 The new effect seen for the DIHP study is an F2 decrease in bw at middose. At the same dose level P0 fertility and F1 sperm count and liver hypertrophy effects are observed, which would very likely be more relevant for classification and labeling

Fentin hydroxide

2.14 In this study a slight F2 pup bodyweight effect is noted at middose which is not noted in the F1. However, in the F1 decreased litter size and increase of several organ weights are noted at this dose level. At the study high dose in the F2 a decreased viability is observed whereas in the F1 increased pup mortality and decreased litter size are noted. The F2 effects do not seem fundamentally different from the F1 effects, and will not have played a decisive role in the classification of fentin hydroxide as developmental toxic, R63.

Fluazifop butyl

2.15 At high dose a P1 fertility (index) effect is noted. No P0 fertility (index) effect is observed. However, in the P0 the number of implantations is reduced, gestational interval is increased, and in the F1 a decrease of the live birth index is already noted (which is not noted in the F2). The effects in the P1 will therefore not have affected the classification for effects on development with R61.

Lindane

2.16 At the highest dose (one dose above the LOAEL for offspring effects according to JMPR) a delay in the onset of teeth and hair growth is observed in the F2, but not in the F1. These effects are seen together with reduced viability index and dec. bw in both the F2 and the F1, and several (serious) kidney and liver effects only in the F1. The specific developmental effects seen only in the F2 did not lead to classification

of lindane for developmental toxicity. Whether this specific F2 effect is attributed to breast feeding only (and the F1 effects not) is not very likely.

Molinate

2.17 In the second study for molinate (Moxon, 1997) at middose decreased litter sizes are observed for the F2. This can however not come as a surprise, as there are several sperm and ovary effects observed in the F1 which should have an marked influence on reproductive performance and possibly litter size. At the highest dose an increased gestational interval is observed for the P1 generation, but so many reproductive parameters are affected in the F1 that classification, specifically as a Cat.3 R62 would not have been dependent on the observation of this gestational interval effect.

Myclobutanil

2.18 At the high dose of the study a decrease in litter size in the F2 is noted. In the F1 (and also in the F2) reduced body weight, a decrease in the number of females delivering litters and an increased nr. of stillborn pups are observed. This additional (F2) effect, at a dose level above the reproductive LOAEL which was based on a minimal increase in proportion of dead pups in both matings of the F1, is not likely to have been decisive for the C&L of myclobutanil as R63.

Nickel sulphate

2.19 At the study low dose an increase in pup mortality and decreased live litter size in the F1b are noted. In the F2a increased malformed fetuses per litter (short rib) are observed. No other P1 or F2 effects are seen in this study, also not at higher doses. It seems logical to assume that the F2a effect has played a role in the classification of nickel sulphate as cat.2 R61 developmental toxic. However, the JMPR summary explicitly notes that the malformations observed at low dose are not considered to be due to nickel because similar effects are not observed at higher doses. At the highest dose the fertility effects which were only noted in the F1b are more pronounced (decreased litter size, increased pup mortality, decreased pup bodyweight) and seen in both cohorts. The classification as developmental toxic will therefore most likely have been based on effects seen in developmental studies, not (this) multi-generation study.

Nitrofen

2.20 The nitrofen study only showed LEL effects in the P2/F3, not in the P1/F2 generations (see analysis part 1). In a classical 2-generation study there would have been no unique P1/F2 effect. The nitrofen P2 body weight effect was dismissed in the previous analysis due to lower initial body weights of rats used for breeding (see part 1 of the analysis).

Nonylphenol

2.21 The F2 shows at middose decreased ovary weight and decreased epididymal sperm density (10%), in the F1 reduced bodyweight gain, histopathological changes in the kidney and delayed vaginal opening are observed. At high dose the decreased epididymal sperm density is still only observed in the F2. These F2 effects will not have changed the Cat.3 reproductive classification for nonylphenol, as the F1 effects would have been sufficient for such a classification.

Octamethylcyclotetrasiloxane

2.22 In this study the P1 was observed to have a decrease in mating performance and in the number of animals producing litters, both at the study LEL and above. This was not noted for the P0. However, the F1

mean live litter size was already reduced (also in the F2) at study LEL. The reduced live litter size in the F1 is thought to be sufficient for classification as Cat.3 R62, especially with the observation of disturbed estrous cycles in the F1 at the study LEL and above, and also increased pituitary gland weight at the highest dose for the F1.

PFOA

2.23 At the highest dose level a gestational interval increase and a decrease in the nr. of implantation sites in the P1 is seen, which is not noted in the P0 generation. However, no (fertility) F2 effects are indicated. At the highest dose also a decrease of the live birth index and the viability index of the F1 is observed, together with body weight and food consumption effects. At one dose below the highest dose several developmental effects (delayed eye opening, delayed pinna unfolding, delay in development of surface righting ability) are observed in the F1, which might have been relevant for classification as R61. The P1 effects at the highest dose will not have played a role in classification for developmental toxicity.

Piperazine

2.24 At middose (LEL) reduced litter sizes are observed in the F1 and F2, and delayed sexual maturation in the F1as well as reduced nr of implantation sites in the P0. At the high dose the P1 shows a decrease in number of pregnancies. This is to be expected with the effects already seen at middose (e.g. reduced number of implantation sites in the P0), and will not have changed the Cat.3 reproductive classification.

9 Substances with unique P1/F2 effects potentially influencing EU C&L

2.25 The 10 studies (related to 9 substances) indicated with a pink background in table 2 are those with a specific P1/F2 effect which might have influenced the C&L discussion. These 10 studies are discussed below in greater detail as the EU Technical Committee on Classification & Labeling meeting minutes archives have been studied to examine if and how these studies and the specific P1/F2 effects may have played a role in the final conclusion for classification.

1-Bromopropane

2.26 Unique effects of 1-bromopropane in the 2-generation study by Wil, 2000 were a non-significant reduction of the fertility in the P1/F2 mating at the low and mid dose and a reduction in number of implantation sites at the mid dose (effects shown in bold in Annex I).

2.27 According to the UK proposal 89/1 and the summary records, the classification with R60 was based on the effects on reproductive organs in the repeated dose toxicity studies and the effects on mating and other fertility parameters in the 2-generation study. The mating effect and the effect on fertility parameters were already observed in the parental animals and in the first mating. The effects in the second mating could be seen as a confirmation in a repeat study. The reduced fertility and reduced number of implantation sites at dose 1 and 2 in the P1 mating was not used as an unique effect. However, UK stated that the presence of effects below 2 mg/ml justified category 2. It is unclear whether this remark related specifically to the reduced fertility and reduced number of implantation sites only observed after the second mating in the 2 generation study at 1.25 mg/ml (dose 2) or also to other effects on sexual function observed in the repeated dose studies and in the P0 of the 2-generation study. The latter interpretation is considered to be the most likely. Therefore, it can be concluded that absence of the second mating in this 2-generation study would have had a minor effect on the argumentation for the classification and would not have affected the classification with R60.

2.28 According to the UK proposal 89/1 and the summary records the classification with R63 is based on the developmental effects observed in the developmental study. Therefore, it can be concluded that absence of the second mating in this 2-generation study would not have affected the classification with R63.

Cadmium chloride

2.29 Unique effects of cadmium chloride in the 2-generation study by Nagymayteni, 1997 are provided in Annex I. As the 2-generation study by Nagymayteni et al. from 1997 was not available for the determination of the classification of cadmium chloride for reproductive toxicity in 1997 and the substance was already classified with R60 and R61, it could not have affected the classification. Further, there were several other studies showing effects on fertility and development warranting classification.

Fenarimol

2.30 In the case of fenarimol a direct follow-up study has been performed by the same test laboratory to confirm the findings of specific P1/F2 effects (Hoffman 1977 and Markham 1978 studies). This second, follow up study could not reproduce the specific effects seen in the P1/F2 in the first study. In the first study (Hoffman, 1977) for fenarimol only F2/F3 effects are observed, without *any* F0/P1 or F1 effects noted. Only two dose levels were tested. A second study (Markham, 1978) was performed for the same company, now using three dose levels. In this study no unique P1/F2 effects are noted, but it is noted that the observed reduction in fertility (index) becomes more pronounced after each successive P0 mating and even more pronounced after the first P1 mating. This in itself might have influenced C&L. However, fenarimol is classified as Cat.3 R62-63 which is the lowest classification for reproductive toxicity possible. The P0 mating effects in the second study would suffice for a classification as Cat.3 R62. No specific effects are noted in these two multi-generation studies that explain the developmental and breastfeeding classification (R63 and R64) of fenarimol. Evidence for that classification must have come from other (type of) studies. This will be determined at a later stage.

Flusilazole

2.31 The same issue with reproducibility in a second study occurs for the Flusilazole study by Pastoors, 1986, which showed effects that could not be reproduced in a 1990 study using identical dose levels. Furthermore the effects in the Pastoors study were only seen in one cohort (F2a) not in the other (see retrospective analysis part I). The background for the classification will be determined at a latter stage.

DEHP

2.32 The new P1/F2 effects in the DEHP study are live birth index and nipple development. The nature of the F1 effects (viability index, anogenital distance) is such that it is questionable if the C&L conclusion would change if these P1/F2 effects (live birth index, nipple development) would not have been observed. At a higher dose level effects on the fertility index are also noted in the P0, and nipple development effects are also seen in the F1. All effects in the DEHP study are summarized in Annex 1.

2.33 The unique effects in the F2 of the 2-generation study by Schilling et al. (2001) could not have been determinative for the classification of DEHP for fertility or development because this study was not available to the TC-C&L during their assessment in 2000 and the substance was classified with R60 and R61. The absence of this study is confirmed by the overview of reprotoductive toxicity studies in 37/99-add25 which only contained the range-finding (Schilling et al., 1999).

Benomyl

2.34 The new P1/F2 effect seen in the Mebus 1991 study for Benomyl shows an age landmark effect – delayed eye opening. Also there was an F2 bodyweight and a litter viability effect, although these only occur together with toxicity seen in the P0 and F1 generations. All effects observed in this study are summarized in Annex 1.

2.35 The classification with R60 for fertility is based on effects on testes seen in repeated dose studies and effects on testes and fertility in mating studies. This classification would not have been affected by the F2 effects in the Mebus study.

2.36 The classification for development with R61 was based on the brain and eye malformations observed in several developmental studies using gavage exposure. This classification would not have been affected by the F2 effects in the Mebus study.

BBP

2.37 In the Aso, 2005 study, at high dose a decrease in fertility index is observed for the P1. However, already at low and mid dose a decrease in epididymis weight in the F1 is noted, as well as a decreased anogenital distance. It is possible that the fertility index effect at high dose gave rise to a Cat.3 R62 classification, where the other effects at low and mid dose are possible reasons for the Cat.2 R61 classification.

2.38 The Tyl, 2004 study shows a testes effect in the F2 at the study LEL where no testes effects are seen in other generations. The effect is limited to missing testes in one male pup. At the high dose there are mating and fertility effects noted in the P1 as well as a decreased litter size in the F2, both of which are not noted in the P0/F1 generations. There are multiple organ and reproductive organ effects as well as developmental landmark effects in the F1. As the only fertility effects in this study are seen in the P1/F2 generation it cannot be excluded that this played a role in the classification of BBP as Cat.3 R62.This study is summarized in Annex 1 to this report.

2.39 The Tyl, 2004 2-generation study, which showed some specific effects in the F2 was provided during the TC-C&L assessment of BBP. However, as clearly shown by the conclusions in the summary records (Annex 2 to this report) this study did not affect the classification because the classification with R61 and R62 was already decided before this study was provided. The other 2-generation study by Aso was published in 2005 and could therefore also not have affected the classification because the substance was already classified with R61.

Fenpropimorph

2.40 At the highest dose in the JMPR summarized study for fenpropimorph there is a retarded development of the fur, and delayed eye opening effect seen in the F2. In the F1 a different developmental effect is noted, retarded unfolding of auricle. An increased % of stillborn pups and a decreased pup weight in the F1 are also noted (not seen in the F2). It seems that for the classification of fenpropimorph as a developmental toxicant the F2 effects were relevant.

2.41 From the summary records and proposals (see Annex 2 to this report) it is clear that the classification of fenpropimorph with Repro Cat 3; R63 was based on the effects seen in the developmental studies in rat and rabbit and some of the effects in the F1 of the 2-generation study but not on the unique effects in the F2 of the 2-generation study.

Tepraloxydim

2.42 In the study for tepraloxydim only decreases in food consumption, bodyweight and bodyweight gain are noted in all generations at the highest dose, except for a developmental landmark effect observed in the F2; a delay in time to eye opening. The classification for developmental toxicity (R63) could have been based on this observation. Fertility effects are not observed in this study.

2.43 The unique effects in the F2 of the 2-generation study may have had some effect on the classification with R63 but the classification was mainly based on the effects in the rat developmental study (A review of the minutes and proposals is made but cannot be provided for these substances as they were confidential).

References

Ambrose, A.M., Larson, P.S., Borzelleca, J.F., Blackwell Smith, R. & Hennigar, G.R. (1971) Toxicologic studies on 2,4-dichlorophenyl-p-nitrophenyl ether (TOK). Toxicol. Appl. Pharmacol. 19:263-275.

Ambrose AM, Larson PS, Borzelleca JF, Hennigar Jr. GR (1976): Long term toxicologic assessment of nickel in rats and dogs. Journal of Food Science and Technology 13:181-187

Aso. A Two-Generation Reproductive Toxicity Study of Butyl Benzyl Phthalate in Rats. The Journal of Toxicological Sciences, Vol.30, Special Issue, 39-58, 2005.

Brown U. (1985) Two-Generation Reproduction Study with RH-53,866 Technical in Rats: Protocol No. 83P-364, Report No. 84R-117. Unpublished study prepared by Research Pathology Services, Inc. 78 p.

Dow Corning Corporation (2001) A two-generation inhalation reproductive toxicity and developmental neurotoxicity study of octamethyltetrasiloxane (D4) in rats. Report No.2001-10000-50855, December 2001.WIL Research Laboratories. An inhalation two-generation reproductive toxicity study of D4-octamethyltetrasiloxane in rats. Study No. 8713. Study Director, D. Stump. Ashland (OH): Study sponsored by Dow Corning. Report No.:2001-10000-50855

Gaines T.B. et al.: Toxicol.Appl.Pharmacol. 26, 118-129 (1973)

Gilles, P. A. and A. G. Richter (Ciba-Geigy Environmental Health Center), 1989. Two generation reproduction study in female rats with R-4572. ICI Americas Report # T-13218. DPR Vol. 228-070, #087658.

Hellwig, J. (1992) Report Reproduction Study with Reg. No. 83 258 (Vinclozolin) in Rats Continuous Dietary Administration over 2 Generations (2 Litters in the First and 2 Litters in the Second Generation): Lab Project Number: 92/11251: 71R0375/88053. Unp

Hellwig, J. et al. (1994) Report: Second reproduction study with Reg. No. 83 258 in rats. Continuous administration over 2 generations. Unpublished report from BASF AG, Ludwigshafen, Germany. Submitted to WHO by BASF AG, Ludwigshafen, Germany.

Hellwig, J.; Deckardt, K.; Gembardt, C. et al. (1997) Report Reg. No. 191819: Two-Generation Reproduction Toxicity Study in Wistar Rats: Continuous Dietary Administration: Lab Project Number: 97/10616: 70R0268/92095: 70R0268/92095F. Unpublished study pre

Hoffman, D.G., Adams, E.R., Markham, J.K., Owen, N.V., Gibson, W.R. & Morton, D.M. (1977b) A multi-generation reproduction study with EL-222 in the rat. Unpublished report No. R-715, R-1345, R-956, R-966 dated November 1977 from Lilly Research Laboratories, USA. Submitted to WHO by DowElanco Europe, Wantage, Oxon, United Kingdom.

King, V. (1991) Lindane: Reproductive Performance Study in Rats Treated Continuously Through Two Successive Generations: Final Report: (Addendum to MRID 422210):Lab Project Number: 91/0948: 91-CIL004-0948: CIL-004-LIND. Unpublished study prepared by Life

Luebker, D.J., Et al. (2005) Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats. 3M.

Markham, J.K., Hoffman, D.G., Owen, N.V. & Morton, D.M. (1978c) A second multi-generation reproduction study with EL-222 (Lilly compound 56722) in the rat. Unpublished report No. R-636,

R-1076, R-217 dated July 1978 from Lilly Research Laboratories, USA. Submitted to WHO by DowElanco Europe, Wantage, Oxon, United Kingdom.

McKee et al. Reproductive Toxicology 21 (2006) 241–252

Mebus, C.A. (1990) Reproductive and fertility effects with DPX-1991-529 (benomyl). Multigeneration reproduction study in rats. Unpublished report from DuPont de Nemours & Co., Haskell Laboratory, Newark, Delaware, USA.

Merkle, J., Freisberg, K.O., Hildebrand, B. & Ruff, M. (1982) Report on a reproduction study with 4-[3-[4-(1,1-dimethylethyl)phenyl]-2-methyl]propyl-2,6(cis)-dimethylmorpholine (Reg. No. 108 406) in rats after oral administration (feeding). Two-generation study. Unpublished report dated 22 February 1982 from Department of Industrial Hygiene and Toxicology, BASF Aktiengesellschaft, Industrial Hygiene and Toxicology, BASF Aktiengesellschaft, Ludwigshafen, Germany. Submitted to WHO by Ciba-Geigy AG, Basel, Switzerland.

Moxon, M. (1997) Molinate: Two Generation Reproduction Study in the Rat: Lab Project Number: RR0708: CTL/P/5409: RR0708/F0. Unpublished study prepared by Central Toxicology Lab. (Zeneca) 4455 p.

Mullin, L. (1990) Reproductive and Fertility Effects with Flusilazole Multigeneration Reproduction Study in Rats: Lab Project Number: HLR/424/90. Unpublished study prepared by E.I. du Pont de Nemours and Co. 1199 p.

Nagymagtenyi L, Schulz H and Desi I (1997) Behavioural and functional neurotoxicological changes caused by cadmium in a three-generational study in rats. Hum. Exp. Toxicol. 16, 691-699.

NTP (1997) Nonylphenol: multigenerational reproductive effects in Sprague-Dawley rats when exposed to nonylphenol in the diet. R.O.W. Sciences study no. 8989-30.

Pastoor, T.P., Rickard, R.W., Sykes, G.P., Kaplan, A.M. & Gibson, J.R. (1986) Long-term feeding (combined chronic toxicity/oncogenicity study) and two-generation, four litter reproduction study in rats with INH-6573. Unpublished report No. HLR 32-86 from Haskell Laboratory for Toxicology and Industrial Medicine, Wilmington, Delaware, USA. Submitted to WHO by E.I. du Pont de Nemours & Co., Inc., Wilmington, Delaware, USA.

Richard, J. (1995) Two generation study by oral route (dietary admixture) in rats. Unpublished report No. 6432 (study No. T5041262) from Centre International de Toxicologie, Evreux, France. Submitted to WHO by Bayer AG, Leverkusen, Germany.

Schilling K, Gembardt C and Hellwig J (2001) Di-2-ethylhexyl phthalate - Two-generation reproduction toxicity study in Wistar rats. Continous dietary administration. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, D-67056 Ludwigshafen, FRG. Laboratory project identification 70R0491/97139. 1183 pages. (referenced as BASF 70R0491/97139 in the IUCLID file)

Tyl RW, Myers CB, Marr MC, Fail PA, Seely JC, Brine DR, Barter RA and Butala JH (2004). Reproductive toxicity evaluation of dietary Butyl Benzyl Phthalate (BBP) in rats. Reprod. Toxicol. 18, 241-264.

WIL Research Laboratories. An inhalation two-generation reproductive toxicity study of 1-bromopropane in rats. Study No. WIL-380001. Study Director, D. Stump. Ashland (OH): Study sponsored by Brominated Solvents Committee (BSOC); 2001

Willoughby, C.R.; Tesh, J.M.; Pritchard, A.L.; Et Al. (1981) Fluazifop Butyl: Effects Upon Reproductive Performance Of Rats Treated Continuously Throughout Two Generations: Lsr Report No. 81/Ily282/130. Final Rept. (Unpublished Study Received Dec 4, 1981)

Wine RN et al. (1997). Reproductive toxicity of di-*n*-butylphthalate in a continuous breeding protocol in Sprague-Dawley Rats. Environ. Health Perspect. **105**(1), 102-107.Wood E and Brooks PN (1994).

Piperazine hydrochloride: Dietary two generation reproduction study in the rat. Report to Akzo Nobel from Safepharm Laboratories Ltd., Derby. (unpublished)

Young, D.L. (1986) A dietary two-generation reproduction study in rats with triphenyltin hydroxide (Code: HOE 0.29664 OF ZD97 0004 technical substance). Final Report project WIL-39022 (A35378) from WIL research laboratories, Inc. Ashland USA. Submitted to WHO by Hoechst AG., Frankfurt-am-Main.

ANNEX 1**:**

Effects tables for studies indicated in the text, where the P1/F2 effects that have not been seen at lower dose levels or in other generations might have influenced Classification and Labelling. These effects are given in bold. The Dose Level (DL) which was determined to be the study reproductive LOAEL is indicated with a *.

SUBSTANCE/ STUDY	DL	F0 PARENTAL	P0 MATING	F1 OFFSPRING	P1 MATING	F2 OFFSPRING	P2 MATING	F3 OFFSPRING
1- bromopropane **Cat.2 R60, Cat.2 R63** **Ref: WIL, 2001**	1				**n.s. red fertility**			
	2*	Dec.prostate w.		red litter w gain; inc.estrous cycle l.	**n.s. red fertility Red. #implanta-tion sites**			
	3	inc estrous cycle, series of reprod. organ w.and histopat.changes	Red.fertility; Red.#implanta-tion sites	inc estrous cycle l.; repr organ weights; red mobile sperm; red #pups born; slightly red pup viability (not cons. treatment rel.); red pup weight	n.s. red fertility Red. #implanta-tion sites	red #pups born; red pup weight		
	4	Same as DL3	0 females became pregnant					

SUBSTANCE/	DL	F0	P0	F1	P1	F2	P2	F3 OFFSPRING

STUDY		PARENTAL	(M)	OFFSPRING	(M)	OFFSPRING	(M)	
Cadmium chloride	1*							**red open field exploration**
Cat.2 R60-61 **Nagymayteni, 1997**	2	lower bw (n.s.)		lower bw (parental) changes in functional parameters of tail nerve		lower bw (parental) changes in functional parameters of tail nerve **changes in latency of evoked potentials (somatosensory)**		changes in funciontal paramenters of tail nerve **changes in latency of evoked potentials (somatosensory)** **changes of ECoG index in somatosensory and visual focus**. **red open field exploration**
	3	lower bw (n.s.)		lower bw (parental) changes of ECoG in somatosensory, visual and auditory focus, changes in latency of evoked potentials (somatosensory, visual and auditory), changes in functional parameters of tail nerve		lower bw (parental) changes of ECoG in somatosensory, visual and auditory focus, changes in latency of evoked potentials (somatosensory, visual and auditory), changes in functional parameters of tail nerve		changes of ECoG in somatosensory, visual and auditory focus, changes in latency of evoked potentials (somatosensory, visual and auditory), changes in functional parameters of tail nerve, **red open field exploration**; **dec rel kidney and spleen w**

SUBSTANCE/ STUDY	DL	F0 PARENTAL	P0 MATING	F1 OFFSPRING	P1 MATING	F2 OFFSPRING	P2 MATING	F3 OFFSPRING
DEHP	1*	focal tubular atrophy		focal tubular atrophy				
Cat.2 R60-61 **Schilling, 2001**	2	focal tubular atrophy		focal tubular atrophy dec. thymus w; dec. spleen w.; red. viability index;		dec. thymus w.; red. viability index,		

				red. anogenital dist.		red. anogenital dist.**; incr. #stillborn p.; nipple dev.**		
	3	focal tubular atrophy	Incr. post implantation loss; decr. fertility;	focal tubular atrophy Dec. bw; anogenital distance; nipple dev; Preputial sep; Vaginal opening; thymus w.; ovarian follicle count.	Incr. post implantation loss; decr. fertility	**Live birth index, dec. testes weight.**		

SUBSTANCE/ STUDY	DL	F0 PARENTAL	P0 MATING	F1 OFFSPRING	P1 MATING	F2 OFFSPRING	P2 MATING	F3 OFFSPRING
Benomyl **Cat.2 R60-61** **Ref. Mebus, 1991**	1							
	2							
	3*	Red testes sperm counts; Atrophy and degeneration of the seminiferous tubules		Red testes sperm counts; Atrophy and degeneration of the seminiferous tubules; Dec. offspring bw; Oligospermia in epididymes		Dec. offspring bw		
	4	Red testes sperm counts; Atrophy and degeneration of the seminiferous tubules; Red testes weight and histopathological changes;		Red testes sperm counts; Atrophy and degeneration of the seminiferous tubules; Red testes weight and histopathological changes; Oligospermia in epididymes; Red mean bw; Red bw gain; Red food cons; Dec offspring bw		**Partially open or unopened eyes were observed** Dec offspring bw,		

SUBSTANCE	DL	F0 PARENTAL	P0 MATING	F1 OFFSPRING	P1 MATING	F2 OFFSPRING	P2 MATING	F3 OFFSPRING
BBP	1							
Cat.2 R61 Cat.3 R62	2*	Inc. kidney weight		Inc kidney, liver and pancreas weight; Dec. pup bw; dec.anogenital dist. (1.89 vs 2.06).		**Missing testis (1 male); Inc uterus weight;** Dec. anogenital dist. (1.99 vs 2.05)		
Tyl, 2004	3	inc liver w inc. kidney w, red ovaries w red. uterus w	red bw during gestation and lactation	dec pup body w/litter, dec male AGD (1.7 vs 2.06), Inc males with 1 or more nipples (19% vs 0%) and 1 or more areolae (32% vs 2.6%), dec bw at weanling, dec abs thymus w, dec abs and rel spleen dec abs and rel testis w, dec abs epididymis w, dec abs ovaries w, dec abs uterus w, Delayed preputial separation (45.2 vs 40.9), delay in vaginal patency (34.1 vs 31.4) red bw inc rel liver w; inc adrenal gland w, inc pancreas w inc pituitary w	**red mating (70 vs 96.7) red fertility (81 vs 100)**	dec pup body w/litter, dec male AGD (1.77 vs 2.05), Inc males with 1 or more nipples (16% vs 0%) and # areolae per male (3.14 vs 0.05), dec bw at weaning, abs thymus w, abs and rel spleen abs and rel. testis w; abs ovaries w, **Red no pups / litter red live pups / litter, missing epididymes, missing seminal vesicle**		

ANNEX II:

EU Technical Committee on Classification & Labelling meeting minutes excerpts on the classification for reproductive toxicity of the substances:

- **1-bromopropane**
- **Benomyl**
- **Benzyl-butyl phthalate**
- **Cadmium chloride**
- **DEHP**
- **Fenpropimorph**
- **Tepraloxydim**

Followed by discussion prepared by Andre Muller (AM), RIVM, The Netherlands, October 2010

1-Bromopropane

Data used: Summary record January 2002

N-PROPYL BROMIDE (~~U057~~U060)
(CAS NO: 106-94-5; EC NO: 203-445-0; ANNEX I INDEX NO: 602-019-00-5)

Classification proposal: [R10 - Repr. Cat. 2; R60 - Repr. Cat. 3; R63 - Xn; R48/20 - Xi; R36/37/38 - R67]
Classification in Annex I, 12th ATP: R10 - Xn;R20

ECBI/89/01 UK, Classification proposal

Reproductive toxicity

NL said that in the two-generation study with high doses no effects were seen, making it a borderline case for classification. **DK** would support Category 2 for fertility on sound evidence. Further they had thought that R63 was borderline, but after checking the studies they agreed that R63 was justified. There was no maternal toxicity in the presence of foetal weight loss. The **Group** provisionally agreed to the proposal put forward by the UK: Repr. Cat. 2; R60 : Repr. Cat. 3; R63. Member States experts were asked to react during the follow-up period if they would like to further discuss the end-points for reproductive toxicity.

(No reaction concerning the classification for fertility and developmental toxicity were received during the follow-up period.)

Conclusion:

The **Group** agreed to classify **n-propyl bromide** as follows:
R10 (to be confirmed - more information expected from the UK) -
Repr. Cat. 2; R60 (provisionally) - Repr. Cat. 3; R63 (provisionally) - Xi; R36/37/38 - R67

Summary record May 2002

N-PROPYL BROMIDE (~~U057~~U060)
(CAS NO: 106-94-5; EC NO: 203-445-0; ANNEX I INDEX NO: 602-019-00-5)

Classification proposal: [R10 - Repr. Cat. 2; R60 - Repr. Cat. 3; R63 - Xn; R48/20 - Xi; R36/37/38 - R67]
Classification in Annex I, 12th ATP: R10 - Xn;R20

ECBI/89/01 UK, Classification proposal
ECBI/89/01 Add.1 UK, Consideration of class. for flammability
ECBI/89/01 Add.2 IND position regarding classification

In **January 2002** the **Group** agreed to classify (2-ethoxy-1-methyl)ethyl acetate as follows: R10 (to be confirmed - more information expected from the UK) - Repr. Cat. 2; R60 (provisionally) - Repr. Cat. 3; R63 (provisionally) - Xi; R36/37/38 - R67. This was made on the basis of the UK proposal that was discussed for the first time at this meeting. The substance would be re-discussed at the next meeting if not possible to conclude during the follow-up period. End-points for follow-up actions are: **R10**, **Repr. Cat. 2; R60** and **Repr. Cat. 3; R63**. **UK** would also send in additional information on R48/20 if they still would support this classification.

Reproductive toxicity: Development

UK said that there was a development toxicity study available that still haven't been considered. They said that in case IND would make the study available soon they would be willing to look into it. **IND** promised to send in the documentation on the new study as soon as possible. Meanwhile, the decision to classify with Repr. Cat. 3; R63 would remain.

Reproductive toxicity: Fertility

IND agreed that there was a positive effect but the question was at what level of classification, category 2 or category 3. They thought that the data rather supported category 3. **UK** argued that as there were effects below 2mg/ml category 2 was justified. **N** added that effects could occur also independent of systematic toxicity. **IND** said that the positive results only had been seen for one species. Systemic toxicity had not been seen at work place but only in epidemiological studies. **UK** said that everything was cited at the data sheet. The IND protocol was not appropriate to test the reproductive endpoints. **UK** would like to wait for the development study to discuss fertility further. The Group agreed that the classification in category 2 would be included in the next ATP proposal. However the new studies would be taken into consideration as soon as available. **UK** reminded that besides the development study also the other one on repeated toxicity should be considered as soon as available.

Conclusion: The **Group** agreed to classify **n-Propyl bromide** as follows: **R10 - Repr. Cat. 2; R60 - Repr. Cat. 3; R63 - Xn; 48/20 - Xi; R36/37/38 - R67**. The labelling would then be with the **Symbols: T**, **R-phrases: 60-36/37/38-48/20-63-67** and **S-phrases: 45-53. No specific concentration limits**. This classification would be reported for future inclusion in an ATP proposal.

ECBI/89/1

Reproductive toxicity

Fertility

Repeated inhalation exposure of rats to n-propyl bromide causes adverse effects on the male reproductive system. Data from the most comprehensive study available reveal reproductive organ weight changes, reductions in epididymal sperm count and motility, increases in the number of morphologically abnormal sperm, and an increase in retained elongated spermatids in stage IX, X and XI seminiferous tubules at 1 mg/l and above. Similar observations (including seminiferous tubule atrophy, atypical spermatids and hypo/aspermatogenesis) were also made in additional repeat inhalation studies in rats. The findings are consistent with an inhibition of spermiogenesis.

In a two-generation inhalation study performed in rats, exposure to n-propyl bromide at concentrations of 1.25 mg/l or greater had significant effects on fertility. Exposure to 3.75 mg/l resulted in complete infertility. At lower levels of exposure, dose-dependent reductions in the numbers of pups born were recorded in both the F_0 and F_1 generations. Reductions in the relative weights of epididymides,

seminal vesicles and prostate were noted following exposure at 1.25 mg/l or above. In this study, the only signs of parental toxicity were, in both generations, reductions in body weight gain of 10% or less and minor changes in the kidney including mild pelvic inflammation. It is very unlikely that this systemic toxicity could have led to the adverse effects seen on fertility.

Female fertility may also be sensitive to n-propyl bromide. In the two-generation study, F_0 animals at 2.5 and 3.75 mg/l showed extended oestrus cycle length, mean relative ovary weight reduction and increases in follicular cysts. Similar findings were seen in the F_1 generation. Additionally, in a 12-week repeat dose study, an increase in the number of irregular oestrus cycles at exposure levels of 1mg/l and above was seen.

These findings in rats are regarded as being relevant to humans and we consider them to be sufficient to justify classification with Repr Cat 2; R60.

Developmental toxicity

A recently conducted developmental toxicity study in rats is available. The results are difficult to interpret, although several findings suggest that classification may be justified.

Firstly, there was an increase in major malformations at the highest dose level (5 mg/l); 4 pups were affected in 3 litters, the malformations being hydrocephaly, mis-shapen cerebral hemispheres, a heart great vessel abnormality and testicular agenesis. No major malformations were seen at the lower and mid exposures and there was a single major malformation (kidney agenesis) recorded in controls. As the malformations seen at 5 mg/l were all different, it is possible that they were spontaneous in origin, but this is not certain.

Mean foetal weights were statistically significantly lower than controls in all n-propyl bromide-treated groups. Reductions in foetal weights were observed at the two highest doses in the presence of maternal toxicity, but were also seen at 0.5 mg/l in the absence of maternal toxicity.

Finally, there was an increased prevalence of foetuses with bent ribs and reduced ossification of the skull at 2.5 and 5 mg/l. At the highest dose, reduced ossification of the ribs, hyoid or 5/6th sternebrae were also observed. A retardation in skull ossification is commonly observed in the presence of maternal toxicity and, given the reductions in maternal body weight gain seen in this study, this may well not have been a direct effect of n-propyl bromide.

Although no firm conclusions about the potential developmental toxicity of n-propyl bromide can be drawn from these studies, we consider them to raise sufficient grounds for classification with Repr Cat 3; R63.

After the meeting in May 2002 there was an extensive discussion for several years with industry which did not result in a change of opinion of the TC-C&L. Therefore, these documents and summary records are not included. Adopted summary records of the TC-C&L are available at http://ecb.jrc.ec.europa.eu/classification-labelling/ in the section documents/adopted summary records. The proposals and follow-up documentation are no longer available via the ECB but are still accessable via the H-class database http://apps.kemi.se/hclass/

<u>Conclusion regarding the additional value of the F1 mating in the 2-generation study for n-propane bromide. (AM)</u>

According to the UK proposal 89/1 and the summary records the classification with R60 is based on the effects on reproductive organs in the repeated dose toxicity studies and the effects on mating and other fertility parameters in the 2-generation study. The mating effect and the effect on fertilty parameters were

already observed in the parental animals and in the first mating. The effects in the second mating could be seen as a confirmation in a repeat study. The reduced fertility and reduced number of implantation sites at dose 1 and 2 in the P1 mating was not used as an unique effect. However, UK stated that the presence of effects below 2 mg/ml justified category 2. It is unclear whether this remark related specifically to the reduced fertility and reduced number of implanatation sites only observed after the second mating in the 2 generation study at 1.25 mg/ml (dose 2) or also to other effects on sexual function observed in the repeated dose studies and in the P0 of the 2-generation study. The last interpretation is considered more likely. Therefore, it can be concluded that absence of the second mating in this 2-generation study would have had a minor effect on the argumentation for the classification and would not have affected the classification with R60.

According to the UK proposal 89/1 and the summary records the classification with R63 is based on the developmental effects observed in the developmental study. Therefore, it can be concluded that absence of the second mating in this 2-generation study would not have affected the classification with R63.

Overall, the second mating of the 2-generation study was not determinative for the classification of n-propane bromide for reproductive toxicity.

Benomyl

Benomyl was classified as Repro Cat 2 R60-61 in the October 2000 meeting of the TC-C&L based on the recommendation of the specialised experts.
Summary record of the meeting of the specialised experts September 2000.
Fertility
Carbendazim (P237).
CAS No.: 10605-21-7 EC No.: 234-232-0 Annex I Index No: 613-048-00-8
Lead Country: Germany
At issue: [Repr. Cat. 2; R60] [Repr. Cat. 3; R62].

Benomyl (P213).
CAS No.: 17804-35-2 EC No.: 241-775-7 Annex I Index No: 613-049-00-3
Lead Country: Germany
At issue: [Repr. Cat. 2; R60] [Repr. Cat. 3; R62].

Reaching no agreement on the appropriate categorisation for fertility effects, the **Pesticides Working Group** had decided to ask the advice of the **Specialised Experts**. On account of the extensive metabolism of benomyl to carbendazim and the complementary evidence from fertility studies with both substances, yielding relevant differences only in the NOELs, the **Specialised Experts** found it appropriate to discuss carbendazim and benomyl together.

The **Specialised Experts** did not specifically discuss negative epidemiological evidence from three studies referred to by Industry during the Introductory Session.

The **Specialised Experts** observed that in a large number of animal studies the two types of oral dosing namely diet in older, and gavage in more recent animal investigations of guideline standard, had a decisive influence on the findings. With the exception of one benomyl study (Du Pont, 1991), the administration of one of the two compounds via diet had not resulted in markedly detrimental effects on reproduction organs or fertility. In contrast, if given by gavage, both substances clearly and dose-dependently impaired spermatogenesis, leading to cellular degeneration in animal testes and irreversible infertility at higher doses. The **Specialised Experts**, asked to consider the weight to be attributed to the

gavage studies, unanimously agreed that exclusion of the gavage data from the overall hazard evaluation is not reasonable and would not be permissible according to the classification rules. They concurred that classification with Category 2 is warranted. Several **Experts** commented that both the plausible mechanism, interaction with the microtubules of the spindle apparatus in germ cells, and toxicokinetics indicate the requirement for Category 2. One **Expert** added the reasoning of Kavlock et al. (1982) that gavage is the more appropriate dosing regimen to deduce effects on humans as it resembles the human feeding behaviour better.

Conclusion on Carbendazim:

The **Specialised Experts** discussed carbendazim and benomyl in parallel. They unanimously agreed on classification of carbendazim in Category 2 for effects upon fertility. The **Specialised Experts** observed that after gavage administration of carbendazim, in more recent studies, testicular changes resulted in infertility, while the substance did not show an effect in older dietary studies. They furthermore noted that in a good quality two-generations dietary study with benomyl oligospermia, testicular atrophy and degeneration had been observed in male rats at high doses (Du Pont, 1991). After short-term inhalation of benomyl male rats showed histopathological changes in the testes and epididymides. Mating of treated males with untreated females resulted in reduced pregnancy (Du Pont, 1978).

Conclusion on Benomyl:

The **Specialised Experts** agreed that benomyl should be considered together with carbendazim, the active metabolite of benomyl. There was unanimous agreement to classify benomyl in Category 2 for effects upon fertility, based on infertility observed in recent gavage studies. The **Specialised Experts** furthermore noted that in a good quality two-generations dietary study with benomyl oligospermia, testicular atrophy and degeneration had been observed in male rats at high doses (Du Pont, 1991). After short-term inhalation of benomyl male rats showed histopathological changes in the testes and epididymides. Mating of treated males with untreated females resulted in reduced pregnancy (Du Pont, 1978).

Development
Carbendazim (P237).
CAS No.: 10605-21-7 EC No.: 234-232-0 Annex I Index No: 613-048-00-8
Lead Country: Germany
At issue: [Repr. Cat. 2; R61] [Repr. Cat. 3; R63].

Benomyl (P213).
CAS No.: 17804-35-2 EC No.: 241-775-7 Annex I Index No: 613-049-00-3
Lead Country: Germany
At issue: [Repr. Cat. 2; R61] [Repr. Cat. 3; R63].

Reaching no agreement on the appropriate categorisation for developmental effects, the **Pesticides Working Group** had decided to ask the advice of the **Specialised Experts**. On account of the extensive metabolism of benomyl to carbendazim and the complementary evidence from developmental studies with both substances, yielding relevant differences only in the NOELs, the **Specialised Experts** found it appropriate to discuss carbendazim and benomyl together.

The **Specialised Experts** did not specifically discuss negative epidemiological evidence from three studies referred to by Industry during the Introductory Session.

The **Specialised Experts** observed that in a large number of animal studies the two types of oral dosing namely diet in older, and gavage in more recent animal investigations of guideline standard, had a decisive influence on the findings. As to results from dietary studies with both compounds, malformations

were absent, but in a recent, good-quality two-generations study with benomyl some fetotoxicity had occurred. In contrast, gavage administration had shown both substances to be potent teratogens. The **Specialised Experts**, asked to consider the weight to be attributed to the gavage studies, unanimously agreed that exclusion of the gavage data from the overall hazard evaluation is not reasonable and would not be permissible according to the classification rules. They concurred that classification with Category 2 is warranted for effects on development. One **Expert** commented that besides the plausible mechanism, interaction with the microtubules of the spindle apparatus, other mechanisms could be involved, as very specific head and eye malformations had been observed. One **Expert** added the reasoning of Kavlock et al. (1982) that gavage is the more appropriate dosing regimen to deduce effects on humans as it resembles the human feeding behaviour better.

Conclusion on Carbendazim:

The **Specialised Experts** discussed carbendazim and benomyl in parallel. They unanimously agreed on classification of carbendazim in Category 2 for effects on the development. The **Specialised Experts** observed that the substances did not show an effect in older dietary studies, but were clearly teratogenic after gavage administration in guideline studies, with predominance of head and eye malformations. Moreover, in a recent, good-quality two-generations dietary study with benomyl the fetuses had been affected. This view for classification was not changed by epidemiological studies, to which one of the presentations by Industry referred.

Conclusion on Benomyl:

The **Specialised Experts** agreed that benomyl should be considered together with carbendazim, the active metabolite of benomyl. They unanimously agreed on classification of benomyl in Category 2 for effects on the development. The **Specialised Experts** observed that the substances did not show an effect in older dietary studies, but were clearly teratogenic after gavage administration in guideline studies, with predominance of head and eye malformations. Moreover, in a recent, good-quality two-generations dietary study with benomyl the fetuses had been affected. This view for classification was not changed by epidemiological studies, to which one of the presentations by Industry referred.

Documents including proposals, comments and summary record of the October 2000 meeting are not available as files.

<u>Conclusion regarding the additional value of the F1 mating in the 2-generation study for benomyl (AM)</u>

The classication with R60 for fertility is based on effects on testes seen in repeated dose studies and effects on testes and fertility in mating studies. This classification would not have been affected by the F2 effects in the Mebus study.

The classification for development with R61 was based on the brain and eye malformations observed in several developmental studies using gavage exposure. This classification would not have been affected by the F2 effects in the Mebus study.

Overall, the second mating of the 2-generation study was not determinative for the classification of benomyl for reproductive toxicity.

BBP

Data used: Summary record September 2002
Benzyl butyl phthalate; BBP (W044)
(CAS No: 85-68-7, EC No: 201-622-7)

Classification proposal: Repr. Cat. 2; R61 - Repr. Cat. 3; R62
Currently not in Annex I.

3rd Priority List, Rapporteur MS: N

ECBI/37/99 Add. 19 Rev. 1 N, benzyl butyl phthalate (W044), ESR substance, health classification proposal, Revision of Enclosure 1
ECBI/37/99 Add. 20 CEFIC, benzyl butyl phthalate (W044), comment on classification proposal health effects
ECBI/37/99 Add. 26 CEFIC / ECPI, benzyl butyl phthalate (W044), comment on classification proposals for reproduction toxicity, on-going studies
ECBI/37/99 Add. 27 ECPI: Interim report on the 2-generation study and comment on the classification of BBP
ECBI/37/99 Add. 28 (room doc.) N: Classification proposal for BBP and additional information on reprotoxicity
ECBI/37/99 Add. 29 CEFIC / ECPI: Benzyl butyl phthalate (W044), cat. 2 not justified
ECBI/37/99 Add. 29 App.1 CEFIC / ECPI: Benzyl butyl phthalate (W044), BBP Metabolism Species Differences
ECBI/37/99 Add. 30 CEFIC, Classification with regard to Developmental Toxicity
ECBI/37/99 Add. 31 Anderson et al., A biomarker approach to measuring human dietary exposure to certain phthalate diesters. Food Add.&Contaminants, in press.
ECBI/37/99 Add. 32 N, Additional information to the classification proposal from Norway
ECBI/37/99 ADD. 33 ECPI, REOPENING OF THE DISCUSSION
ECBI/37/99 Add. 34 D, Proposal for the CMR classification of Benzylbutylphthalate

In **November 2000** the **Group** provisionally agreed to classify benzyl butyl phthalate with Repr. Cat. 3; R62 : Repr. Cat. 2; R61, and to finalise the discussion when the result of the new two-generation study would be available. In **May 2001** the provisional agreement from the last meeting to classify benzyl butyl phthalate with Repr. Cat.3; R62 : Repr. Cat.2; R61 was confirmed. In **September 2001 IND** announced that a 2-generation study performed according to OECD guidelines would be available in December. The study might provide further insight into developmental effects. **N** reminded the Group that the study was designed for risk assessment and not for classification. Furthermore, the animals analysed so far showed effects. **UK** and **F** agreed with N that enough data was available for classification. The new study would not provide further information for hazard. **IRL** and **NL** wanted to wait for the study and see the documents. The **Group** reconfirmed the classification for benzyl butyl phthalate with Repr. Cat. 3; R62 : Repr. Cat. 2; R61. At the next meeting the decision was to be taken whether development had to be rediscussed based on the new data. In **January 2002, NL** and **IRL** who wanted to look into the new study prior a final decision both agreed that the picture not had changed and that category 2 was justified. The Group re-confirmed the already taken decision to classify with Repr. Cat. 2; R61. None of the Member States made a reservation to this decision. BBP would then be classified as follows: Repr. Cat. 2; R61 - Repr. Cat. 3; R62.

BBP was concluded already in January 2002. There was new documentation but **IND** had asked to get a second possibility to discuss the 2-generation rat study, which had been summarised in January 2002 but the actual study had then not been circulated to all participants in the Group. The study had now been circulated to the Group, but none of the MS found any reason for additional discussions but agreed to the previous decision to classify with Repr. Cat. 2; R61.

IND explained the contents of the distributed documentation. 20 Studies were undertaken from which the results gave rise to complex information. **IND** had evaluated the data, and created a document to give a better overview. The paper presented, provided the most appropriate studies. The results of metabolism studies were also detailed in the assessment. The results showed that Cat. 3 would be most appropriate according to IND, when taking the EU criteria into account. For Cat. 2 there would be needed a strong proof of toxicity, which was not the case. Cat. 2 would be a step too far according to **IND** because there was only developmental toxicity when maternal toxicity occurred in 9 of 9 cases. The **rapporteur MS (N)** had come to a different conclusion. **IND** requested further discussion on developmental toxicity now that all the data had been made available.

ECB had received one reaction prior to the meeting from D, which wanted to keep the already agreed classification. **N** commented that BBP was discussed for a long time, also at the TM. There was only 1 human study performed with 6 people and a lot of rat studies. There were great polymorphisms in humans, so 6 people would not be enough to draw the conclusions that IND did.

The **Group** did not consider further discussion necessary and BBP would remain with the already agreed classification as Reprotoxic Cat. 2 R61 and Cat. 3 R62.

Conclusion:
The **Group** agreed to classify benzyl butyl phthalate as follows: **Repr. Cat. 2; R61 - Repr. Cat. 3; R62.** The labelling would then be with the **Symbols: T; R-phrases: 61-62 and S-phrases: 45-53.** This classification would be included in the next draft ATP.

Conclusion regarding the additional value of the F1 mating in the 2-generation study for BBP (AM)

The new 2-generation study is the same study (EURAR-1) which showed some specific effects in the F2. However, as clearly shown by the conclusions above this study did not affect the classification because the classification with R61 and R62 was already decided before this study was provided. The other 2-generation study by Aso was published in 2005 and could therefore also not have affected the classification because the substance was already classified with R61.

Overall, the second mating of the 2-generation study was not determinative for the classification of BBP for reproductive toxicity.

CADMIUM CHLORIDE

Data used:**Summary records October 1997**
Cadmium chloride (C023), (048-008-00-3).
Proposal: Carc. Cat. 2; R[45][49] : [Repr. Cat. 2; R60-61] : [Muta. Cat. 3; R40]: [T+; R26] : T; [R25 -]48/23/25 : N; R50-53.

ECBI/43/95 - Add. 38	ECB, List of cadmium entries in Annex I
ECBI/43/95 - Add. 60	UK comments on ECBI/43/95 - Add. 38
ECBI/46/95 - Add. 33	N, classification proposal for cadmium chloride.

Cadmium fluoride is problematic for **S** (Accession Treaty) and **N** and **I** (EEA). It has been agreed that as much of the classification proposal for this substance is based on the classification of cadmium chloride, the two substances will be discussed together. The classification of cadmium chloride was introduced into Annex I as R: 45-23/25-48 in the 7th ATP, and this was updated to the present format as Carc. Cat. 2; R45; T; R48/23/25 in the 12th. ATP. Effects on the environment were discussed in March 1996 and the classification N; R50-53 was agreed.

The substance is currently classified as Carc. Cat. 2; R45 which **N** proposed be retained and the **Group** agreed. There was no need to alter the Category 2 classification from R45 to R49. With respect to the classification for reprotoxic effects, **N** had proposed Repr. Cat. 2; R60-61 for both developmental and fertility effects. For R60, **N** stated there was extensive atrophy in both rat testes and ovaries. In addition, there were increased numbers of resorptions observed. In response, the **UK** noted that the routes of exposure used in some of the animal studies were not ones which the **UK** considered relevant for humans. Only studies which used oral and inhalation routes should be taken into account. Furthermore, some of the studies were contradictory and therefore classification with Category 3 was more relevant. **FR** expressed doubts between Category 2 and 3 and asked to consider the issue at the next meeting following consultation with their experts. **IRL** supported the classification with Repr. Cat. 2; R60.

With respect to developmental effects and R61, **N** noted that such effects were observed at levels between 5 and 20 mg/kg/body weight on brain, kidneys etc. In addition, there were also effects such as lower levels of key enzymes. The **UK** again felt that further discussion was needed on which studies and routes of exposure were relevant. Furthermore, the issue of maternal toxicity had also to be considered and they asked for further information on this point. **S** strongly supported the Category 2; R60-61 as the cadmium ion was the responsible species. It was agreed to return to a discussion on reprotoxicity at the next meeting.

For mutagenic effects, **N** had proposed Muta. Cat. 3; R40 as there were a range of positive *in vivo* and *in vitro* tests. There was also some evidence to suggest that the substance might be an *in vivo* somatic cell mutagen, however, at best it was a weak effect and so **N** did not propose Category 2. The **UK** however, stated that the substance was a borderline Category 2 or 3 mutagen. If mutagenicity in somatic cells has been demonstrated, then Category 2 was warranted because the substance appears to be able to reach the germ cells. The **UK** asked for more data on the mutagenic effects. **IRL**, **DE** and **NL** all supported Category 3.

Classification for acute toxicity with T+; R26 and T; R25-48/23/25 were agreed also. A discussion would be required on lower specific concentration limits pending agreement on the outstanding proposals.
Conclusion:

The **Group** agreed to classify the substance *provisionally* as Carc. Cat. 2; R45; [Repr. Cat. 2; R60-61]; [Muta. Cat. 3; R40]; T+; R26; T; R25-48/23/25 and N; R50-53. Further discussion was needed on reprotoxicity and mutagenicity. **N** was asked to provide additional information on maternal toxicity and somatic cell effects.

Summary records May 1998

Cadmium chloride (C023) (048-008-00-3)
Proposal: Carc. Cat. 2; R45; [Repr. Cat. 2; R60-61]; [Muta. Cat. 3; R40]; T+; R26; T; R25-48/23/25 and N; R50-53.
Concentration limits: 10%; 1%; 0.1% and 0.01%.

ECBI/43/95 - Add. 38	ECB, List of cadmium entries in Annex I
ECBI/43/95 - Add. 60	UK comments on ECBI/43/95 - Add. 38
ECBI/46/95 - Add. 33	N, classification proposal for cadmium chloride (inc. potency).
ECBI/46/95 - Add. 40	N, additional information on cadmium chloride and cadmium fluoride.

Cadmium chloride is problematic for **SE** (Accession Treaty) and **N** and **I** (EEA). The classification of cadmium chloride was introduced into Annex I as R: 45-23/25-48 in the 7th ATP, and this was updated to the present format as Carc. Cat. 2; R45; T; R48/23/25 in the 12th. ATP. Effects on the environment were discussed in March 1996 and the classification N; R50-53 was agreed. In October 1997, it was provisionally agreed to classify the substance as Carc. Cat. 2; R45; [Repr. Cat. 2; R60-61]; [Muta. Cat. 3; R40]; T+; R26; T; R25-48/23/25 and N; R50-53. Further discussion was needed on reprotoxicity and mutagenicity. **N** was asked to provide additional information on maternal toxicity and somatic cell effects.

UK noted that this substance can cause aneuploidy and reach the germ cells, and therefore proposed Muta Cat 2. **IRL** and **SE** supported this view, but the **Group** asked for more time to reach a final decision between Cat 2 and Cat 3. **DE** favoured Muta Cat 3.

The issue of specific concentration limits for carcinogenicity of this substance was not discussed.
Conclusion:
The **Group** agreed to classify cadmium chloride as Carc Cat 2; R45 : Repr. Cat. 2; R60-61: [Muta Cat 2; R46] [Muta. Cat. 3; R40] : T+; R26 : T; R25-48/23/25 and N; R50-53. Symbols T+, N. R-phrases 45-[46]-60-61-25-26-48/23/25-[40]-50/53. S-phrases [45-53-]60-61. The **Group** has still to decide whether specific limits should be set for the carcinogenicity of this substance; a limit of 0.01 % has been proposed by **SE**.

Summary records July 1998
Cadmium chloride (C023) (048-008-00-3)
Proposal: Carc. Cat. 2; R45; [Repr. Cat. 2; R60-61]; [Muta. Cat. 3; R40]; T+; R26; T; R25-48/23/25 and N; R50-53.
Concentration limits: 10%; 1%; 0.1% and 0.01%.

ECBI/43/95 - Add. 38	ECB, List of cadmium entries in Annex I
ECBI/43/95 - Add. 60	UK comments on ECBI/43/95 - Add. 38
ECBI/46/95 - Add. 33	N, classification proposal for cadmium chloride (inc. potency).
ECBI/46/95 - Add. 40	N, additional information on cadmium chloride and cadmium fluoride.

Cadmium chloride is problematic for **SE** (Accession Treaty) and **N** and **I** (EEA). The classification of cadmium chloride was introduced into Annex I as R: 45-23/25-48 in the 7th ATP, and this was updated to the present format as Carc. Cat. 2; R45; T; R48/23/25 in the 12th. ATP. Effects on the environment were discussed in March 1996 and the classification N; R50-53 was agreed. In October 1997, it was provisionally agreed to classify the substance as Carc. Cat. 2; R45; [Repr. Cat. 2; R60-61]; [Muta. Cat. 3; R40]; T+; R26; T; R25-48/23/25 and N; R50-53. Further discussion was needed on reprotoxicity and mutagenicity. **N** was asked to provide additional information on maternal toxicity and somatic cell effects.

UK noted that this substance can cause aneuploidy and reach the germ cells, and therefore proposed Muta Cat 2. **IRL** and **SE** supported this view, but the **Group** asked for more time to reach a final decision between Cat 2 and Cat 3. **DE** favoured Muta Cat 3.

The issue of specific concentration limits for carcinogenicity of this substance was not discussed.

Conclusion:

The **Group** agreed to classify cadmium chloride as Carc Cat 2; R45 : Repr. Cat. 2; R60-61: [Muta Cat 2; R46] [Muta. Cat. 3; R40] : T+; R26 : T; R25-48/23/25 and N; R50-53. Symbols T+, N. R-phrases 45-[46]-60-61-25-26-48/23/25-[40]-50/53. S-phrases [45-53-]60-61. The **Group** has still to decide whether specific limits should be set for the carcinogenicity of this substance; a limit of 0.01 % has been proposed by **SE**.

Proposals

The provided proposals consisted of ECBI 46/95-add33 and add40. They were provided in September and December 1997. The 2-generation study considered for possible effects on the classification due to unique effects in the F2 was Nagymayteni, 1997 was published in 1997 and was not included in the proposals.

Conclusion regarding the additional value of the F1 mating in the 2-generation study for cadmium chloride. (AM)

As the 2-generation study by Nagymayteni et al. from 1997 was not available for the determination of the classification of cadmium chloride for reproductive toxicity in 1997 and the substance was already classified with R60 and R61, it could not have affected the classification. Further, there were several other studies showing effects on fertility and development warranting classification.

Overall, the second mating of the 2-generation study was not determinative for the classification of cadmium chloride for reproductive toxicity.

DEHP

Data used: The classification for effects on reproduction was not discussed in the meeting of October 1999. Summary records January 2000

bis(2-ethylhexyl) phthalate; DEHP (D090), (EC No 204-211-0, CAS No 117-81-7, Index No 607-...).

Proposal: [Carc. Cat. 3; R40] [no classification] : [Repr. Cat. 2; R60-61] [Repr. Cat. 3; R62-63] : [R64].
Concentration limit: [C ≥ 5%: for Carc. Cat. 3; R40].
Rapporteur: S.

ECBI/59/98	D, new classification proposals (D083-D092)
ECBI/37/99 – Add. 2	S, di(2-ethylhexyl phthalate) (DEHP; D090), ESR substance, health classification proposal

ECBI/37/99 – Add. 3 S, di(2-ethylhexyl phthalate) (DEHP; D090), ESR substance, proposal of specific concentration limits
ECBI/37/99 – Add.5 Rev.1 F, revision of tabled comparison of test results on toxicity DEHP (D090), DIDP (F020), DINP(F021)
ECBI/37/99 – Add. 6 F, Phthalates and other substances: article concerning reproduction toxicity (profiles of malformations, male rat) L.E. Gray jr. et al., 1999
ECBI/37/99 – Add. 7 S, The relevance of Peroxisome Proliferators-induced liver tumours in rodents / Summary of current data and five review articles
ECBI/37/99 – Add. 8 S, DEHP (D090), ESR substance, DEHP-induced testicular tumours in rats
ECBI/37/99 – Add. 10 CEFIC, DEHP (D090), position paper on proposed classification
ECBI/37/99 – Add. 13 CEFIC, article in press (Arch.Toxicol): Ruth A Roberts. Peroxisome proliferators and species differences
ECBI/37/99 – Add. 14 CEFIC, article submitted (Arch.Toxicol.): S.C. Hasmall et al. Species differences in response to diethylhexylphthalate
ECBI/37/99 – Add. 15 CEFIC, Summary Paper - RH. McKee
ECBI/37/99 – Add. 16 CEFIC, publ. article (Toxicol Sci.1999): D.J. Caldwell, et al. on alpha 2u globulin accumulation in male rat kidneys following high doses of diisononyl phthalate.
ECBI/37/99 – Add. 17 CEFIC, publ. article (Regul. Toxicol. Pharmacol. 1999): D.J. Caldwell. Review of mononuclear cell leukemia in F-344 rat bioassays and its significance to human cancer risk: A case study using alkyl phthalates.

The substance is on the 2nd ESR Priority List with **S** as rapporteur.
[parts on carcinogenicity removed]

The evidence for effects on reproduction was treated separately for each of the phthalates. **S** introduced the available data on fertlity effects of DEHP (details in the **S** classification proposal ECBI/37/99 – Add. 2). **Industry** disagreed referring to the arguments listed in Add. 10. The **S** proposal to classify with Repr. Cat. 2; R60 was accepted by all **Member States** except **UK**, **IRL** and **D**. **UK** could not agree to Category 2 at the moment, they said, as they had doubts on the human relevance of the rodent data. Effects in rodent species occurred at high doses only, toxicokinetics indicated species differences and the mouse data were conflicting. Their current position was Category 3; R62. **IRL** and **F** acknowledged the Category 2 criteria were met by the evidence in the rat, nevertheless, they had a reservation pending more toxicokinetics data. **D** had not yet formed a final position concerning all phthalates. The German „Advisory Group for Toxicology on CMR effects" was to meet in the near future for this purpose.

Some additional comments were made. **NL** shared the view that Repr. Cat. 2; R60 was justly derived from the animal data, but, they added, good quality information on toxicokinetics might make them re-consider this position. **NL** furthermore felt that DIDP and DINP should not be classified for fertility effects. **DK** emphasised their support for Category 2, because unlike **UK** they did not see the effects to occur at exceedingly high doses. Neither was there any documented indication of a lower sensitivity of humans. The **Group** *provisionally* agreed to classify with Repr. Cat. 2; R60 and wanted to continue the discussion at the next meeting.

Concerning developmental toxicity, **S** gave a detailed account of the substance-specific information, which lead to their proposal to classify with Repr. Cat. 2; R61. Overall, DEHP was clearly an antiandrogenic agent, and most critical for malformations to be detected was exposure during the late gestation, early postnatal period. This applied to male rats and mice at doses $\geq$ ca. 100 mg/kg/d. In a rat study testicular damage in male offspring was observed down to 3-4 mg/kg/d. **NL**, **IRL** and **F** supported **S** based on the animal data. **UK** interpreted the evidence as clear effects in rodents at high doses, but the difference in toxicokinetics to primates lead them to propose classification in Category 3; R63. **IRL** wanted to further examine the available toxicokinetics information. **D** had not yet formed a final position

and referred to the meeting of the German „Advisory Group for Toxicology on CMR effects" in this context. The **Group** *provisionally* agreed to classify with Repr. Cat. 2; R61 and wanted to continue the discussion at the next meeting.

Due to time constraints, the discussion of the S **proposal to classify with R64 for harm to the breast-fed baby had to be postponed to the next meeting.**

Conclusion:

The **Group** *provisionally* agreed to classify DEHP with Repr. Cat. 2; R60-61 and to continue the discussion at the next meeting. Due to time restraints the discussion of harm to the breast-fed baby had to be postponed. The **Group** also agreed to include DEHP in their request for advice to the **Specialised Experts** concerning carcinogenicity of all phthalates under discussion by the **Working Group**. **S** volunteered to take the lead for all phthalates and offered to host an extra meeting of the **Specialised Experts** for this purpose later this year.

Summary records May 2000

bis(2-ethylhexyl) phthalate; DEHP (D090), (EC No 204-211-0, CAS No 117-81-7, Index No 607-...).

Proposal: [Carc. Cat. 3; R40] [no classification] : [Repr. Cat. 2; R60-61] : [R64].
Rapporteur: S.

ECBI/59/98	D, new classification proposals (D083-D092)
ECBI/37/99 – Add. 2	S, di(2-ethylhexyl phthalate) (DEHP; D090), ESR substance, health classification proposal
ECBI/37/99 – Add. 3	S, di(2-ethylhexyl phthalate) (DEHP; D090), ESR substance, proposal of specific concentration limits
ECBI/37/99 – Add. 5	Rev.1 F, revision of tabled comparison of test results on toxicity DEHP (D090), DIDP (F020), DINP(F021)
ECBI/37/99 – Add. 6	F, Phthalates and other substances: article concerning reproduction toxicity (profiles of malformations, male rat) L.E. Gray jr. et al., 1999
ECBI/37/99 – Add. 7	S, The relevance of Peroxisome Proliferators-induced liver tumours in rodents / Summary of current data and five review articles
ECBI/37/99 – Add. 8	S, DEHP (D090), ESR substance, DEHP-induced testicular tumours in rats
ECBI/37/99 – Add. 10	CEFIC, DEHP (D090), position paper on proposed classification
ECBI/37/99 – Add. 13	CEFIC, article in press (Arch.Toxicol): Ruth A Roberts. Peroxisome proliferators and species differences
ECBI/37/99 – Add. 14	CEFIC, article submitted (Arch.Toxicol.): S.C. Hasmall et al. Species differences in response to diethylhexylphthalate
ECBI/37/99 – Add. 15	CEFIC, Summary Paper - RH. McKee
ECBI/37/99 – Add. 16	CEFIC, publ. article (Toxicol Sci.1999): D.J. Caldwell, et al. on alpha 2u globulin accumulation in male rat kidneys following high doses of DINP
ECBI/37/99 – Add. 17	CEFIC, publ. article (Regul. Toxicol. Pharmacol. 1999): D.J. Caldwell. Review of mononuclear cell leukemia in F-344 rat bioassays and its significance to human cancer risk: A case study using alkyl phthalates.
ECBI/37/99 – Add. 22	S, di-"isodecyl" phtalate (DIDP) and di-"isononyl" phtalate (DINP), F020 and F021, reproductive toxicity, and general comments on the possibility of standard reproductive toxicity tests to detect antiandrogens
ECBI/37/99 – Add. 23	S, Comments on the discussion of reproductive toxicity of DEHP (and DBP, BBP, DINP and DIDP)
ECBI/37/99 – Add. 24	CEFIC, DIDP and DINP, response to ECBI/37/99 - Add. 22
ECBI/37/99 – Add. 25	S, DEHP (D090), change in classification proposal to "no classification" for carcinogenicity

[part on carcinogenicity removed]
The substance is on the 2nd ESR Priority List with **S** as rapporteur.

On effects on reproduction the **S** proposal to classify with Repr. Cat. 2; R60 was accepted by all **Member States** except **UK**, **IRL** and **D**. **Industry** disagreed as well. **UK** could not agree to Category 2 at the moment, as they had doubts on the human relevance of the rodent data. Effects in rodent species occurred at high doses only, toxicokinetics indicated species differences and the mouse data were conflicting. Their current position was Category 3; R62. **IRL** and **F** acknowledged the Category 2 criteria were met by the evidence in the rat, nevertheless, they had a reservation pending more toxicokinetics data. **D** had not yet formed a final position concerning all phthalates. In additional comments **NL** shared the view that Repr. Cat. 2; R60 was justly derived from the animal data, but, they added, good quality information on toxicokinetics might make them re-consider this position. **NL** furthermore felt that DIDP and DINP should not be classified for fertility effects. **DK** emphasised their support for Category 2, because unlike **UK** they did not see the effects to occur at exceedingly high doses. Neither was there any documented indication of a lower sensitivity of humans. The **Group** *provisionally* agreed to classify with Repr. Cat. 2; R60 and wanted to continue the discussion at the next meeting. Concerning developmental toxicity substance-specific information had lead **S** to propose Repr. Cat. 2; R61. Overall, DEHP was clearly an antiandrogenic agent and most critical for malformations to be detected was exposure during the late gestation, early postnatal period (observed in male rats and mice at doses $\geq$ ca. 100 mg/kg/d). In a rat study testicular damage in male offspring was observed down to 3-4 mg/kg/d. **NL**, **IRL** and **F** supported **S**. **UK** interpreted the evidence as clear effects in rodents at high doses, but the difference in toxicokinetics to primates lead them to propose classification in Category 3; R63. **IRL** wanted to further examine the available toxicokinetics information. **D** had not yet formed a final position. The **Group** *provisionally* agreed to classify with Repr. Cat. 2; R61 and wanted to continue the discussion at the next meeting.Due to time constraints, the discussion of the **S** proposal to classify with R64 for harm to the breast-fed baby had to be postponed to the next meeting. – In February 2000 the **Environment Group** agreed not to classify bis(2-ethylhexyl)phtalate with R51 for toxicity to aquatic organisms. They further agreed to postpone the discussion on biodegradability and classification with R53 to their next meeting.

S explained they had withdrawn their proposal to classify DEHP for carcinogenicity subsequent to the re-evaluation of the phthalates by IARC, with inclusion of recent mechanistic information. IARC had allocated DEHP to Class 3 (not classifiable…), different from the original **S** proposal. **S** now wanted to advocate the IARC conclusion, despite gaps of knowledge on the mechanism of action and maintenance of their position that the differences between rodents and humans were quantitative, not qualitative. For **S** the liver tumours and the mononuclear cell leukemia (MCL) was not of high concern anymore, but there were still questions in relation to the Leydig cell tumours (LCT). **S** asked **Industry** to provide more details from their re-evaluation of the Berger study. **S** added that an additional consideration was the low carcinogenic potency of DEHP, which had contributed to their original opinion that the case was borderline. The **Group** considered all phthalates on the agenda in parallel.

UK and **D** welcomed the clarifications provided by **S** and said that the phthalates were an important case, in that the tumours produced in animals can be discounted in terms of classification. **N** said some test results were available on BBP not sufficient for classification. **UK** and **N** wanted to submit short justifications in writing to be attached to the minutes of the meeting. **S** referred to summary justifications in ECBI/37/99 – Add. 25. **F** related to the **Group** that they, also, would not take into consideration liver tumours anymore. However, there was still some concern about the high frequency and fast appearance of MCL for DINP. **Industry** referred to the arguments provided in the IARC Monograph 77. The **Group** concluded that none of the phthalates on the agenda should be classified for carcinogenicity.

As to effects on fertility, **F** drew the attention of the **Group** to the similarities of the reproduction test results between DEHP and DBP and the doses where atrophies were observed. According to this comparison, **F** preferred DEHP to be classified in Category 3 like DBP. **IRL** had similar concerns, but

could accept Category 2 based on the marmoset data and toxicokinetics information. According to the latter, **UK** pointed out, differences existed between the phthalates. **Industry** referred to pharmacokinetic differences between rodents and primates. Rodents were not appropriate species to derive a concern for human. Pharmacokinetics studies were ongoing to be finalised by the end of this year, and a fertility study in primates was under discussion. **Industry** suggested the instalment of a small Working Group, with S, NL, N and F, to clarify some of the open issues. **S** said that pharmacokinetics had not been established for the marmoset and the effective relevant metabolite was present in rats and humans. Furthermore, testicular toxicity had been observed in non-rodents, like the ferret and to a minor degree in the hamster. Hence **S** did not see the point in Industry's view that the available information on pharmacokinetics showed no relevance of rodent data for humans. The **S** classification proposal for Repr. Cat. 2; R60 found the support of **B, DK, IRL, N, NL, A, P** and **FIN**. **IRL** added that if a view across all phthalates needed to be taken to arrive at an agreement, they preferred deferment to the **Specialised Experts**, which was supported by **I** and **E**. **D** had not yet arrived at a firm position but could accept the majority vote. So did **EL, F** and **UK**. **E** wanted to wait for the results of the ongoing toxicokinetics studies, but could accept the majority agreement.

The **S** proposal to classify with Category 2 for effects on the development was supported by the majority of **Member States**, including **B, DK, F, IRL, N, NL, A, P**, and **FIN**. **D, EL** and **UK** could follow the majority agreement. **E** preferred deferment to the **Specialised Experts**, which was supported by **I**. **I** added they had a preference for Category 2.

S withdrew their proposal to classify with R64.
Conclusion:

The **Group** agreed to classify DEHP with Repr. Cat. 2; R60-61. Symbol T. R-phrases 60-61. S-phrases 53-45. No Note, no specific concentration limits. This proposal would be sent to **DG ENV** for possible inclusion in a future TPC.

Repro part from 37/99 add25
Toxicity for reproduction *(4.2.9. in the RAR)*

The reproductive health effects of DEHP are presented in more detail in the complete Risk Assessment Report (section 4.1.2.9 and table 4.1.2.9). Testicular effects have been observed in several repeated dose toxicity studies in rats and are described in more detail in the chapter on repeated dose toxicity (section 4.1.2.6.1). Only the studies used for the classification of the reproductive effects of DEHP are presented in this document.

Effects on fertility; Gonadal effects

Exposure to DEHP causes reduced fertility and other adverse reproductive effects in rats and mice of both sexes. In male rats, DEHP induces severe testicular effects, including testicular atrophy (i.a. Gray et al., 1977; NTP, 1982; Agarwal et al., 1986a,b; Gray and Gangolli, 1986; Parmar et al., 1986; 1995; Moore, 1996; Poon et al., 1997). There is a partial reversibility of DEHP-induced testicular atrophy after cessation of exposure to DEHP in adult rats, if the exposure is not too high and long lasting.

Developing male rats have been found to be more sensitive to DEHP-induced testicular toxicity than sexually mature animals (i.a. Gray and Butterworth, 1980; Sjöberg et al., 1985a,b). The onset of the lesions in young animals is also more rapid. The higher sensitivity in developing and sexually immature rats is supported by recently published data (Arcadi et al., 1998) (see below). Irreversible testicular effects were observed in pups exposed pre- and postnatally to DEHP in drinking water at 32.5 or 325 µl/litre (roughly corresponding to 3.0-3.5 mg/kg/day and 30-35 mg/kg/day, respectively). Only minor histological damage of the testes was observed in adult rats (vacuolization of Sertoli cells accompanied by seminiferous tubules filled by cellular deposit in one out of four rats) at 325 µl/litre.

Monoethyl hexylphthalate (MEHP) is believed to be the active metabolite of DEHP affecting the testes and reproductive functions both *in vivo* and *in vitro*. The possible role of other metabolites is, however, not fully elucidated.

Several *in vivo* and *in vitro* experiments have demonstrated that the Sertoli cell is the main target of DEHP-induced testicular toxicity producing subsequent germ cell depletion. Study results have also shown that DEHP and MEHP may exert a direct effect on Leydig cell structure and function (Jones et al., 1993).

In a study performed according to OECD guidelines and in compliance with GLP, groups of 10 young male rats (105-130 g at initiation of dosing) per dose level were given 0, 5, 50, 500, or 5 000 ppm (0, 0.4, 3.7, 37.6, or 375.2 mg/kg b.w.) for 13 weeks (Poon et al., 1997). Dose-dependent testicular effects, shown as a high incidence (7/9) of Sertoli cell vacuolation in the absence of other systemic effects, were seen from 500 ppm in the diet (equivalent to 37.6 mg/kg b.w.) in sexually immature rats. At the highest dose level, a high incidence of atrophy of the seminiferous tubules with complete loss of spermatogenesis was found in addition to a higher incidence of cytoplasmic Sertoli cell vacuolation (9/10). This progressive increase in vacuolisation of Sertoli cells plus injury and loss to germinal epithelium and spermiogenesis in a treatment-related fashion is regarded as strong evidence for a DEHP related effect. The presence of multiple, small vacuoles in the basal Sertoli cell cytoplasm has also been found to be a prominent feature of early response to phthalate exposure in young rats (Creasy et al., 1983) and was early thought to be a nonspecific response of Sertoli cells to a variety of insults (Fawcett, 1975). This view is also supported by other cases where Sertoli cell vacuoles precede the degeneration of germ cells (Courtens and Plöen, 1999). A LOAEL of 37.6 mg/kg b.w. was derived from the Poon et al. study.

DEHP also adversely affects the number of fertile matings in mice. In a continuous breeding study an oral LOAEL of 0.1% in the diet (200 mg/kg b.w./day) was identified for impaired fertility (Lamb et al., 1987). At the highest dose level, 0.3% in the diet (600 mg/kg b.w/day), no pairs were fertile. A diet of 0.3% DEHP caused an increased liver weight (both absolute and relative) and significantly reduced weights of the reproductive organs in parental animals of both sexes (testes, epididymis, prostate, and seminal vesicles in males and ovaries, oviducts, and uterus in females). All but one of the high-dose males showed some degree of bilateral atrophy of the seminiferous tubules. In addition, this dose level also caused decreased sperm motility and sperm concentration and an increased incidence of abnormal sperm forms. DEHP did not significantly decrease body weight gain at 0.3% DEHP.

In a complementary crossover mating trial, females given 0.3% DEHP (600 mg/kg b.w./day) were more seriously affected than males (Lamb et al., 1987). None of the females were able to produce pups. The fertility index was 0% (0/16) for females and 20% (4/20) for males compared to 90% for the control group (18/20).

There are indications that oral dosing of DEHP causes hypo-oestrogenic anovulatory and polycystic ovaries in adult female rats (Davis et al., 1994a,b). There also are indications that DEHP treatment alters the oestrus cycle and causes concentration changes of testosterone and oestradiol as shown in ovary cell cultures with cells obtained from cycling female rats administered DEHP *in vivo*. No NOAEL or LOAEL has, however, been established for these effects.

Both *in vivo* and *in vitro* study results indicate that DEHP can interfere with the endocrine system. Due to the effects on the Leydig cells as measured by a decreased testosterone output, it cannot be excluded that DEHP may exert an antiandrogen effect (Jones et al., 1993). The data are, however, limited and too sparse for an evaluation. The results of recently performed *in vivo* studies in rats exposed to DEHP (Schilling et al., 1999) or dibutylphthalate (DBP) (Mylchreest and Foster, 1998) support the hypothesis that exposure to phthalates may interfere with male specific differentiation factors among which the action of androgens is the most important.

Results from a 90-day oral study using marmosets (4/sex/group, from 13 or 14 months of age) did not show any testicular toxicity at doses higher than those producing effects in adult rats (Kurata et al., 1998). The marmosets were given daily doses of 0, 100, 500, or 2 500 mg/kg DEHP (purity not specified) in corn oil The animals studied had, however, reached sexual maturity. The absence of testicular findings in the marmoset, a small non-human primate, does not necessarily contradict the findings in studies with rats. This difference in response may be a reflection of the difference in sensitivity in developing, pre-pubertal animals in comparison with sexually mature males. Apparently, non human primates absorb a smaller percentage of a high oral dose of DEHP in comparison to rodents, although there seems to be differences among ape strains. However, data are lacking concerning the similarity in the metabolism of DEHP in marmosets and humans. Old World monkeys, such as the Rhesus monkey, are more similar to man with regard to metabolism and physiology than are the smaller New World monkeys, such as the marmoset.

Conclusion: Developing and prepubertal male rats have been found to be much more sensitive to gonadal effects following exposure to DEHP than adults. In some instances, the onset for the occurence of the testicular lesion is also more rapid in young animals.

There are sufficient data from well performed oral rat and mouse studies showing effects on fertility in males and females and also serious effects on the testicles in males. The effects have been noted in the absence of other toxic effects or at around the same dose levels as other toxic effects but the reproductive effects are not considered a secondary consequence of the other toxic effects. These data are considered adequate to support the possibility that these effects can occur in humans. Hence, DEHP should be classified for effects on fertility in **Category 2, R 60**.

Developmental effects

In Wistar rats, DEHP was embryotoxic and teratogenic when given orally in doses close to the maternal toxic dose (BASF, 1995; Hellwig et al., 1997). The study was performed according to OECD Guideline 414 and GLP principles. DEHP (99.8% pure) was administered as an oily solution to 9-10 pregnant female rats/group by stomach tube at doses of 40, 200, or 1 000 mg/kg b.w. on day 6 through 15 of gestation. On day 20 of pregnancy, all females were sacrificed and assessed by gross pathology. At 1 000 mg/kg b.w., slightly reduced maternal food consumption was noted. Reduced uterus weight was assessed as to be associated with the high embryolethality (see below). The corrected body weight gain did not show any differences of biological relevance. Statistically increased relative kidney and liver weights were considered directly related to DEHP. At 1 000 mg/kg b.w. severe developmental effects were observed: statistically significantly increased implantation loss (about 40%), statistically significantly lower number of live foetuses /dam, and statistically significant lower number of live foetuses/dam and decreased foetal body weights, a drastically increased incidence of external, soft tissue, and skeletal malformed foetuses/litter (in total 70.1% of the foetuses/litter) predominantly of the tail, brain, urinary tract, gonads, vertebral column, and sternum. There also were an increased percentage of foetuses/litter with soft tissue and skeletal variations and skeletal retardations. The LOAEL for maternal and developmental toxicity in this study was 1 000 mg/kg b.w. per day.

Arcadi et al. (1998) exposed female Long-Evans rats (12 rats/dose group) daily to drinking water containing DEHP at 32.5 or 325 µl/litre from day 1 of pregnancy to day 21 after the delivery. The water intake was roughly calculated to correspond to 3.0-3.5 and 30-35 mg/kg DEHP/day during pregancy; during suckling this value was increased by at least 30%, due to increased water intake. Body weight gain and gross appearance in the dams were not affected by the treatment. No further maternal toxicity data are available. Perinatal exposure produced no significant changes in body weight gain in the pups. A statistically significant reduction in kidney weight (absolute and relative) at both dose levels was observed, accompanied by histopathological findings (shrinkage of renal glomeruli with signs of glomerulonephritis, dilation of renal tubuli and light fibrosis) between week 0 and 4 of age. The alterations were less

pronounced at week 8. The increased liver weight was not dose related and was considered not to be related to the exposure level. A highly significant and dose-dependent reduced testicular weight (absolute and relative) was observed and did not appear to reduce with growth. Histologically, severe testicular damage was noted including gross disorganization of the seminiferous tubule structure, detachment of the spermatogonial cells from basal membrane and absence of spermatocytes in both exposure groups. At the end of the observation period, 8 weeks after delivery, there were still severe histopathological changes in the testes of the pups.

Three-week-old female pups exposed perinatally to 325 µl/litre of DEHP showed a significantly increased time necessary to perform the beam walking test indicating neurobehavioural effects of DEHP.

These study results indicate a low LOAEL for developmental effects in rats in the absence of overt toxicity in the dams.

In a study comparable to a guideline study and performed according to GLP principles, DEHP caused embryotoxic and teratogenic effects in mice at oral dose levels below those producing observable evidence of toxicity to the dams (Tyl et al., 1988). DEHP was administered at dietary levels of 0, 0.025, 0.05, 0.10, or 0.15% DEHP (0, 44, 91, 190.6, or 292.5 mg/kg b.w. per day; >99% pure) to groups of 1-CR outbred mice (30-31 per group) throughout gestation (days 0-17). Reduced maternal body weight gain was noted in the two highest dose groups, mainly due to reduced gravid uterine weight. There were no treatment-related effects on the number of corpora lutea, implantation sites per dam, the percent pre-implantation loss, and sex ratio of live pups. The number and percent of resorptions, late foetal deaths, and dead and malformed foetuses were all significantly increased from 0.1% DEHP. Foetal weight and the number of live foetuses per litter was significantly reduced from the same dose level. Both the percentage of foetuses with malformations and the percentage of malformed foetuses per litter was significantly increased from 0.05% DEHP. The observed external malformations included unilateral and bilateral open eyes, exophthalmia, exencephaly, and short, constricted, or no tail. Visceral malformations were localised predominantly in the major arteries. Skeletal defects included fused and branched ribs and misalignment and fused thoracic vertebral centra. An oral LOAEL for developmental toxicity was identified as 0.05% in the diet (equivalent to 91 mg/kg b.w./day). The LOAEL for maternal toxicity was 0.10% (190.6 mg/kg b.w./day).

In a continuous breeding study in mice, comparable to a guideline study and performed according to GLP principles, an oral LOAEL for maternal and developmental toxicity of 200 mg/kg b.w./day was identified (NTIS, 1984; Lamb et al., 1987) (see above). DEHP (> 99% pure) was given to CD-1 mice (20 animals of each sex per dose group and 40 control animals of each sex) at dietary levels of 0, 0.01, 0.1, or 0.3% (equivalent to 0, 20, 200, or 600 mg/kg b.w. per day, respectively). Both male and female mice were exposed during a 7-day premating period and were then randomly grouped as mating pairs. The dosing continued during the 98 day cohabitation period and thereafter for 21 days during which final litters were delivered and kept for at least 21 days. Exposure to DEHP produced a dose-dependent and significant decrease in the number of litters as well as the number and proportion of pups born alive from 0.1%. No pairs were fertile at 0.3%. A diet of 0.3% DEHP caused an increased liver weight (both absolute and relative) as well as effects on the reproductive organs in parental animals of both sexes (see above).

In a dietary 2-generation study (comparable to a guideline study and performed according to GLP principles) in CD-1 mice, DEHP was given in the diet at 0.0, 0.01, 0.025, and 0.05% (equivalent to 0, 19, 48, and 95 mg/kg b.w., respectively) to CD-1 mice (NTIS, 1988). DEHP treatment did not affect the number of implantation sites per dam, the percent fertile matings, the pregnancies with live litters on pregnancy day 1, or the percent viable litters through gestation to postnatal day 4.

The F1 generation was mated within dose groups at sexual maturity and F2-offsprings were evaluated for viability and growth at postnatal day 4. For F1-litters, the percentage of prenatal mortality was increased at the high dose (9% versus 26,4%). During the neonatal period, the percent of viable pups was significantly decreased at 0.05% DEHP. No other effects of DEHP were observed upon growth, viability, age of acquisition for developmental landmarks (incisor eruption, wire grasping, eye opening, testes decent or vaginal opening, or spontaneous locomotor activity) on postnatal days 14, 21 or 50. per day. Treatment-related lesions were not observed in the dams and no maternal LOAEL was established. The NOAEL for parental toxicity and for F2-offspring was 0.05% DEHP (95 mg/kg b.w. per day), the highest dose tested. The LOAEL for F1 offspring was 0.05% (95 mg/kg b.w. per day) (NTIS, 1988).

Only one developmental study is available concerning the effects of exposure to DEHP by inhalation (6 hours) (Merkle et al., 1988). The effects reported were not regarded as exposure related, since no dose dependency was observed. The number of corpora lutea, uterine weights, body weights, living and dead implants, early and late resorptions, dead foetuses, pre- and postimplantation losses were unchanged compared to controls. No LOAEL could therefore be established. The NOAEL for maternal and developmental toxicity in rats was 300 mg/m^3 (the highest dose tested).

Conclusion: Studies in rats have shown serious developmental effects including malformations below or at around the same dose levels causing slight maternal toxicity. Well performed oral studies in mice have shown developmental effects at dose levels not causing maternal toxicity. Hence, DEHP should be classified for developmental toxicity in **Category 2, R 61**.

Post-natal effects

It has been documented that DEHP and its metabolites are secreted into the milk of rats orally exposed to DEHP during the lactation period and transferred to suckling pups. Dostal et al. (1987b) found a milk/plasma ratio of >200 for DEHP, which indicates an efficient extraction of DEHP from the blood plasma into the milk. Higher amounts of DEHP (216 ± 23 µg/ml) than MEHP (25 ± 6 µg/ml) were transferred through the milk following three high daily doses of DEHP (2 000 mg/kg). The plasma contained <0.5 µg/ml DEHP and 75 ± 12 µg/ml MEHP. DEHP has been found in human mother´s milk at concentrations of 71-160 µg/kg (Gruber et al., 1998).

Irreversible testicular damage have been observed in male pups exposed prenatally and during suckling at dose levels not affecting the dams (Arcadi et al., 1998) (see above). This study design does not allow to assess if these effects are the results of prenatal or of postnatal exposure or a result of the whole exposure period. However, phthalate esters are thought to exert their primary effect in the Sertoli cell of the testis. The sensitive period of Sertoli cell division spans late gestation until postnatal days 14-16 in the rat (Gondos and Berndtson, 1993) and exposure during this period may therefore result in deleterious effects on the testis. This supports that the lactational exposure has contributed to the toxicity observed in the study by Arcadi et al.

A daily high dose of DEHP (2 000 mg/kg b.w.) to female albino rats throughout the lactation period (21 days) caused a significant decrease in the activity of acid phosphatase and sorbitol dehydrogenase in the pups (Tandon et al. ,1990). No maternal toxicity was observed. The decrease of the activity of acid phosphatases may suggest an injury to germ cells, as the acid phosphatases are present in the lysosomes of the germ cells. A decrease in sorbitol dehydrogenase indicates the deterioration of germinal epithelium. Biochemical alterations caused by the exposure to DEHP during early life may thus affect the functional development of the testis. However, data are lacking for these effects at lower dose levels.

Conclusion: DEHP has caused toxic effects when transferred to suckling pups. As DEHP has been found in human mothers milk, classification and labelling with **R 64** (may cause harm to breastfed babies) is proposed.

Conclusion regarding the additional value of the F1 mating in the 2-generation study for DEHP (AM).

The unique effects in the F2 of the 2-generation study by Schilling et al. (2001) could not have been determinative for the classification of DEHP for fertility or development because this study was not available to the TC-C&L during their assesment in 2000 and the substance was classified with R60 and R61. The absence of this study is confirmed by the overview of reprotoxicity studies in 37/99-add25 which only contained the range-finding (Schiling et al., 1999).

Overall, the second mating of the 2-generation study was not determinative for the classification of DEHP for reproductive toxicity.

FENPROPIMORPH

Data used:
Summary records April 2001
Fenpropimorph (P554), (613-124-00-0)
Proposal: [Repr. Cat. 3; R63] : Xn;R20 : Xi;R38 : N; R51-53

ECBI/71/95 Add. 98 I: fenpropimorph – classification as category 3 (R63) for developmental toxicity
Current classification in Annex I (24 ATP): Xn; R20 : Xi; R38 : N; R51-53.
Reprotoxicity
I informed the group that fenpropimorph was reclassified with Repr.Cat.3; R63 on the national level due to new data. **DK** mentioned that an expert group was evaluating fenpropimorph at the moment. A document would be ready for the next meeting. **D** as well was re-evaluating the substance and classified with Repr.Cat.3; R63 on the national level. **NL**, based on the same rabbit study warranting R63 for I, came to the conclusion that classification for reprotoxicity was not justified. The effects seen during organogenesis were only seen in presence of maternal toxicity. **B** mentioned that the substance was discussed in 1995. In their opinion the current data would not lead to re-classification. **S** preferred to wait with the discussion until the evaluation from DK was available.
Other endpoints

NL asked to consider Xn; R22 due to the oral LD50 and C; R35, due to a publication on dermal damage. Xn; R20 could be deleted.

Conclusion

The **Group** decided to discuss reprotoxicity of fenpropimorph again at the next meeting, taking into consideration the DK evaluation. **MS** are asked to check the NL proposal to add Xn; R22 and C; R35 to the current classification and to dismiss Xn; R20.

Summary records Februari 2002
Fenpropimorph (P554)
(CAS No: 67564-91-4, EC No: 266-719-9, Index No in Annex I: 613-124-00-0)
Proposal: [Xn;R22 - C;R35 - Repr. Cat. 3; R63] - N; R51-53
Current classification in Annex I (24th ATP): Xn;R20 - Xi; R38 - N; R51-53

ECBI/71/95 Add. 98 I: fenpropimorph – classification as category 3 (R63) for developmental toxicity

ECBI/71/95 Add. 99 DK proposal to classify fenpropimorph with Repr. Cat. 2; R61
ECBI/71/95 Add. 100 BASF comment on skin irritation
ECBI/71/95 Add. 101 BASF regarding reproduction toxiciy (MS only)

In April 2001 the **Group** decided to continue the discussion on developmental effects of fenpropimorph at the next meeting, taking into consideration the DK evaluation. **I** and **D** supported classification with Repr. Cat. 3; R63 based on a new rabbit study, while **NL** and **B** did not see that this new data would lead to re-classification. For other endpoints **NL** asked MS in addition to consider Xn; R22 due to the oral LD50 and C; R35, due to a publication on dermal damage. Xn; R20 could then be deleted. **MS** were asked to check the NL proposal concerning the acute toxicity and the corrosivity.

[other endpoints deleted]

Reproductive toxicity

The **DK** evaluation for reproductive toxicity of the substance had now been made available to the Group. They suggested classifying the substance in category 2 for development. **EL** agreed with the DK proposal, as they also assumed that this molecule would have the same kind of action as triazole. **NL** said that this was a difficult case. They agreed that at least category 3 would be necessary, but they had only looked at the summary of the rabbit study and could therefore not say whether category 2 would really be appropriate. Before agreeing to a decision in category 2, they would like to see the full rabbit study. **B** was in favour of category 3. The effects of most concern were seen in rabbit but those effects were only present together with marked maternal toxicity. **D** was also in favour of category 3 on the basis of the same argument as put forward by B. **UK** remarked that there also had been an increased death of litters in rats, also in absence of maternal toxicity. They therefore thought that category 2 was appropriate. They could however agree that it would be useful to see the full study before reaching conclusion. **S** said that they supported category 2 also on the basis of developmental effects observed at dose levels where no maternal effects were expressed. **UK** further pointed out that there was an issue of cleft palate in presence of maternal toxicity. They requested whether there was a correlation and whether cleft palate should be dismissed when there was proven maternal toxicity. They felt that this would be an issue to discuss by Specialised Experts to give an advice to the Group how to treat these cases. **UK** also asked IND to provide them with the 2-generation rat study.

It was then concluded that the full rabbit study was needed before making a final decision. **IND** promised to send the study. The discussion would then continue at the next meeting.

Conclusion
The Group agreed to classify the substance with **Xn; R22 - N; R51-53**. Irritancy/corrosivity would be discussed at the next meeting when new test results from an *in vitro* test will be made available by Industry. Reproductive toxicity (development) would be further discussed after that the MS have looked into the full rabbit and the 2-generation rat study.

Summary records Januari 2003
Fenpropimorph (P554)
(CAS No: 67564-91-4, EC No: 266-719-9, Index No in Annex I: 613-124-00-0)

Proposal: [Xn;R22 - C;R35 - Repr. Cat. 3; R63] - N; R51-53
Current classification in Annex I (24th ATP): Xn;R20 - Xi; R38 - N; R51-53

ECBI/71/95 Add. 98	I: fenpropimorph – classification as category 3 (R63) for developmental toxicity
ECBI/71/95 Add. 99	DK proposal to classify fenpropimorph with Repr. Cat. 2; R61
ECBI/71/95 Add. 100	BASF comment on skin irritation
ECBI/71/95 Add. 101	BASF regarding reproduction toxiciy (MS only)
ECBI/71/95 Add. 102	BASF: Skin corrosivity test
ECBI/71/95 Add. 103	BASF: Assessment of skin irritation potential

In **April 2001** the **Group** decided to continue the discussion on developmental effects of fenpropimorph at the next meeting, taking into consideration the DK evaluation. **I** and **D** supported classification with Repr. Cat. 3; R63 based on a new rabbit study, while **NL** and **B** did not see that this new data would lead to re-classification. For other endpoints **NL** asked MS in addition to consider Xn; R22 due to the oral LD50 and C; R35, due to a publication on dermal damage. Xn; R20 could then be deleted. **MS** were asked to check the NL proposal concerning the acute toxicity and the corrosivity.

In **February 2002** the **Group** agreed to classify the substance with **Xn; R22 - N; R51-53**. Irritancy/corrosivity would be discussed at the next meeting when new test results from an *in vitro* test will be made available by Industry. Reproductive toxicity (development) would be further discussed after that the MS have looked into the full rabbit and the 2-generation rat study.

The documents were presented by **ECB**. Skin irritation and corrosivity were open issues.

There was one room document from **EL**, supporting the **DK** proposal.

Reprotoxicity, R63

B supported Repr. Cat. 3 because of the rabbit studies. The effects are mainly observed in litters from the mothers the more severely affected. **ES** supported Cat. 3 as well, while **S** found enough support for Cat. 2 because of the rat studies. There was however a combination with decreased food uptake.

ECB suggested a round table discussion.

FIN had concern about two studies warranting Repr. Cat. 2. and thought there might be relevance to humans. Therefore **FIN** supported Cat. 2. In presence of little maternal toxicity there was reprotoxicity, and therefore **UK** suggested that specialised experts should have a look at this substance. **ECB** was not sure if it would be worthwhile to wait for an **SE** meeting because the **Group** wanted to finalise the substance.

During the round table discussion there was a majority of the **Group** in favour of classification with Repr. Cat. 3; R63. The **Group** wanted to finalise Fenpropimorh as Repr. Cat. 3, R63 now and address the issue to **SE's** later. When the maternal toxicity expert group would have come ahead the **Group** would come back to the substance when there would be a need for it.

[irritancy deleted]

The substance was concluded with Repr. Cat. 3, R63, Xi; R38, Xn; R22, N; R51-53.

Conclusion:

The substance would go to the 29th ATP with the classification: **Repr. Cat. 3, R63, Xi; R38, Xn; R22, N; R51-53. Symbols: Xn, N; R-phrases: R22-38-63-51/53; S-phrases: S(2)-36/37-46-61.** Fenpropimorph would be used as an example for the maternal toxicity working group. If the experts would advise to classify with Repr. Cat. 2; R61, **ECB** would change the classification for reprotoxicity without any formal request by any **MS**.

71/95-ADD99 (PARTLY)
SUMMARY AND CONCLUSIONS

The developmental toxicity of fenpropimorph has been examined in a number of studies. In rats, four studies have been performed: a peri-postnatal study, a pre-postnatal screening study, a two-generation study, and a prenatal study. In rabbits, two studies have been performed. Both were prenatal studies. In addition, information about a possible mechanism for developmental toxicity and information about a related substance is available.

Rat studies

The rat studies show some evidence of developmental toxicity.

There was some evidence of developmental toxicity in the peri-postnatal study in rats at 40 mg/kg. This effect was independent of maternal toxicity and included significantly decreased pup survival index as well as transiently decreased body weight in male pups. At 10 mg/kg there were no effects.

In the pre-/postnatal screening study in rats, signs of developmental toxicity (decreased body weight) and maternal toxicity (decreased body weight and body weight gain, increased serum cholinesterase activity) were found at all dose levels. Since there was no dose level without signs of maternal toxicity, the results of the study do not allow a comparison of the sensitivity of adults with the sensitivity during development. Effects were found in adults as well as offspring at 5 mg/kg.

The 2-generation study in rats showed some signs of developmental toxicity including increased number of stillbirths, decreased body weight gain in pups, and slight delays in physiological development of pups. The effects seen in the pups, most pronounced in the F_1 pups, together with virtually no observed effect in the adult animals (serum cholinesterase not investigated) indicate some developmental effect of fenpropimorph. Developmental effects were found at 1.99 mg/kg. No effects were found at 1.01 mg/kg bw per day.

The prenatal toxicity study in rats showed clear foetal malformations at the highest dose level, 160 mg/kg which was also very toxic to the dams. At 40 mg, with slight maternal toxicity, a possible foetal toxicity cannot be excluded. At 10 mg/kg no foetal effects were found.

Rabbit studies

The two prenatal studies in rabbits give clear evidence of developmental toxicity.

In the most recent study from 1993, increased incidence of limb position anomalies and shortened bones of the limbs was present at 30 mg/kg. In addition, other malformations such as cleft palate, exencephaly, gastro- and cranioschisis, and diaphragmatic hernia were observed. There was some degree of maternal toxicity, but there is no background for assuming that the specific malformations found were caused by maternal toxicity. At 15 mg/kg no foetal toxicity was found.

In the older study from 1979 developmental toxicity, i.e. increased number of resorptions and limb position anomalies (pseudoankylosis) was found at 36 mg/kg. There were also some weak signs of maternal toxicity at this dose level, but there is no background for assuming that the limb position anomalies could be due to maternal toxicity. A possible developmental effect in the absence of signs of maternal toxicity at 12 mg/kg cannot be excluded since malformations/anomalies of the limbs (including one with pseudoankylosis) were found in two foetuses in this group. At 2.4 mg/kg no foetal toxicity was found.

Additional information

Concerning a possible mechanism for developmental toxicity, literature suggests that fenpropimorph interferes with the biosynthesis of cholesterol in mammalian cells as well as sterol synthesis in fungal cells. Limited in vivo data suggest that high doses of fenpropimorph reduce blood cholesterol levels in rats. The limited data that indicate a possible interference by fenpropimorph with biosynthesis of sterol in mammalian cells show that the inhibition occurs at an earlier synthetic step than in fungi, and that the inhibition in mammalian cells is much weaker than in fungi. It is known that inhibition of biosynthesis of cholesterol causes developmental defects/congenital malformations in rodents and man. In humans, inhibition of cholesterol synthesis is known to cause various syndromes in the foetus and developing child. These syndromes include Smith-Lemli-Opitz syndrome, CDPX2 and CHILD syndrome. Among other defects, affected children show malformations of the face. A direct link between fenpropimorph, inhibited cholesterol synthesis and developmental toxicity has not been shown. However, the mechanism would be relevant for man.

The structurally related fungicide tridemorph is a known developmental toxicant in mice and rats and is classified as toxic to reproduction in category 2. The malformations caused by tridemorph include cleft palate, cleft vertebral centra, dilated renal pelvis with hydroureter and syndactyly and oligodactyly.

Conclusions

Fenpropimorph causes developmental toxicity in rats and especially in rabbits. The data in rabbits provide clear evidence of developmental toxicity as limb anomalies and malformations are found in two studies. It is proposed to classify fenpropimorph as a reproductive toxicant in category 2 (Substances which should be regarded as if they cause developmental toxicity to humans). The mechanism behind the malformations in rabbits is not known.

Fenpropimorph interferes with the biosynthesis of cholesterol in mammalian cells as well as sterol synthesis in fungal cells. In humans, inhibition of cholesterol synthesis is known to cause various syndromes in the foetus and developing child. A direct link between fenpropimorph, inhibited cholesterol synthesis and developmental toxicity has not been shown. However, the mechanism would be relevant for man.

The overall NOAEL is established to 1.01 mg/kg/day based on the signs of developmental toxicity (decreased body weight gain in pups and increased number of stillbirths in F1) found in the 2-generation study in rats.

Conclusion regarding the additional value of the F1 mating in the 2-generation study for n-propane bromide. (AM)

From the provided summary records and proposals it is clear that the classification of fenpropimorfph with Repro Cat 3; R63 was based on the effects seen in the developmental studies in rat and rabbit and some of the effects in the F1 of the 2-generation study but not on the unique effects in the F2 of the 2-generation study.

Overall, the second mating of the 2-generation study was not determinative for the classification of fenpropimorph for reproductive toxicity.

TEPRALOXYDIM

The summary records and proposals cannot be provided as these were confidential.

Conclusion regarding the additional value of the F1 mating in the 2-generation study for tepraloxydim. (AM)

The unique effects in the F2 of the 2-generation study may have had some effect on the classification with R63 but the classification is mainly based on the effects in the rat developmental study.

Overall, the second mating of the 2-generation study was not determinative for the classification of tepraloxydim for reproductive toxicity.

APPENDIX 2: ANALYSIS PROCEDURE FOR THE RETROSPECTIVE DATA ANALYSIS

1. Risk assessment report summaries of 498 multigeneration studies representing 438 substances were incorporated in the existing ToxRefDB format of the USEPA multigeneration study database.
2. The database aimed at comprehensiveness at the level of the substances. Thus, the aim was that each substance for which at least one multigeneration study was performed is represented in the database. Moreover, for each substance entered, at least the most critical multigeneration study as considered in the risk assessment report, was entered in the database. Additional studies were incorporated wherever feasible, which explains the difference between 438 substances and 498 studies in the database.
3. The database collection was carried out by RIVM-Netherlands (NL) in close collaboration with USEPA, Canada PMRA and Germany BfR who all provided input for the database. Several additional study reports were included as provided by Notox-NL.
4. On the basis of discussions in the Expert Group, studies considered important for the retrospective analysis were identified from the completed database using two parallel approaches, both starting from the entire database.
5. First, in the Part I analysis, studies were identified that showed a lower LEL in the P1/F2 generations as compared to the corresponding P0/F1 generations. LELs were compared between generations as well as within generations considering premating and postmating parameters separately (see figure 1 in the Part I report for detailed explanation).
6. Second, the Part II analysis focused on substances carrying a classification for reproductive toxicity under EU regulation. For these substances, studies were identified which showed (a) different type(s) of reproductive effect in both generations, independent of the dose level at which they occurred in each of the generations.
7. Studies identified in Parts I and II were analysed in more detail both as to their interpretation in the risk assessment reports (Part I) or in the EU Technical Committee on Classification & Labeling (Part II), as well as regarding the study reports when available.
8. The analysis procedure was discussed extensively with experts from member countries and stakeholders involved both before and during the performance of the analysis. To this end, both regular teleconferences as well as two face-to-face meetings in Amsterdam took place during the course of the retrospective analysis.
9. The development of the database could be followed by Expert Group members as it was posted on the OECD clear-space server since May 2010, and updated regularly.
10. The entire procedure aimed at transparency with regular input from all involved aiming at a consensus analysis outcome.
11. RIVM-NL produced the Part I and Part II reports of the analyses performed as explained above. Workload and deadlines did not allow a round of comment and revision of these reports. Therefore, they should be considered solely as the responsibility of the RIVM authors indicated on the cover of the documents.

APPENDIX 3: BOUNDARIES OF THE RETROSPECTIVE DATA ANALYSIS

The retrospective analysis has been designed to assess the impact of the P1/F2 generation of the 2-generation reproduction toxicity study on hazard characterization for Risk Assessment and Classification and Labelling. This analysis was done to determine whether or not the assessment of a second generation offspring would be warranted in the EOGRTS protocol. This evaluation should also incorporate consideration of differences in study design between the 2-generation reproductive toxicity study and the EOGRTS. For transparency, this annex provides information on the boundaries of the retrospective analysis.

Rules for the selection of the studies in the Database:

- Most studies were selected on the basis of the existence of a publicly available Risk Assessment (RA) report. A few studies, for which a public RA report was not available, were also included in the Database (DB);
- When several studies evaluating the same chemical were identified in the RA report, those judged critical to the RA report were always included;
- Studies that were not or were less in agreement with TG 416 were not included in the DB in case more robust/reliable studies were available for the same chemical;
- The vast majority of studies are conducted in rats; therefore this analysis is based on studies performed on rats only. Studies performed on other species than rats were not included in the DB.

Rules used for the analysis:

- Effects reported are those that were deemed relevant for inclusion in the existing RA reports. Magnitude, incidence, severity and other types of effects were not taken into consideration when selecting the studies from the database but if available in the RA reports, this information was used in the interpretation phase for the selected studies;
- The original study reports were further scrutinized when possible for studies that were picked up on the basis of their LELs;
- LELs and LOAELs used are those set in the existing RA report selected to be used for that substance;
- LELs are based on any effects, including reproductive and non reproductive effects;
- Part I of the retrospective analysis considered all end points assessed, irrespective of whether they should be considered as reproductive end points or general toxicity end points: concerns

have been expressed as to whether the consideration of reproductive end points without general toxicity parameters would lead to different conclusions as to the specific sensitivity of P1/F2 vs P0/F1. However, this approach is subject to prior discussion about how to separate reproductive from general toxicity parameters; e.g. should body weight effects in an adult F1 in a 2-generation study be considered general or reprotoxicity in view of the exposure protocol? The same question can be asked for liver weight or any other parameter considered in general toxicity studies. Therefore, there is no clear separation between reproductive and general toxicity parameters;

- Part II of the analysis was limited to those substances in the DB that carried an EU classification for reproductive toxicity;

- Due to time restrictions the analysis to identify different studies showing the same LEL in P1/F2 vs P0/F1 but might have a different magnitude, incidence or severity of an effect was not done.

Differences in the scope of the US study and the NL study:

- In the US analysis (Reaves et al., Appendix 4), F2 offspring effects were compared with F1 offspring effects, and P1 reproductive effects with P0 reproductive effects. This analysis was done with the aim to assess relative reproductive parameter sensitivity between generations and with that to evaluate whether or not triggers in the first generation could be identified that might be used to decide on the breeding of a second generation.

- At the October 2009 Paris expert meeting, it was decided that in addition, a retrospective analysis incorporating all findings in the study was necessary to decide upon the overall impact of the P1/F2 generation on study interpretation for hazard assessment both for purposes of risk assessment and for classification and labeling.

- The NL analysis therefore compared P1/F2 versus P0/F1 for all effects reported, including reproductive as well as non-reproductive endpoints as defined in the ToxRef DB format. In addition, as EU classification may rely partly on the type of effect observed, all substances in the database carrying an EU classification for reproductive toxicity were analysed for generation-specific findings occurring irrespective of the dose level at which they occurred.

- The interpretation of a multigeneration study as to the reproductive toxicity of the substance tested takes into account whether an endpoint(s) affected should be considered as a specific reproductive endpoint(s) or a general toxicity endpoint(s). The distinction between the two is subject to expert judgment on a case-by-case basis. Combining the Reaves and the NL analysis outcomes allows for considering this issue for studies present in both analyses, as Reaves et al analyzed reproductive and offspring parameters only, whereas the NL analysis considered all parameters affected in the study in a weight-of-evidence approach.

APPENDIX 4: 2009 RETROSPECTIVE ANALYSIS OF TWO-GENERATION REPROTOXICITY DATA

A Retrospective Analysis of 350 Multi-Generation Reproductive Toxicity Rat Studies to Support the Proposed Extended One-Generation Reproductive Toxicity Test Guideline

ME Reaves[1], J Kidwell[1], E Mendez[1], V Dellarco[1], D Dix[2], M Martin[2], R Cooper[3], T Stoker[3]

[1]US EPA Office of Pesticide Programs (OPP); US EPA Office of Research and Development (ORD), [2]National Center for Computational Toxicology, [3]National Health and Environmental Effects Research Laboratory

1. Executive Summary 4
2. Introduction 5
3. Design/Analysis 5
Data Source: 5
Tools: 5
Data Input: 5
Data Quality Control: 6
Data Extraction: 6
4. Criteria of Effects for Retrospective Analysis 7
5. Retrospective Analysis Results 8
A. 9 Literature Studies 8
Figure 1. A flow diagram of the F_1 LOAEL vs F_2 LOAEL comparison of the 9 literature studies 9
Table 1. Study details for the Parental Generation (P), the F_1 generation (F_{1A}), and the F_2 generation (F_{2A}) for the 9 Industrial compounds 9
B. OPP/PMRA Pesticide Studies 14
Figure 2. Results of the 341 Pesticide Studies in the Retrospective Analysis 16
1. F_2 LOEL/LOAEL Only 16
Table 2. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring or reproductive LOAELs based solely on the F_2 generation (F_2 only) 18
2. $F_1 \geq F_2$ LOEL/LOAEL Comparison 26
Table 3. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL > F_2 LOAELs 27
3. $F_1 = F_2$ LOEL/LOAEL but with unique F_2 effect or C&L concerns 37
Table 4. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL = F_2 LOAELs but with a unique F_2 effect 38
6. Occurrence of 1st and 2nd Generation Sensitive or Unique Effects 52
Figure 3. 1st vs. 2nd Generation Sensitive or Specific Endpoints 52
7. C&L Concerns but No Quantitative Concerns 53
Table 5. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive LOAEL/LOELs less sensitive than F_2 but with C&L concerns 53
Table 6: List of potential reproductive endpoints considered for triggering an F_2 generation* 56
Table 7: List of potential offspring endpoints considered for triggering an F_2 generation* 57
8. Results of Trigger Analysis 58
Table 8. The incident of specific triggers and false positive rate from the 341 pesticide studies in the retrospective analysis 58
9. Conclusions from this Retrospective Analysis 59
Acknowledgements 60
References 60

List of Abbreviations

ACSA	Agricultural Chemical Safety Assessment
ADME	Adsorption, Distribution, Metabolism, and Excretion
C&L	Classification & Labeling
DER	Data Evaluation Record
DHT	Dihydrotestosterone
FSH	Follicle Stimulating Hormone
HDT	Highest Dose Tested
ILSI/HESI	International Life Science Institute/Health and Environment Sciences Institute
PPS	Preputial Separation
VO	Vaginal Opening
RP	Reproductive Performance
GD	Gestational Day
GI	Gestational Interval
AGD	Anogenital Distance
LOAEL	Lowest Observable Adverse Effect Level
LOEL	Lowest Observable Effect Level
LD	Lactational Day
LDT	Lowest Dose Tested
MDT	Mid Dose Tested
NAS	National Academy of Sciences
NCCT	National Center for Computational Toxicology
NHEERL	National Health and Environmental Effects Research Laboratory
NOAEL	No Observable Adverse Effect Level
NOS	Not Otherwise Specified
ns	not statistically significant
OECD	Organization for Economic Co-operation and Development
OPP	Office of Pesticides Programs
OPPTS	Office of Prevention, Pesticides, and Toxic Substances
ORD	Office of Research and Development
PND	Postnatal Day
REACH	Registration, Evaluation, Authorisation and Restriction of Chemical substances
ToxRefDB	Toxicity Reference Database
F_1	First generation of reproduction study
F_2	Second generation of reproduction study
P	Parental generation of reproduction study
*	$p \leq 0.05$
**	$p \leq 0.01$
***	$p \leq 0.001$

1. Executive Summary

The US EPA's Office of Pesticide Programs (OPP) and the Office of Research and Development (ORD) conducted a retrospective analysis of the multi-generation reproduction toxicity database to ascertain the contribution of the observed effects only in the second generation (F_2) to hazard identification and characterization as well as classification and labeling under the REACH program of the European Union (EU). The analysis was originally conducted in 2008, however, this revised analysis contains additional studies from the USEPA as well as Health Canada's Pest Management Regulatory Agency (PMRA). This analysis continues to support and to confirm that if an F_2 generation is not produced the Extended One-Generation Reproductive Toxicity Test Guideline as proposed by the International Life Science Institute/Health and Environment Sciences Institute (ISLI HESI) Agricultural Chemical Safety Assessment (ASCA) in Cooper et al. (2006) would not fail to identify critical sensitive endpoints or lower Lowest Observable Adverse Effects Levels (LOAELs). EPA's relational toxicity database ToxRefDB was used for this analysis. This retrospective analysis evaluated the toxicity profile of 350 studies (341 pesticides [21 PMRA, 320 OPP], and 9 industrial compounds [OECD guideline]) including those with known reproductive toxic effects. Reproductive and offspring type effects were evaluated for not only quantitative sensitivity (i.e., effects at lower doses) of the F_2 generation but also qualitative sensitivity (i.e., effects not seen in the F_1 generation or "unique" effects) of the endpoint identified in the F_2 generation and its importance for risk assessment. Based on comments from the Organization for Economic Co-operation and Development (OECD) Expert Panel meeting in October 2008, this retrospective analysis also evaluated whether any endpoints essential to classification and labeling would be missed by not mating the F_1 animals to produce the second generation. Furthermore, the proposed reproductive and offspring triggers (specified endpoints to mate F_1 animals to produce the F_2 generation) outlined in the Extended One-Generation Reproductive Toxicity Test Guideline were used in this analysis to determine how many animals would be saved from the 341 pesticide studies.

Results of the revised retrospective suggest that only 2 out of 350 (0.6%) studies (fenarimol and fenbutatin oxide) would have quantitatively been misinterpreted for risk assessment. (*i.e.,* the lowest LOAEL identified in the study came from the F_2 generation and the NOAEL was used as a point of departure in the risk assessment). F_2 effects for fenarimol consisted of decreased litter size (1.2 MKD). However, a special study for fenarimol identified decreased mating, epididymal weight and dystocia at a higher dose. This information in conjunction with marginal F_1 effects would likely provide classification and labeling information and may have triggered the mating of F_1 animals to produce the F_2 generation for fenarimol. For fenbutatin oxide, decreased pup weight was at a lower dose in the F_2 (16 MKD) compared to the F_1 (34 MKD).

Further review of many studies without triggers suggests that only 13 studies of the 341 (3.8%) pesticide studies would have missed potential endpoints important for classification and labeling. Finally, the trigger analysis supports the saving of almost 165,000 animals. In summary, this revised analysis further confirms that the draft OECD Extended One-Generation Reproductive Guideline would likely not fail to identify critical sensitive endpoints or lower NOAELs in the F_2 generation nor fails to identify potential endpoints important classification and labeling while also significantly reducing the number of animals required to perform a comprehensive reproductive toxicity study.

2. Introduction

This revised retrospective analysis has been updated since the 2008 retrospective analysis with additional multi-generation reproduction studies from the Office of Pesticide Programs (OPP) of the US Environmental Protection Agency (USEPA) and Health Canada's Pest Management Regulatory Agency (PMRA). In this revised retrospective analysis there are an additional 175 studies since the 2008 analysis. The 2009 analysis encompasses a total of 341 pesticide studies, consisting of 21 PMRA studies and 320 OPP studies. The 2009 analysis also includes the same 9 literatures studies of industrial compounds as from the 2008 analysis.

3. Design/Analysis

Data Source:

Over the past 30 years, the Office of Pesticide Programs of the US EPA has collected a large number of studies conducted under GLP standards with consistent protocols. The data collected from these studies can, therefore, be compared on a similar basis. The collection of toxicity studies provides an ideal environment for performing these types of retrospective analyses. The current retrospective analysis evaluated 341 multi-generation reproductive toxicity studies for pesticides and 9 reproductive studies for industrial compounds conducted according to US EPA or OECD guidelines. For the multi-generation retrospective analysis on pesticides, 309 of the 341 (91%) reproduction toxicity studies were conducted according to the pre-1998 US EPA guideline while 32 of the 341 (9%) studies were post-1998 guideline studies. The major differences between the pre- and post-1998 guidelines is that in the pre-1998 guideline endpoints evaluating sexual maturation, andrology, estrous cyclicity, and anogenital distance (AGD) were not required. The majority of the 9 literature studies for the industrial compounds were conducted according to OECD guidelines (Test No. 416, 2001). Therefore, there are a total of 41 studies (32 pesticide and 9 industrial) in this retrospective analysis that were evaluated most closely with the current proposed Extended One-Generation Reproductive Toxicity Test Guideline.

Tools:

To facilitate analysis of the 350 different data sets, the data from each study was entered into a structured and curated database called ToxRefDB (Toxicity Reference Database; http://www.epa.gov/ncct/toxrefdb). ToxRefDB is ideal for tabulating information from these multi-generation reproduction guideline *in vivo* toxicity studies. Specifically, the Data Evaluation Records (DERs) of the (OPP) and PMRA reviews of the reproduction studies submitted by the registrants were collected for 341 chemicals. Every DER file was then indexed according to the study and chemical specifics as outlined in Martin *et al.* 2009a.

Data Input:

The dose-response information from each DER/literature citation of the reproduction toxicity studies was filed into ToxRefDB. This is important since the effects observed at doses other than the LOAEL may contribute to the evaluation of whether the F_1 needed to be mated to produce an F_2 generation. This provided information regarding effects at all doses in the P, F_1 and F_2 generations for comparison. It should be noted that dose-response data was entered into

ToxRefDB for second matings within a generation (e.g., F_{1B}, F_{2B}) as well. The toxicity profile of the chemical as well as the biological significance of effects in the P generation was essential since parental effects could potentially "trigger" the mating of the F_1 animals to generate an F_2 generation. In this approach, statistically significant (designated as "*") effects occurring in the reproductive studies but lacking a dose-response or biological significance were not included in the LOAEL consideration. This weight-of-the-evidence approach is used by the Agency (USEPA) in review of all pesticide toxicity studies. The additional 9 studies on industrial chemicals were also entered in ToxRefDB using the same criteria applied to the 341 studies on pesticides. In summary, effects from all generations (P, F_1, F_2), and if available, second matings (F_{1B}, F_{2B}) from all 350 studies were available for evaluation in the current retrospective analysis.

The suite of effects entered into ToxRefDB was consistent based on the development of a toxicity-based controlled vocabulary. The development of a controlled vocabulary within ToxRefDB was necessary for the standardization of data captured across various studies and study types performed over roughly 30 years. Specifics of the standardized terminology are described in Martin *et al.* 2009a, and available at http://www.epa.gov/ncct/toxrefdb. The terminology for establishing the parental, offspring, and reproductive LOAELs were captured and normalized across the reproductive toxicity studies for this retrospective analysis (Martin *et al.* 2009b).

Data Quality Control:

Quality control (QC) consisted of 100% cross-checking of studies, systematic updates of ToxRefDB to ensure consistency across the studies, expert review of data outputs, and external review by stakeholders. All data entered into ToxRefDB have undergone cross-checking, which entailed a second person validating each entered value based on the source information (primarily DERs). Systematic quality control involved querying the database for potential inconsistencies (e.g., male only effects being assigned to female treatment groups, or systemic LOAEL being set at multiple dose levels) along with updating vocabularies and related records. Expert review was performed on all data outputs from the multi-generation studies captured in the data tables of this document. In addition to internal QC, an ongoing process allowing USEPA stakeholders the opportunity to review ToxRefDB records is in place. The companies or registrants that sponsor the USEPA data or support the registration of the chemical are reviewing the accuracy of the data relative to DERs and other risk assessment documents. The majority of multi-generation studies have been reviewed by registrants, and comments from these reviews indicate greater than 99% accuracy in capturing treatment-related effects from DERs. The USEPA stakeholder review process has facilitated the inclusion of information from additional studies, DERs, and other risk assessment documents to be collected and entered into ToxRefDB.

Data Extraction:

The ToxRefDB provided a framework such that focused queries were generated for the strategies of this retrospective analysis. Specifically, the stored reproductive toxicity data within ToxRefDB were extracted for analysis by chemical and endpoints for the defined set of 350 reproductive studies. The data output files that were used in the retrospective analysis consisted of rows of chemical information such as CAS registry number, PC Code, chemical name, and MRID along with columns of endpoints or effects and a cross-section identifying the lowest dose at which the endpoint/effect was observed (LOAEL or LOEL) according to the DER. These data

spreadsheets provided suites of effects at multiple dose levels for each generation of the reproductive toxicity study (i.e., parental, F_1 generation, F_2 generation). These data spreadsheets were available in excel format such that sorting and filtering of the data were possible for the retrospective analysis.

4. Criteria of Effects for Retrospective Analysis

This retrospective analysis focused on the potential difference in effects (Lowest Observable Effect Level [LOEL] or Lowest Observable Adverse Effect Level [LOAEL]) of the F_1 generation from the F_2 generation. This analysis also focused on effects that potentially may be missed if the F_1 generation is not mated and that are important for classification and labeling (C&L) under the REACH program of the European Union (EU). Furthermore, this analysis used data generated using a study design similar to similar to the proposed Extended One-Generation Reproductive Toxicity Test Guideline. For example, the proposed Extended One-Generation Reproductive Toxicity Test Guideline does not include second matings (i.e., F_{1B}) or a third generation, therefore, this analysis did not rely on information from any second matings or third generations available from the 350 studies. Therefore, the quantitative and qualitative comparison was based on the F_{1A} and F_{2A} generations. In addition, the trigger analysis was performed solely on effects appropriate as triggers in the P and F_{1A} generations, not triggers from the F_{1B} generation, since these F_{1B} effects would not typically be available while conducting the proposed Extended One-Generation Reproductive Toxicity Test Guideline. A list of current proposed triggers is presented in Tables 6 and 7.

Another strategy used in this retrospective analysis was the separation of offspring from reproductive type of effects/endpoints. For example, a comparison of offspring effects (e.g., pup weight, viability index, lactation index) in the F_{1A} to the F_{2A} generation was performed separately from a comparison of reproductive effects (e.g., litter size, fertility, reproductive organ weights) in the F_{1A} and F_{2A} generations. This strategy supported a focused and concerted evaluation of both offspring and reproductive effects. A list of effects for both the offspring and reproductive analyses were first defined for consistency in the retrospective analysis. Some effects identified in the reproductive studies could be considered by some scientists as both reproductive and offspring type effects. However, for consistency in the retrospective analysis, ambiguous effects (e.g., could be considered either offspring or reproductive in nature) were discussed among team members and consensus reached about how a specific effect would be treated. The following section highlights the potential list of reproductive and offspring effects that were examined in this retrospective analysis. It should be noted that this list is not exhaustive of all effects, but serves as an example of common reproductive and offspring effects that may have been observed in the available reproductive toxicity studies.

Reproductive Endpoint Criteria:

The reproductive effects defined in this retrospective analysis included any effect that may impact the reproduction system. Reproductive effects may occur in the P, F_1, or F_2 generation. Examples may include: changes in reproductive performance (pregnancy rate, fertility index, mating index, implantations, gestational interval, litter size/weight, litter loss), sexual maturation (vaginal opening, preputial separation) anogenital distance, changes in the reproductive

tract (gross, histopathology, malformations, weights), sex ratio, estrous cycle length and periodicity, sperm measures (epididymal sperm counts, motility, morphology, testicular spermatid counts), dystocia, and reproductive hormone levels (testosterone, estradiol). Reproductive effects did NOT include pup viability, pup weights or other offspring types of effects. Furthermore, effects of the reproductive tract even in the offspring were considered reproductive effects, not offspring effects.

Offspring Endpoint Criteria:

The offspring effects defined in this analysis included any effect that impacts the pup (offspring). A consistent list of defined endpoints for offspring effects for the analysis was developed. Offspring effects may be observed in either the F_1 or F_2 generations. Examples of potential offspring effects that may be observed in reproductive studies include: behavioral effects (righting reflex, startle reflex), clinical chemistry, clinical signs, pup mortality (cannibalism), stillbirth, developmental landmarks (tooth eruption, pinna detachment, unfolding, eye opening), lactation index, live birth index, organ weights (except reproductive), malformations (gross, histopathology, except reproductive tract), pup body weight (gain), and viability/viability index.

5. Retrospective Analysis Results

A. 9 Literature Studies

Quantitative Analysis of F_1 and F_2 LOAEL/LOELs for 9 Industrial Compounds

The 9 industrial compounds were chosen from the literature since they represent a class of known reproductive toxicants. The studies from the literature were comprehensive in their evaluation and most closely followed the current proposed Extended One-Generation Reproductive Toxicity Test Guideline such that VO/PPS and AGD were well studied. Sperm parameters were also available for a majority of these studies. The study design of these 9 chemicals is important to note since the results would best predict the study results of the proposed Extended One-Generation Reproductive Toxicity Test Guideline.

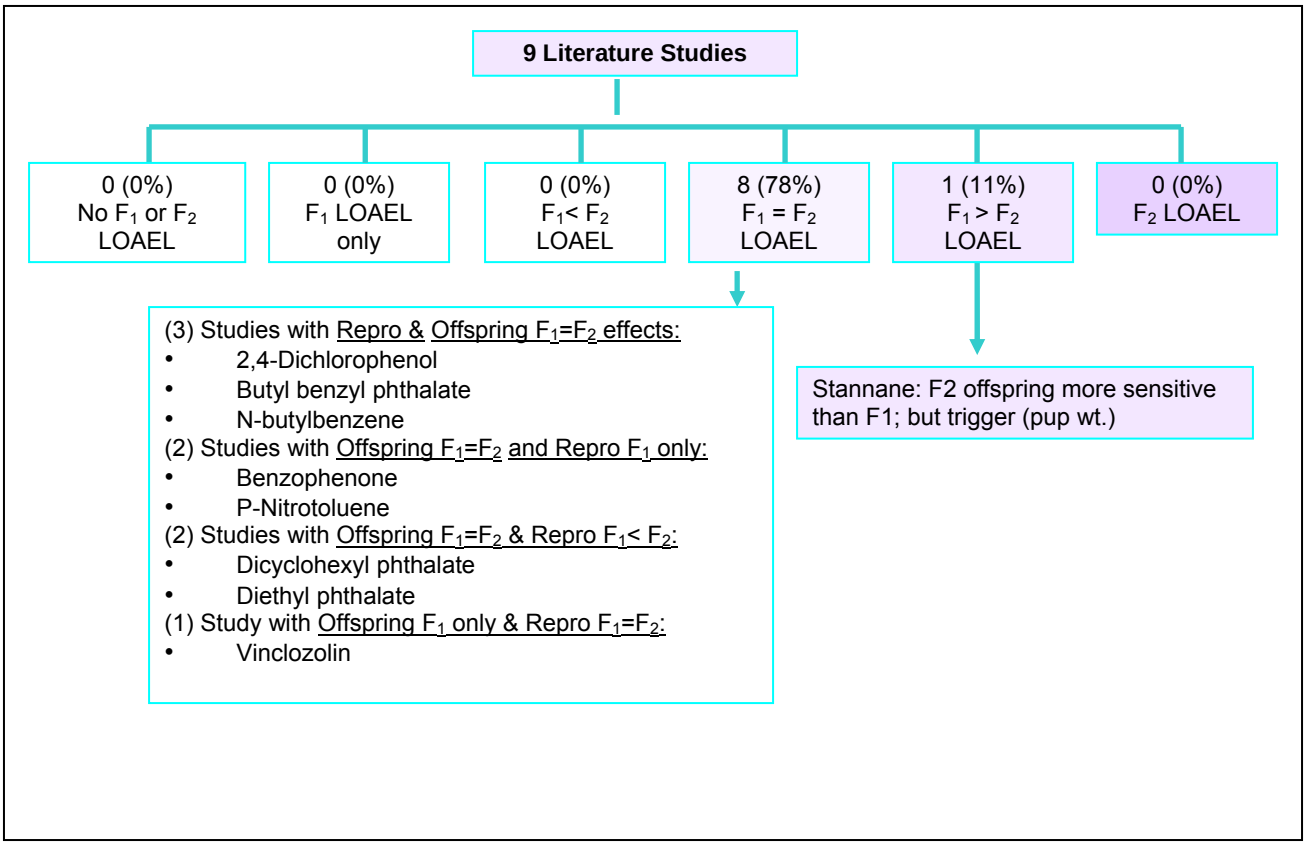
These 9 industrial compounds were evaluated to determine if reproductive or offspring effects occurred in the F_{2A} generation at doses below or similar to the F_{1A} generation. For consistency with the other studies in the retrospective analysis, LOAEL/LOELs for the industrial compounds were established according to the policies used for the pesticide studies. It should be noted that 2 studies are available for vinclozolin; a literature study and an OPP pesticide study. The studies for vinclozolin have been separated according to the source of the data.

Results

None of the 9 studies for the industrial compounds identified a reproductive or offspring effect that was solely in the second generation. Only one study (stannane) was identified in ToxRefDB with an F_2 effect that was at a lower dose in the second generation (F_1 LOAEL > F_2 LOAEL). Pup weight loss in the F_1 generation, however, would likely have triggered the mating of F_1 animals to produce a second generation. Therefore, this F_2 effect would not have been missed according to the proposed Extended One-Generation Reproductive Toxicity Test Guideline. The

reproductive and offspring effects in the F_1 would also have provided information for classification and labeling. The remaining 8 studies identified offspring or reproductive effects in both the F_1 and F_2 generation or only in the F_1 generation. In summary, there were no concerns for risk assessment or classification and labeling by performing the Extended One-Generation Reproductive Toxicity Test Guideline for these 9 industrial compounds. The F_1 and F_2 LOAEL groups for the 9 industrial compounds are presented in Figure 1 and Table 1 below. It should be noted that the green highlights in Table 1 indicate the dose level and endpoints that served as the basis for the determination of increased F_2 sensitivity or F_2 unique effect.

Figure 1. A flow diagram of the F_1 LOAEL vs F_2 LOAEL comparison of the 9 literature studies



Green highlights in Table 1 below indicate the dose level and endpoints that served as the basis for the determination of increased F2 sensitivity or F2 unique effect

Table 1. Study details for the Parental Generation (P), the F_1 generation (F_{1A}), and the F_2 generation (F_{2A}) for the 9 Industrial compounds

Chemical	P effects	F_1 effects	F_2 effects	F_2 triggered?
F_1 LOEL > F_2 LOEL				
Stannane	No P LOAEL	**F_1 Repro LOAEL LDT** LDT (0.25 MKD): -↓ ab. epididymis wt.	**F_2 Repro LOEL MDT** MDT: -↓rel. prostate	Trigger (pup wt) No C&L concerns

Table 1. Study details for the Parental Generation (P), the F_1 generation (F_{1A}), and the F_2 generation (F_{2A}) for the 9 Industrial compounds

Chemical	P effects	F_1 effects	F_2 effects	F_2 triggered?
		-↓ ab. testes wt. MDT (1.25 MKD): -↓testes wt. HDT (6.25 MKD): -↓prostate wt. -↓epididymis -↓spermatid -↑abnormal sperm **F_1 Offspring LOAEL MDT** MDT: -↓ **pup wt.** HDT: -↓pup wt.	-↓spermatid HDT: -↓testes -↓epididymis -↓E_2 -↓spermatid -↓rel. prostate -↑LH **F_2 Offspring LOAEL LDT:** -↓pup wt. PND4 only MDT: -↓pup wt. HDT: -↓pup wt.	
F_1 LOEL = F_2 LOEL				
2,4-dichlorophenol	**P LOAEL HDT** HDT (543/768 MKD): -↓bw -↓food -↑ staining -enlarged/discolored mammary (14/24*** vs 0/23) Also MDT (134/194 MKD): -↑ rel. adrenal -enlarged mammary (7/24** vs 0/23) LDT (33/49 MKD): -enlarged mammary (9/24*** vs 0/23)	**Repro LOELs:** LDT: -enlarged/discolored mammary (13/22*** vs 2/23) MDT: -enlarged/discolored mammary (15/23*** vs 2/23) -↑ abs. uterus (offspring 25%*) HDT: -enlarged/discolored mammary (18/23*** vs 2/23) -↓PPS (days):(42.2* vs 41.2 -↓VO (bw): 95.2*** vs 108.0 -↑ rel. testis -↑ abs. uterus (offspring 41%***) -↑ rel. uterus(parental 42%**) -↓ ab. ovary (parental 17%***) -↓ implantations (10.2* vs 12.7) **F_1 Offspring LOAEL** HDT: -↓ pup wt. LD7-21 (7-16%***) -%delay eye open LD14 (M: 43%***; F: 31%***) -↓ spleen -↓ thymus **F_1 Adult LOAEL HDT** HDT: -↑kidney -↑pituitary -↑staining -↑brain -↓bw -↓food -↓spleen	**F_2 Repro LOELs HDT:** -↑uterus (20%**) **F_2 Offspring LOAEL HDT** HDT: -↓thymus -↓ brain -↓spleen -↓pup wt. LD7-21 (8%-15%***) -%delay eye open LD14 (M: 43%**; F: 44%***)	No Triggers P LOAEL = F_2 LOAEL No C&L concern

Table 1. Study details for the Parental Generation (P), the F_1 generation (F_{1A}), and the F_2 generation (F_{2A}) for the 9 Industrial compounds

Chemical	P effects	F_1 effects	F_2 effects	F_2 triggered?
		-↓thymus -↓liver Also MDT: -↓ abs. liver		
Benzophenone	**P LOAEL MDT** LOEL LDT (6.4/8.4 MKD): -liver hypertrophy -↑rel. liver wt. MDT (29/38 MKD): -↑ rel./ab. liver wt. -↑ rel./ab. kidney wt. -liver hypertrophy -kidney regeneration HDT (130/167 MKD): -↑ testes wt. -↑ rel./ab. liver wt. -↑ rel./ab. kidney wt. -liver hypertrophy -kidney regeneration -↑ rel. brain wt. -↑ rel. spleen wt.	**F_1 Repro LOAEL LDT** LDT**:** -↓ **AGD** MDT: -↓AGD HDT: -↑ rel. testes wt. -↑ rel. ovary wt. **F_1 Offspring LOAEL HDT** HDT: -↓pup wt. -↓ spleen wt. - delay pinna unfolding -↑ rel. brain wt. **F_1 Adult LOELs** LDT: -liver hypertrophy MDT: -liver hypertrophy -kidney regeneration -↓ ab. pituitary wt. -↑ rel. kidney wt. HDT: -↓ bw -↓ rel. spleen wt. -↓ ab. pituitary wt. -↑ rel./ab. kidney wt. -↑ kidney dilatation -↑kidney regeneration -↑ rel./ab. liver wt. -↑ liver hypertrophy -↑ rel. brain wt.	**No F_2 Repro LOELs** **F_2 Offspring LOAEL HDT:** -↓ rel. brain wt. -↓ pup wt. -delay pinna unfolding	Trigger P LOAEL < F_2 LOAEL No C&L concern
Butyl benzyl phthalate	**P LOELs** MDT (200 MKD): -↑kidney -↑liver -↑ salivation HDT (400 MKD): -↓epididymis (6%*) -↑testes hyperplasia -↑epididymis depletion -↑kidney -↑liver -↑salivation	**F_1 Repro LOELs:** LDT (100 MKD): -↑**AGD** (1.08** vs 0.98, rel BW) MDT: -↓epididymides (12%*) -↑AGD (1.07* vs 0.98, rel BW) HDT: -↓seminal vesicle -↓**PPS** (14/24 (58.3**) vs 23/24 (95.8)) -↑AGD (1.06* vs 0.98, rel BW) -↓fertility (13/20 (65%) vs 16/21 (76.2%)) -↓testes size (6/24* vs 0/24) -↑epididymis (16%**) -↑testes atrophy (9/24* vs	**F_2 Repro LOELs:** LDT: -↓AGD (4.2** vs 4.75; rel bw: 1.96** vs 2.12) MDT: -↓AGD (4.39 vs 4.75; rel bw: 1.94** vs 2.12) HDT: -↓AGD (4.08 vs 4.75; rel bw 1.87** vs 2.12) **F_2 Offspring LOAEL** HDT: -↓spleen	Trigger P LOEL > F_2 LOEL No C&L concerns

Table 1. Study details for the Parental Generation (P), the F_1 generation (F_{1A}), and the F_2 generation (F_{2A}) for the 9 Industrial compounds

Chemical	P effects	F_1 effects	F_2 effects	F_2 triggered?
		1/24) -↑testes hyperplasia (5/24* vs 0/24) -↑testes soft (4/24 vs 0/24) **F_1 Offspring LOAEL** HDT: -↓spleen **F_1 Adult LOELs**: MDT: -↑salivation -↑liver HDT: -↑thyroid -↑liver -↑salivation		
Dicyclohexyl phthalate	**P LOAEL HDT** HDT (402/511 MKD): -thyroid hypertrophy -kidney hyaline droplets -↑ rel./ab/ thyroid wt. -↑ rel./ab. liver wt. -liver hypertrophy	**F_1 Repro LOAEL LDT** LDT (18/21 MKD): -↓ ab. prostate wt. MDT (90/107 MKD): -abnormal sperm -↓ ab. prostate wt. HDT: -abnormal sperm -delay nipple development -testes atrophy -delay **AGD** -↓ rel./ab. prostate wt. **F_1 Offspring LOAEL HDT** HDT: -↓ **pup wt**. -↓ spleen wt. -↓ thymus wt. -↑ brain wt. **F_1 Adult LOAEL HDT** HDT: -↓ bw -↑ rel. liver wt. -↑ thyroid hypertrophy -↑ liver hypertrophy -↑ kidney hyaline droplets	**F_2 Repro LOELs** MDT: -delay AGD HDT: -delay nipple development -delay AGD **F_2 Offspring LOAEL HDT** HDT: -↓ pup wt. -↓ spleen wt -↑ rel. brain wt.	Trigger F_1 LOAEL < F_2 LOEL P LOAEL < F_2 LOAEL No C&L concern
Diethyl phthalate	**P LOAEL HDT** MDT (197/255): -abnormal sperm -↓ testosterone HDT (1016/1297 MKD): -↑ P450 -↑ rel./ab. liver wt. -↓ ab. adrenal wt. -↓ testosterone -↓ ab epididymis	**F_1 Repro LOAEL MDT** MDT: -abnormal sperm HDT: -abnormal sperm -↓ rel. seminal vesicle wt. -↓ ab. prostate wt. -↓ ab. uterus wt. - delay VO **F_1 Offspring LOAEL HDT** HDT: -↑rel. brain wt. -↑ liver wt. -↑ pituitary wt. -↓ spleen wt.	**F_2 Repro LOAEL HDT** HDT: -↓ rel./ab. uterus wt. **F_2 Offspring LOAEL HDT** HDT: -↓ thymus wt. -↓ pup wt. -↓ spleen wt. -↑ liver wt. -↓ adrenal wt. -↑rel. brain wt.	Trigger (pup wt.) F_1 LOAEL< F_2 LOAEL P LOAEL= F_2 LOAEL No C&L concerns

Table 1. Study details for the Parental Generation (P), the F_1 generation (F_{1A}), and the F_2 generation (F_{2A}) for the 9 Industrial compounds

Chemical	P effects	F_1 effects	F_2 effects	F_2 triggered?
		-↓ thymus wt. -↓ adrenal wt. -↓ **pup wt.** -↓ kidney wt. **F_1 Adult LOAEL HDT** HDT: -↓ ab. adrenal wt. -↑ rel./ab. liver wt. -↑ rel./ab. kidney wt.	-↓ kidney wt.	
N-butylbenzene	**P LOAEL MDT** LDT (30 MKD): -↑ rel./ab. liver wt. MDT (100 MKD): -↑ rel./ab. liver wt. -salivation HDT (300 MKD): -salivation -↑ rel./ab. liver wt. -↑ rel./ab. kidney wt. -kidney hyaline droplets -liver hypertrophy -kidney basophilia	**F_1 Repro LOAEL HDT** HDT: -↑ ovary wt. **F_1 Offspring LOAEL HDT** HDT: -↑ rel./ab. thymus wt. **F_1 Adult LOAEL MDT** MDT: -kidney hyaline droplet -↑ rel. kidney wt. -salivation HDT: -salivation -↑ kidney wt. -↑ liver wt. -kidney hyaline droplet/basophilia -↑adrenal wt.	**F_2 Repro LOAEL HDT** HDT: -↑ **Uterus wt.** **F_2 Offspring LOAEL HDT** HDT: -↑ rel. thymus. Wt.	No trigger F_2 unique (uterus vs. ovary wt) P LOAEL < F_2 LOAEL No C&L concern
p-Nitrotoluene	**P LOELs** LDT (40 MKD): -↑ rel./ab. liver wt. -↑ rel. kidney wt. MDT (80 MKD): -↑ rel./ab. kidney wt. -↑ rel./ab. liver wt. HDT (160 MKD): -↑ rel./ab. kidney wt. -↑ rel./ab. liver wt. -↑ hematopoietic cell -discolored spleen -spleen hemosiderosis -kidney hyaline droplet -↓T4	**F_1 Repro LOEL** HDT: -delay **AGD** -delay **VO** **F_1 Offspring LOAEL HDT** HDT: -↓ **viability** index (PND4) Also ↓ brain wt. Also MDT: -↓ **viability** index **F_1 Adult LOELs** LDT: -↑ rel./ab. liver wt. MDT: -↓ ab. brain wt. -kidney hyaline droplet -↑ rel. liver wt. HDT: -spleen hemosiderosis -↓ ab. brain wt. -↓ ab. heart wt. -↑ kidney wt. -↑liver wt. -↑ pituitary wt.	**No F_2 Repro LOEL** **F_2 Offspring LOAEL HDT** HDT: -↓ viability -↓ brain wt.	Triggers P LOEL < F_2 LOAEL No C&L concerns

Table 1. Study details for the Parental Generation (P), the F_1 generation (F_{1A}), and the F_2 generation (F_{2A}) for the 9 Industrial compounds

Chemical	P effects	F_1 effects	F_2 effects	F_2 triggered?
		-kidney discolored --kidney hyaline droplet		
Vinclozolin	**P LOAEL HDT** -MDT (11.5/12.8 MKD): -adrenal pathology -liver hypertrophy -↓T4 HDT (57.3/64.0 MKD): -↓ bw (Females only) -↑ rel. liver wt. -adrenal pathology -pituitary hypertrophy -↑ rel./ab. adrenal wt. -↓T3 & T4 -↑ rel./ab. pituitary wt. -↑ rel. kidney wt. Also Repro Effects HDT: -↑ testes hyperplasia -↑ rel./ab. testes wt. -↑ testes atrophy -↓ rel./ab. epididymis wt. -↑ ovary vacuolization & hyperplasia	**F_1 Repro LOAEL MDT** MDT: -↓rel./ab. epididymis wt. -↓ prostate secretory fluid -↓ rel./ab. prostate wt. -PPS -abnormal nipple development -↑ rel. ovary wt. HDT: -↓rel./ab. epididymis wt. -AGD -PPS -↑ LH -↑ FSH -↓ seminal vesicle wt. -↓ rel/ab prostate wt. -↓ prostate secretory fluid -↓ rel./ab. testes wt. -testes hyperplasia -↑ DHT -↓ fertility -abnormal nipple development -↑ rel. ovary wt. -ovary hyperplasia **F_1 Offspring LOAEL HDT** HDT: -↓ pup wt. -↓ rel./ab. brain wt. **F_1 Adult LOELs** MDT: -adrenal pathology -liver hypertrophy HDT: -↑ rel./ab. pituitary wt. -adrenal pathology -liver hypertrophy -pituitary hypertrophy -↑ rel. kidney wt. -↓T3 & T4	**F_2 Repro LOAEL MDT** MDT: -AGD -abnormal nipple development HDT: -AGD -abnormal nipple development -↓rel./ab. epididymis wt. **No F_2 Offspring LOEL**	Triggers F_1 repro= F_2 repro P LOAEL = F_2 repro No C&L concerns

B. OPP/PMRA Pesticide Studies

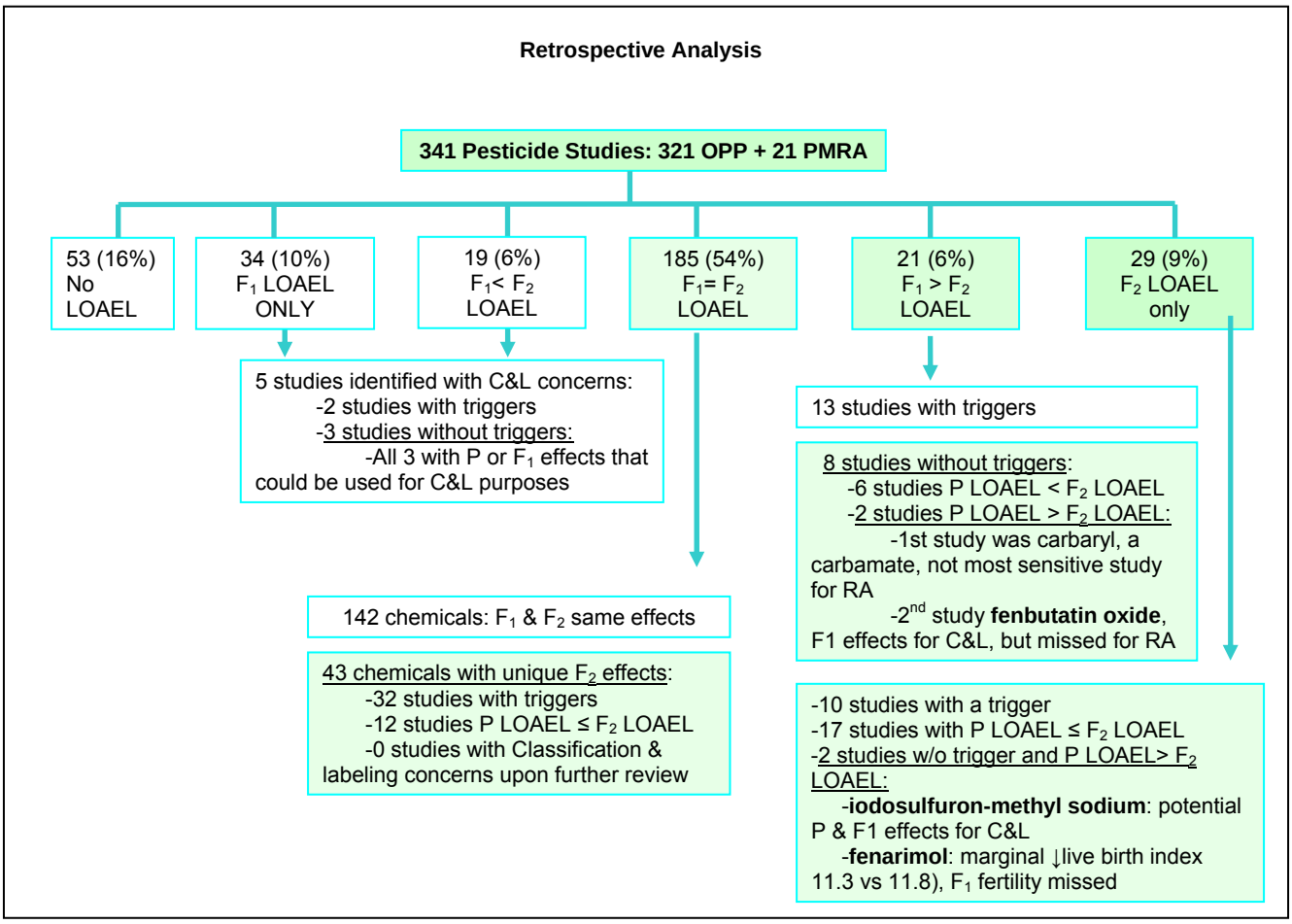
Comparison of F_{1A} and F_{2A} LOAEL/LOELs for 341 Pesticides

As mentioned previously, the Agency evaluated both the quantitative (i.e., dose level) and qualitative (i.e., different or "unique" effects) LOAEL and LOEL differences in the F_{1A} and F_{2A}

generation for both reproduction and offspring effects. Furthermore, for consideration of the REACH program, the retrospective analysis also took into consideration any effects that would be necessary for classification and labeling (C&L). A C&L concern was identified for a study if a new or different effect (unique) occurred in the F_2 and not in the F_1 generation. Classification and labeling concerns were also identified for any F_1 effect that was evaluated after the decision for the second mating would occur. For example, a change in F_1 gestation interval would be a C&L concern since this effect would have been missed if the F_1 animals were not mated to produce the second generation. It should also be noted that no conclusions were drawn regarding the critical nature of the effects used for C&L. For example, a 7% decrease in pup weight was considered as critical as hypospadias. Ultimately the C&L decision is determined based on a weight of the evidence for the chemical. Thus, this analysis simply presents effects from all generations to better inform the suite of effects occurring in the reproductive study.

This revised retrospective analysis encompasses 341 pesticide studies, which includes 320 OPP pesticides and 21 PMRA pesticides. Of the 341 studies, 309 studies were pre-1998 guideline (91%) and 32 studies were post-1998 guideline (9%). This report will first summarize the studies that identified effects in the F_2 generation only followed by the studies where the F_2 generation was more sensitive than the F_1 generation (F_1 LO(A)ELS > F_2 LO(A)ELs). The studies with similar LOEL/LOAELs (F_1=F_2) but with unique F_2 effects are also be presented. Finally, the report summarizes the results of the trigger analysis that was performed.

Figure 2. Results of the 341 Pesticide Studies in the Retrospective Analysis



1. F_2 LOEL/LOAEL Only

The first quantitative comparison was for reproductive and/or offspring effects in the F_{1A} generation compared to the F_{2A} generation. This comparison resulted in 29 pesticide studies (29/341 = 9%) in which an effect (LOAEL or LOEL) for reproductive or offspring was based solely on the F_2 generation. This group represents a situation in which no reproductive or offspring effects and therefore no LOAEL or LOELs were identified in the F_1 generation at the time of the study review. It should be noted that study reviews may not have included effects considered non-adverse due to Agency policies at that time. This retrospective analysis reviewed these studies further by examining effects listed in tables in the study review document. These study details are presented in the Tables of this report.

A trigger analysis resulted in 10/28 studies with triggers either in the P or F_1 generation (acequinocyl, bensulide, butylate, dichlorvos, forchlorfenuron, hexaconazole, metasystox R, propoxycarbazone, TCMTB, and trichlorfon). The remaining 19 studies with effects in the F_2 generation only were compared quantitatively with the parental LOAEL/LOELs. From this

parental comparison, only 2 studies would have been missed (iodosulfuron-methyl sodium and fenarimol) quantitatively (*i.e.,* P LO(A)EL > F_2 LO(A)EL), based on the conclusions in the last study evaluation. However, upon reviewing the details of both studies more closely, there were effects in the P (↑seminal vesicle weights) and F_1 (↓pup weight) generation for iodosulfuron-methyl sodium that would likely have triggered an F_2 generation and provided information for classification and labeling. Thus, re-evaluation of iodosulfuron-methyl sodium suggests an F_1 LOAEL = F_2 LOAEL conclusion. The iodosulfuron-methyl sodium reproduction study was not used for risk assessment; the chronic dog was used based on gross and histopathological changes in the hematopoietic system. Upon closer review of fenarimol, there was a marginal decrease in F_1 liveborn litter size (11.3 vs 11.8) that was not statistically significant. This endpoint did not require F_1 mating and is therefore a potential endpoint for classification and labeling. There was also a significant decrease in F_1 fertility, however, this endpoint would not have been available from a one-generation study, thus missed for classification and labeling. The fenarimol reproduction study was used for the USEPA intermediate dermal/inhalation and chronic dietary risk assessment. However, a special study with fenarimol that identified decreased mating, epididymal weight and dystocia (LOAEL 35 MKD) was used for the short term risk assessment. This special study in conjunction with the fenarimol reproduction study may have provided adequate information for classification and labeling. In summary, when evaluated under the current standards, fenarimol is one of two studies (fenbutatin-oxide, F_1>F_2) out of 341 pesticide studies with identified effects in the second generation that would have contributed to the risk assessment and potentially used for regulatory purposes. Details of the F_2 only studies are provided in Table 2. It should be noted that the green highlights in Table 2 indicate the dose level and endpoints that served as the basis for the determination of increased F_2 sensitivity or F_2 unique effect. Fenbutatin oxide is presented in Table 3.

For classification and labeling concerns, 13 studies were identified with F_2 effects that may be important for classification and labeling. Pup weight, however, was the only F_2 effect for 5 of the 13 studies. If pup weight alone is not considered sufficient evidence to warrant classification and labeling, then 7 studies (cyhalothrin, endosulfan, fluroxypyr, formetanate, fenpropathrin, parathion-methyl, propiconazole) would potentially have information missing for C&L by not mating the F_1 animals. Details of these studies are provided in Table 2. Green highlights in Table 2 indicate the dose level and endpoints that served as the basis for the determination of increased F2 sensitivity or F2 unique effect.

Table 2. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring or reproductive LOAELs based solely on the F_2 generation (F_2 only)

Chemical	P effects	F_1 effects	F_2 effects	F_2 triggered?
F_2 only, no triggers, and P LOAEL > F_2 LOAEL				
1. Iodosulfuron-methyl sodium	**No P LOAEL** ↑seminal vesicle wts.13%* @HDT (346MKD) (LEL)	**No F_1 LOAEL** but: HDT (346/390 MKD): -↓5% litter size Day 0 -↓7% litter size Day 4 -Pup deaths Day 0-4: 22 vs. 13 controls -↓9% pup wt. gain Day21 (no * performed)	**F_2 LOAEL HDT** -HDT: Viability, live birth index @ HDT -↓12-14% litter size Day 0-4 -#live pups ↓12-14% Day 0-4 -15 HDT vs. 9 control pup deaths Day0-4 -↓5% pup wt. gain	No trigger in ToxRefDB However, further review suggests trigger (pup wt.) No P LOAEL No C&L concern
2. Fenarimol	**No P LOAEL** No P effects	**No F_{1A} LOAEL** but: HDT (2.5/3.2 MKD): -↓F_1 fertility 35% vs. 83% controls * -liveborn litter size (11.3^{ns} vs. 11.8 controls) -#live pups Day 1: 202^{ns} vs 337 -#live pups Day 21: 183^{ns} vs 299 -Mean litter size: Day 1: 11.2 vs 11.6 Day 21: 10.2 vs 10.3 -(12/204)^{ns} 5.9% pups born dead vs. 5.3% (18/341)	**F_{2A} LOAEL HDT** HDT: -(8/82)^{ns} 10% pups born dead vs. 4.3% (11/254) controls; yet -↑liveborn litter size 11.7^{ns} vs. 10.2 controls -progeny survival index: 78%^{ns} vs 91% Day 1 **F_{2B} LOAEL MDT and used for risk assessment** MDT (1.2 MKD): -↓liveborn litter size (9.0* vs 10.9; 17%) HDT: -↓liveborn litter size (7.9* vs 10.9; 28%)	No trigger No P LOAEL Current policy would suggest F_1 fertility as LOAEL Potential C&L concern (Note: a special study with decreased mating, epididymal wt, and dystocia also available)
Offspring or Repro F_2 only but triggers				
3. Acequinocyl	**P LOAEL @ MDT** MDT: F_1 adult males: **hemorrhage** at MDT [1/25 post weaning day 2-3] and HDT [3/25 pnd 2-7]	**F_1 Offspring LOAEL @ MDT** MDT**: hemorrhagic effects**, swollen body parts [Similar effects at 1500 ppm but at increased frequency] HDT: 1500 ppm (HDT) increased mortality (F_1 pups) 17.1% vs 1.2% control Day 22-30, 6.9% vs 0 control D31-56 no stats **No F_1 Repro LOEL**	**F_2 Offspring LOAEL MDT** MDT: **hemorrhagic effects**, cyanosis, swollen body parts, protruding eyes, clinical signs behavioral effects , delays in pupil development and increased mortality post weaning (F_2 pups)[4.3% vs 0.6% d22-30] HDT: [Similar effects MDT listed above but at increased frequency] -increased mortality (F_2 pups) 32% vs 0.6% D22-30, 5.8% vs 0% on d31-35 no stats **F_2 Repro LEL at HDT** HDT: -↓PPS on d47-49 33-21%: -↓VO on d43 & d44 19% & 16%,	**Trigger** No C&L concern P LOAEL = F_1 LOAEL = F_2 LOAEL for offspring P<F_2 LEL for repro

Table 2. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring or reproductive LOAELs based solely on the F_2 generation (F_2 only)

Chemical	P effects	F_1 effects	F_2 effects	F_2 triggered?
			delayed testes descent on D25-28	
4. Bensulide	**P LOAEL @ HDT** HDT: ↓plasma ChE 54-76%** ↓RBC ChE 32-57%** ↓brain ChE 68%** ↓fertility: Males: 21 of 28 siring litters; Females: 24 of 28 pregnant MDT: ↓plasma ChE 21-43%**	**No F_1 LOAEL** but: ↓ChE @Low, Mid, HDT F_1: -↓plasma 28%**, 30-47%**, 62-80** -↓RBC 0%, 11%*, 42-63%** -↓brain 0%, 0%, 42%** Also HDT ↓pup wt but not part of LOAEL: Day 1: 4% Day 4: 9% Day 14: 9-10% Day 21: 9-9.4% (*not performed)	**F_2 LOAEL HDT** ↓viability HDT: Day0-21 survival index 61% LD0-4 viability index 74% No changes in pup wt.	**Trigger** No C&L concern Under current policies the cholinesterase effects in the F_1 would have been considered as part of a LOAEL
5. Butylate	**P LOAEL @ MDT** (50 MKD) MDT: -↓food (4-7%); -↓bw (5-8%); -↑liver wt (5%) HDT (200 MKD): -↓bw (10-14%*); -↓food(8-20%); -↑liver wt(12%)	**F_1 Repro LOAEL** @ HDT: **↓litter size (15%*)** **No F_1 Offspring LOAEL** but HDT: ↓pup wt (8-12%*)	**F_2 LOAEL @ MDT** ↓pup wt. (8-11%*) ↓pup wt. (21%*) HDT HDT: ↓litter size (7%*)	**Trigger** P LOAEL = F_2 LOAEL No C&L concern
6. Dichlorvos	**P LOAEL @ MDT** (1.9/2.3 MKD) MDT: ↓ChE (14-26%* brain, 29-39%* RBC) HDT (7.2/8.3 MKD): 53-59%* brain, 57-61%* RBC)	**F_1 Repro LOAEL @ HDT** (7.2/8.3 MKD) -↓fertility index (55 vs. 71) -↓pregnancy index (55 vs. 81) -↓lactation index (80 vs. 100) **-↓estrous length** (4.87 vs. 4.96) **and periodicity** (as 19 vs. 25 controls of % cycling; 13 vs. 4 control with abnormal cycles) **No F_1 Offspring LOAEL but** -↓8%* pup wt. PND21	**F_2 Offspring LOAEL @ HDT** -↓pup wt.(9%^{ns}), -↓survival index (86.5 vs. 96.4 controls)	**Triggers** Current policy F_1= F_2 P LOAEL < F_2 LOAEL No C&L concern
7. Forchlorfenuron	**P LOAEL @ MDT** MDT: -↓FC: F_1 adults (3-10%) -↓bw: F_1 adults: (4-10%)**, males: (6-9%)** females premating HDT: -↓bw 5-8%, P males; 9-15% P females;	**Offspring F_1 LOAEL @ MDT** MDT: -↓pup bw/bwg (5-111%/11-14%)** lactation d4-21 -↓ size HDT: -↓pup bw/bwg (11-60%/63-74%)** lactation d1-21 -↓size	**Offspring F_2 LOAEL @ HDT** HDT: -↓pup bw/bwg (15-63%/ 62-83%) LD1-21 -↓ size **-↓lactation index (4%)** **F_{1A}**	**Trigger** No C&L concern P LOAEL <F_2 repro LOAEL

Table 2. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring or reproductive LOAELs based solely on the F_2 generation (F_2 only)

Chemical	P effects	F_1 effects	F_2 effects	F_2 triggered?
	26-71% F_1 males; 26-67% F_1 females premating 12-15% P females during GD0-20 and 16-24% during lactation D1-21 -↓FC 34% during lactation d1-21 -↓FC 17-73% F_1 males premating; 14-67% females premating -↑testes** (P males) -↓testes (F_1 males)	**-↓lactation index (4%) F_{1A}**	**Repro LOAEL @ HDT** HDT: -↓litter size F_{2A} (14%)**	
8. Hexaconazole	**P LOAEL @ HDT** HDT (50 MKD): -liver (fatty, discolored, enlarged pattern, hypertrophy) -adrenal vacuolization	**Offspring F_1 LOAEL @ HDT** HDT: -↓pup wt. gain (16-17%**) Day 1-21 -fatty liver **No F_1 Repro LOAEL**	**Offspring F_2 LOAEL @ HDT** HDT: -liver (fatty, discolored, vacuolization) **F_2 Repro LOAEL @ HDT** -↓litter size F_{2A} (18-20%)*	**Trigger** No C&L concern P LOAEL <F_2 repro LOAEL
9. Metasystox R (Oxydemeton-methyl)	**P LOAEL @ HDT** (2.1 MKD) -↓**fertility index: 57%** -100% epididymal vacuole -F_0 F bw (10-75%) gestation & lactation periods -↓ovary wt. (14-24%) LDT: ↓ChE brain (7-11%), RBC (10-75%) No NOEL for ChE Inhibition	**F_1 Repro LOAEL @HDT** HDT: -↓**estrous corpora lutea (65-84%)**, -↑**estrous length** (15-35%) -↓fertility index (43%) -100% epididymis vacuole. -↓testes wt (4-11%) -↓ovary wt. (14-24%) - F_1 F bw (10-75%) gest.& lactation periods -↓Adult Male bw (4-5%)	**F_2 Offspring LOAEL @ HDT** ↓pup wt., ↓viability HDT: ↓live litter size (33%) ↓pup wt. LD (14-31%) LDT: ↓ChE RBC (10-14%), brain (8-12%), plasma (24-29%)	**Triggers** P LOAEL= F_2 LOAEL No C&L concern
10. Propoxycarbazone	**P LOAEL @ HDT** (1231/1605 MKD) Stomach vacuolization, intestine dilatation -#**F0 diestrous/metestrous**: 12 vs. 6^{ns} control	**Repro LOAEL based on**: HDT: - F_1 post-implantation loss: (14.3%** vs. 7.4% control); historical controls: 4.00-13.80% But also LEL: -# F_1 **diestrous/metestrous** 18* vs. 10 control **No F_1 Offspring LOAEL**	**F_2 Offspring LOAEL @HDT** ↓live birth index ↓ F_2 mean live litter size: -Day 0: 9.82±1.48 vs. 11.07± 2.82^{ns} -Live birth index: 100% vs. 98.4% controls -Day 0 M&F pup wt. similar to control -Viability index: 97.8% vs. 99.3% controls -LD28 index: 89.5% vs. 95%	**Trigger** P LOAEL = F_2 LOAEL No C&L concern

Table 2. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring or reproductive LOAELs based solely on the F_2 generation (F_2 only)

Chemical	P effects	F_1 effects	F_2 effects	F_2 triggered?
11. Trichlorfon	**P LOAEL @ LDT** LDT (P and F1 adults): -↓plasma (24%) and brain (12%) ChE MDT: -↓plasma (30-47%), rbc (27%), brain ChE (38%) HDT: -↓plasma (40-75%), rbc (17-25%m, 20-43%f), brain (12%)m, 59%) ChE -chronic pneumonia	**F_1 Offspring LOAEL @ HDT** HDT: -↓pup bw on days 7 (10%), 14 (18%) and 21 (26%)* -dilated renal pelvis -↓birth index (9%^{ns}) Also Repro LOEL: -↓Litter size (7%^{ns})	**Offspring F_2 LOAEL @ HDT** HDT: -↓pup bw on days 14 (12%) and 21 (19%)* -↓birth index (13%)* -dilated renal pelvis **Repro F_2 LOAEL @ HDT** -↓Litter size (20%)*	**Trigger** P LOAEL< F_2 LOAEL No C&L concern
12. TCMTB	**No P LOEL**	**No F_1 Repro LOEL** **ToxRefDB indicated no F_1 Offspring LOEL** **However, study review indicates:** HDT: -↓ pup wt Day 21 (9-10%)	**No F_2 Repro LOEL** **F_2 Offspring LOAEL HDT** HDT: -↓ pup wt. Day 21 (10%*)	Trigger (pup wt) ToxRefDB suggests F_2 only Current policy suggests F_1= F_2 No C&L concern
Offspring or Repro F_2 only, no triggers, but P LOAEL ≤ F_2 LOAEL				
13. 2-phenyl phenol	**P LOAEL @ MDT** (140 MKD) MDT: -↓bw (7.5%) gestation -↓bw (8%) pre-mating -↑Rel. Kidney wts (11.5%) -Urinary calculus & cellular alteration HDT (490 MKD): -Urinary calculus & cellular alteration -ovarian cysts, not examined in all females, not* in those examined., but ↑ovary wts.	**No Repro LOAEL** **No F_1 Offspring LOAEL** but, HDT: ↓pup wts (8%) Day21^{ns}	**F_2 LOAEL @ HDT** HDT: -↓pup wt. (6%*) Day 14, -↓pup wt. (12%**) Day 21	No trigger P LOAEL < F_2 LOAEL No C&L concern if use P ovary wts. Current policy F_1= F_2
14. Carfen trazone-ethyl	**P LOAEL @ HDT** (343/387 MKD) HDT: -↑Liver wt. (6-17%* - liver histo F_o & F_1, -↓bw gain M (9-13%** pre-mating, -M hematology changes; -bile duct hyperplasia	**No Repro LOAEL** **No F_1 Offspring LOAEL** but, MID: -↓epididymides 7&9%** HDT: -9-11% ↓pup wt M&F -↓ F_1 adult M bw 9-10%** - ↑liver wt F 13%*; - ↓ab. testes wts - ↓epididymides 10%*; considered not treatment related due to high control wts in F_1, similar to F_0	**F_2 LOAEL @ HDT** HDT: -↓Pup wt. (12-15%** LD 7, 14, 21)	No trigger P LOAEL= F_2 LOAEL Current policy F_1= F_2 No C&L concerns

Table 2. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring or reproductive LOAELs based solely on the F_2 generation (F_2 only)

Chemical	P effects	F_1 effects	F_2 effects	F_2 triggered?
		controls		
15. Clomazone	**P LOAEL @ 3rd dose** (100MKD) 3rd: Females: -↓bw F_0 weeks 4-8 pre-mating (6-9% *) - ↓bw gain pre-mating (26%**); gestation (8%*) and lactation (8-6%*); 4thFemales: (200 MKD): -↓bw F_0 weeks 2-8 pre-mating (6-9%*) & ↓bw gain (26%**) gestation 9%* and lactation 10%** ; -↓food (4-20%*)	**No Repro LOAEL** **No F_1 Offspring LOAEL** 3rd Adult females: -↓bw gestation (8%*) & lactation 8%*, -↓food M&F(10-15%*); -Males dilated & distended pelvis of kidney 4th Adult females: -↓bw gestation (11%**) and lactation (9%*); ↓food M&F(4-20%*); %*); -Males dilated & distended pelvis of kidney -No pup wt. changes	**F_2 LOAEL @ 3rd dose** 3rd: -↓pup wt. M&F (3.4%, 4.0% PND21ns) 4th: -↓pup wt. M&F (both 8%* PND21)	No trigger P LOAEL= F_2 LOAEL C&L concern
16. Cyhalothrin	**P LOAEL @ HDT** HDT: -↓bw/bwg during premating and gestation (8%*); --↓bw/bwg F_1 adult (7-9%* F_1 male; 8-9%** F_1 females)	**Offspring LOAEL @ HDT** HDT: -↓pup bw/bwg F_{1A}: 1-14%ns	**Repro LEL @ HDT** HDT: -↓litter size (F_{2A}) (20%*))	No trigger C&L concern P LOAEL = F_1 LOAEL = F_2 LOAEL
17. Endosulfan	**P LOEL @ MDT** MDT: -↑liver wt (8%)** female F_{1B} [no histopath] -↑heart (5%) P male HDT: -↓bwg wks 0-1 (67% of controls)*** females -↓bwg wks 0-4 (91% of controls)* females -↑kidney (11%)**, liver (7%)*, heart (7%)** wt: P male -↑brain (2%)*, liver (10%)** wts P female -↑kidney wt (12%)** F_{1B} male -↑liver wt (12%)***, F_{1B} female	**F_1 Offspring LOEL @ HDT** HDT: -↑pituitary wt (28%) F_{1A} [no histopath)	**F_2 Repro LOEL @ HDT** HDT: -↑uterus wt (21%) F_{2A} [no histopath]	No trigger C&L concern P< F_1 and F_2 Old DER, LELS
18. Fenamidone	**P LOAEL @MDT** (64/84 MKD) Mid: -↓brain wt (7%**) F_1 females HDT (328/460 MKD): -↓bw (4-10%*) -↓bw gains (20-53%**); --↓food con./efficiency (11-15%*)	**No Repro LOAEL** **No F_1 Offspring LOAEL** Adult HDT: -↓bw 7-10%** pre-mating	**F_2 LOAEL @MDT** Mid: -↓pup wt (21% females LD21*, 17% males LD8* when sexes combined); -↓ ab female brain wts (9%**) HDT: -↓ ab. Female brain wts	No trigger P LOAEL = F_2 LOAEL C&L concern

Table 2. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring or reproductive LOAELs based solely on the F_2 generation (F_2 only)

Chemical	P effects	F_1 effects	F_2 effects	F_2 triggered?
			(11%**)	
19. Fenpropathrin	**P LOEL @ MDT** (8.9/10.1 MKD) Mid: -↓bw F (20%) weeks 0-26 HDT (27/32 MKD): -2 F deaths; F tremors (23); -↓bw F (21%) weeks 0-26; -↑thyroid wt (17%**)M -↓liver wt.(9%**) F; no histo	**No F_1 Repro or Offspring LOAEL** but Mid: -↓ adult bw gain 8-10%^{ns} wks 4-13 HDT: -↓pup wt. (5% PND12^{ns}) -↓pup wt. (6% PND21^{ns} -↓litter size Day 0 (12.1 vs. 13.2, 8%^{ns}) -litter size Day 21 (7.5 vs. 7.4 controls)	**F_2 Repro & Offspring LOAEL @ HDT** HDT: -↓pup wt 9% PND8^{ns} -↓pup wt 8% PND12^{ns} -↓pup wt 9% PND21* -↓litter size(7.3 vs. 7.9; 7%* Day21)	No trigger P LOEL < F_2 LOAEL Current policy F_1= F_2 C&L concern
20. Fluroxypyr	**P LOAEL @ HDT** (750/1000 MKD) HDT: -↓bw (6-9%)/gain(7%), -3 deaths due to renal failure	**No Repro LOAEL** **No F_1 Offspring LOAEL** but HDT: -↓pup wt. 9-12%^{ns} Day1-4	**F_2 LOAEL @ HDT** HDT: -↓pup wt.(8-15%*), -↓**viability** (10.4 vs. 12 Day 1); *not performed	No trigger P LOAEL= F_2 LOAEL Current policy F_1= F_2 C&L concern Unique F_2
21. Foremetanate-HCL	**P LOAEL @ HDT** (22.75 MKD) HDT: -↓bw gain LD1-21 (52%**) -↓brain ChE 22%** -↓whole blood ChE 22%**	**No Repro LOAEL** **No F_1 Offspring LOAEL** but HDT: -↓ viability index LD4 (92 vs. 95 controls) LD21 (90 vs. 93 controls); -↓ litter size LD1 (12.6 vs. 14.4 controls)	**F_2 LOAEL @ HDT** HDT: -↓ viability index LD4 (83 vs. 89 controls) -↓ viability index LD21 (74 vs. 87 controls) , -↓pup wt. LD1 (3.4%**)	No trigger P LOAEL= F_2 LOAEL C&L concern
22. Oryzalin	**P LOAEL @ HDT** (125/149 MKD) HDT: -urogenital staining all doses	**No Repro LOAEL** **No F_1 Offspring LOAEL** but F_1 adults HDT: -↓bw/gain 8-10% males ^{ns} 8-15% females ^{ns} F_1 pup wt HDT: -PND7: ↓12%^{ns} -PND14: ↓9%^{ns} -PND21: ↓8%^{NS} Pup wt not considered since no *.	**F_2 LOAEL @ HDT** ↓pup wt. F_2 pup wt HDT: -PND7: ↓10% -PND14: ↓9% -PND21: ↓12%*	No trigger P LOAEL= F_2 LOAEL Current policy F_1 = F_2 No C&L concern

Table 2. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring or reproductive LOAELs based solely on the F_2 generation (F_2 only)

Chemical	P effects	F_1 effects	F_2 effects	F_2 triggered?
23. Parathion-methyl	**P LOAEL @ HDT** (2.3 MKD) F_0 females: ↓bw gain -3g vs. 19 g control -ChE not measured	**No F_1 offspring LOAEL** **No Repro LOAEL** Adult HDT: F_1 females: ↓bw gain -7g vs. 19 g control; -↓bw (7-11%) 2 mos. post-weaning -ChE not measured	**F_2 LOAEL @ HDT** ↓viability index: -# dead pups (Day 0-4): 14* vs. 5 control; -7 vs. 4 control litters with dead pups (Day 4); -Litters not standardized Day 4. -ChE not measured. -Also noted many pups from both F_1 and F_2 litters found at necropsy with intestinal worms (impact not known).	No trigger P LOAEL= F_2 LOAEL C&L concern
24. Pendimethalin	**P LOEL @ MDT** MDT: -↓bwg: P1 males (5-7.2%)** wks 5-25 -↓bwg: P1 females (4.7-6.7%)**, wks 5-9 premating -↓bwg: P1 females (33%)**lactation -↓FC: P1 males (6.2-8.9%)** wks 1-9 -↓FC: P1 females (7.7-8.2%)** wks 1-9 -↓FC: P1 females (8.7-10%)** gestation HDT: -↓bwg: P1 males (5-8.6%)** wks 5-25 -↓bwg: P1 females (10-13%)**, wks 5-9 premating -↓bwg: P1 females (15%)** gestation -↓bwg: P1 females (29%)* lactation -↓FC: P1 males (7.1-17.8%)** wks 1-15 -↓FC: P1 females (13.2-14.4%)** wks 1-9 -↓FC: P1 females (13.5-17%)** gestation	**F_1 LOEL @ MDT** MDT: -↓pup bw F_{1A} (7-11%)** lactation days 7, 14, 21 HDT: -↓pup bw F_{1A} (10-20%) lactation days 7, 14, 21 **No repro LOEL**	**Offspring F_2 LOEL @ MDT** MDT: -↓pup bw F_{2A} (8-9%) *,**, lactation days 14, 21 HDT: -↓pup bw F_{2A} (11-20%)** lactation days 7, 14, 21 **Repro F_2 LOEL @ HDT** HDT: -↓litter size F_{2A} (21%)	No trigger C&L concern P= F_1= F_2 offspring P< F_2 repro
25. Picolinafen	**P LOAEL @ MDT** (19/22 MKD) Hemolytic anemia, splenic hemosiderosis, extramedullary hematopoiesis HDT (39/44 MKD): Hemolytic anemia, ↑spleen wt, splenic hemosiderosis, extramedullary hematopoiesis,	**F_1 adult @ MDT** Hemolytic anemia, splenic hemosiderosis, extramedullary hematopoiesis, ↑spleen wt **F_1 pups not subjected to hematology evaluations** **No Repro LOAEL**	**F_2 LOAEL @ MDT** MDT: slight hemolytic anemia (↓RBC counts 8-10%, ↓HGB 6-9%, ↓HCT 7-9%), PND21 HDT: anemia (↓RBC counts 13-17%**, ↓HGB 8-9%, ↓HCT 9-13%*), PND21	No trigger P LOAEL = F_2 LOAEL F_1 pups not evaluated like F_2 pups No C&L concern

Table 2. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring or reproductive LOAELs based solely on the F_2 generation (F_2 only)

Chemical	P effects	F_1 effects	F_2 effects	F_2 triggered?
26. Propazine	**P LOAEL @ HDT** (50 MKD) HDT: -↓bw M (12-18%) -↓bw F (7-8%**)	**No Repro LOAEL** **No F_1 Offspring LOAEL** but HDT: -↓bw adult M (12%*) -↓bw adult F (7-10%**) -↓pup wt. (3.4-4.5% PND21)^{ns}	**F_2 LOAEL @ HDT** HDT: -↓pup wt. (11-12%* PND21)	No trigger P LOAEL= F_2 LOAEL C&L concern
27. Propiconazole	**P LOAEL @ MDT** MDT (48/52 MKD): ↑liver hypertrophy (cell swelling 19/45 vs 11/45 controls) and clear cell change (4/45 vs 1/45) HDT (238/263 MKD): -↓ bw (6-18%)$^{*\,\&\,**}$, P and F_1 females -↓bwg (15-23%)** pre-mating, gestation, lactation for P and F_1 females -↓FC (12-17%) -liver hypertrophy (43/45** vs 11/45 cellular swelling; 15/45** vs 1/45 clear cell change)	**F_1 Offspring LOAEL @ HDT** HDT**:** -↓pup bw (9%, 16%, 22%, 25%**, PND 4, 7,14, 21) **F_1 Adult @ MDT** MDT: ↑liver hypertrophy (cell swelling 20/45** vs 0/45 controls) and clear cell change (15/45* vs 4/45) HDT: -↑liver hypertrophy (cell swelling 44/45** vs 0/45 controls) and clear cell change (21/45** vs 4/45)	**F_2 Offspring LOAEL @HDT** HDT: -↓pup bw (12%, 21%, 22%,2 8%*, PND 4, 7,14, 21) -↓live birth index (26%)** -↓viability (26%**) -↓Lactation index (15%*-21%*) **F_2 Repro LEL @ HDT** HDT: -↓litter size (22%)* F_{2A}	No trigger P < F_2 LOAEL C&L concern
28. Rimsulfuron	**P LOAEL @ HDT** (830M/1021F MKD) HDT: -↓bw gain (M 13%* Day0-7; F 10%* Day 0-70), -↓food (F 10%* GD0-14)	**No Repro LOAEL but Repro LEL @ HDT** -↑rel. testes wt (11%*) **No F_1 Offspring LOAEL** but Offspring HDT: - F_1 pup wt ↓3.3% PND14 Adult HDT: -↓ adult bw gain (M 14%* Day0-105; -↓food efficiency (M 6%* Day 0-105; F 12%*Day 0-7)	**F_2 LOAEL @ HDT** HDT: -↓pup wt PND7 8%* -↓ pup wt. PND14 5.5%* -↓pup size	No trigger P LOAEL = F_2 LOAEL C&L concern
29. Tribenuron-methyl	**P LOAEL @ MDT** (19/21 MKD) F_0 Mid: -↓bw gain M&F pre-mating (7%* & 7%); F_0 HDT (75/88 MKD): -↓bw gain M & F pre-mating (19%* & 29%*); -↓bw gain M mating 23.5%*	**No Repro LOAEL** **No Offspring LOAEL** but Mid: -F_1 adult ↓bw gain F pre-mating (13*); HDT: -↓pup wt. 9% PND21* -↓bw gain M & F pre-mating (16%* & 25%*) & M mating (17.5%*)	**F_2 LOAEL @ HDT** HDT: -↓pup wt. 10% PND21*	No trigger P LOAEL < F_2 LOAEL Current policy F_1= F_2 No C&L concern

2. $F_1 \geq F_2$ LOEL/LOAEL Comparison

Only 21 studies of the 341 pesticide studies (6%) demonstrated a quantitatively more sensitive LOAEL/LOEL in the F_2 generation compared to the F_1 generation. This indicates that the effects occurring in the second generation occurred at a lower dose than effects in the F_1 generation. These 21 studies were further evaluated to determine if potential triggers (as identified by Cooper et al.) would be identified in the F_1 or P generation such that an F_2 generation would have been produced.

Triggers were identified for 13 of the 21 studies. Consequently, for these chemicals the F_1 generation would have been mated to produce an F_2 generation. Further examination of these 13 studies with triggers revealed that 4 of the 13 studies had F_2 LOAELs that were lower (more sensitive) than the parental generation. This indicates that if the triggers were not applied 4 of the 13 studies would have been missed quantitatively for risk assessment.

The remaining 8 studies of the 21 that did not have a trigger were further evaluated to determine if the parental LOAEL would protect for the F_2 LOAEL or LOEL. The parental comparison resulted in only two studies that had a parental LOAEL that was higher - and therefore less protective - than the F_2 LOAEL (carbaryl and fenbutatin oxide). First, carbaryl is an n-methyl carbamate (NMC), in which cholinesterase inhibition is the most sensitive endpoint. Therefore, the doses used in the multi-generation study are greater than doses used for risk assessment since cholinesterase inhibition was not assessed in the study. The carbaryl reproductive study was not used for the Agency's risk assessment. For fenbutatin oxide, there was pup weight information in the F_1 generation (46 MKD) that may have been useful for classification and labeling purposes. The point of departure from this study – based on the F_2 pup weight decrements (15 MKD) – was used in the fenbutatin oxide risk assessment. <u>This was only the second example out of 350 studies in which the F_2 generation was lower than the F_1 or P generation (15 MKD F_2 LOAEL vs 30 MKD Parental LOAEL) and used for risk assessment</u>.

As for C&L concerns, there were only 2 studies out of the 21 that identified F_1 or F_2 information that may be important (fenbutatin oxide and acifluralin) for labeling. For acifluralin, decrease pup weight and viability were identified in the mid and high-dose of the second generation whereas only pup weight was identified at the high-dose in the first generation. If the F_1 pup weight was used for C&L then the F_2 would not have been missed. For fenbutatin oxide, decrease pup weight gain occurred at a lower dose in the F_2 compared to the F_1. Thus, the F_1 pup weight may have been used for classification and labeling.

The 21 studies with an F_2 generation more sensitive is provided in Table 3. It should be noted that the green highlights in Table 3 indicate the dose level and endpoints that served as the basis for the determination of increased F_2 sensitivity or F_2 unique effect.

Table 3. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL > F_2 LOAELs

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
F_1 LOAEL > F_2 LOAEL, no trigger, P LOAEL > F_2 LOAEL				
1. Carbaryl	**P LOAEL @ HDT** (94/111 MKD) HDT: -↓bw Day 0-70 (4-5%**) -↓bw gain Day 0-7 27%**females; Day 0-70 21%** males -↓bw GD 0-20 (6%**) -↓bw LD 0-21 (4-7%**) -↓ food consumption (8-9%* Day 14-35	**No F_1 Repro LOAEL** but HDT: -wt adjusted PPS 44.2 vs 41.4*** -wt adjusted VO 33.0 vs 30.3 -↓ ab epididymides wt. (5%*) **F_1 Offspring LOAEL @ HDT** HDT: -↓pup wt. (11-15%**) F_1 Adults HDT: -↓bw Day 0-70 (8-21%***) -↓bw gain Day0-7 (16%**males, 9%* females) -↓bw GD 0-20 (8-11%*) -↓bw LD 0-21 (7-14%**) -↓food consumption (9-10** Day 0-70)	**F_2 Repro LOAEL @ MDT** (31/37 MKD) MDT: -↓litter size Day 4 (12.7 vs 15.4) HDT: -↓litter size Day 4 (12.5 vs15.4) **F_2 Offspring LOAEL @ MDT** MDT: -↓viability index (92 vs 98.3) -↓lactation index (94.4 vs 98.6) HDT: -↓viability index(88.9 vs98.3) -↓lactation index (90 vs 98.6) -↓pup wt (9-14%**)	No Trigger P LOAEL > F_2 LOAEL F_1 C&L info No C&L concern
2. Fenbutatin oxide	**P LOAEL @ 4th dose** (30/34 MKD) 4th dose: -↓bw Day 0-119 (818%*) -↓bw gain Day 0-119 (25-35%) -↓bw GD 0-21 (6-12%*) -↓bw LD 0-21 (16%*) -↓food consumption Day 0-70 (12-13%*) -↓food efficiency Day 0-70 (25-32%*)	**No Repro LOAEL** **F_1 Offspring LOAEL @ 4th Dose** 4th dose Offspring: -↓pup wt. Day 4-21 (6-24%*) -↓pup wt. gain Day 0-21 (28%ns) 4th dose Adults: -↓bw Day 0-105 (21-23%*) -↓bw gain Day 0-105 (20-23%) -↓bw GD 0-21 (9-10%*) -↓bw LD 0-21 (17-22%*) -↓food consumption (12-20%*) -↓food efficiency (Day 0-105 (11-12%*)	**F_2 LOAEL @ 3rd dose** (15/17 MKD) 3rd dose: -↓pup wt. Day 14-21 (9-10%*) -↓pup wt. gain Day 0-21 (11%ns) 4th dose: -↓pup wt. Day 4-21 (13-29%*) -↓pup wt. gain Day 0-21 (33%ns)	**No Trigger** P LOAEL > F_2 LOAEL Miss for RA Potential C&L concern
F_1 LOAEL > F_2 LOAEL, no trigger, P LOAEL ≤ F_2 LOAEL				
3. Acifluralin	**P LOAEL @ MDT** (50 MKD) Mid:	**No Repro LOAEL** **F_1 LOAEL @ HDT** HDT:	**F_2 LOAEL @ MDT** Mid: -↓**Viability** PND4 96.6 vs	No trigger P LOAEL = F_2 LOAEL Potential C&L concern F_2 unique (viability)

Table 3. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL > F_2 LOAELs

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
	-↓ bw gain Females 36%** GD6-10 -kidney dilatation HDT (250 MKD): -↓ bw (4-7%*) -kidney pelvis dilatation	-↓pup wt. (6-19%) birth, LDs 7, 14, 21. -didn't look at kidney histo in HDT (no lesions in the low and mid-doses) Adult HDT: -↓ bw (10-25%*) -kidney pelvis dilatation -kidney hydronephrosis	98.8 controls -Kidney pelvis dilatation HDT: -↓Pup wt. (11-26%) birth, LDs 7, 14, 21. -↓**Viability** PND4 96.3 vs 98.8 controls -Kidney pelvis dilatation	
4. Diethyl-hexyl phthalate	**P LELs:** 6th Dose (391 MKD): -↑ab liver (43% males, 27% females) -↑rel. liver (45% M; 36% F) -↑rel kidney(14% M; 12% F) -liver hypertrophy (10/10 males, 9/10 females) 7th Dose (543 MKD): -↓bw (6% M; 12% F) -↑ ab. liver (47% M; 36% F) -↑rel liver (55% M, 53% F) -↑ab kidney (11% M) -↑rel kidney (17% M; 15% F) -↓ ab right cauda epididymis (19%) -↓ ab right epididymis (16%) -↓right testis (23%) -↑adrenal vacuolation (6/10 males vs 1/10) -liver hypertrophy (9/10 males, 10/10 females) -epididymis histopath (4/10 males) -↓ sperm velocity (11.3%) -↓# spermatids (30.6%) -↑days to deliver	**F_1 Adult LELs:** 5th Dose (48 MKD): -↑ab liver (15% males) -↑rel. liver (8% M) -↑rel kidney (38% M) 6th Dose: -↑ab & rel kidney (25%/33% M; 29%/39%F) -↓ab. ventral prostate (28%) -↓ab/rel epididymis (35%/30%) -↓ab/rel right testis (51%/47%) 7th Dose: -↓bw (8% males, 11% F) -↓bw (PND4-21 10-20% F) -bw mating (16-19%) -↑ab/rel liver (14%/43% M; -/38%F) -↓ab ventral prostate (32%) -↓ab/rel right epididymis (54%/42%) -↓ab/rel right cauda epididymis (44%/34%) -↑ab/rel pituitary (19%/48%M) -↑rel kidney (18%M) -↑rel adrenal (31%M) **F_1 Offspring LELs:** 6th Dose: -↓ # live male pups (20%) -↓ male AGD (7%) -delayed VO (3 days) -delayed testicular descent (3 days) -delayed PPS (3.5days) 7th Dose: -↓ # live male pups (26%) -↓ # live pups (21%) -↓ pup wt (8-13%) -↓ male AGD (16%) -delayed VO (8days) -delayed testicular descent (6 days) -delayed PPS (11days) **F_1 Repro LELs:** 7th Dose:	**F_2 Offspring LELs:** 6th Dose: -↓viability (19-20% during lactation) -↓live birth (9%) -↓male AGD (13-18%) -↓pup wt. (11%) -delayed VO (6 days) -delayed testicular descent (3 days) -delayed PPS (7 days) 7th Dose: -no litters/pups **F_2 Repro LELs** 6th Dose:	No Trigger P LEL = F_2 LEL No C&L concerns

Table 3. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL > F_2 LOAELs

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
		-no litters -↑cycle length (4.4 days vs 4.0 days)	-AGD -↓ litter size	
5. Diphenylamine	**P LOAEL @ LDT** (40/46 MKD) LDT: -spleen congestion, discolored, hemosiderosis -liver hypertrophy MDT (115/131 MKD): -↓bw gain Female pre-mating 0-20 (17% *not performed) -spleen congestion, discolored, hemosiderosis -liver hypertrophy HDT (399/448 MKD): -↓bw gain pre-mating 1-20 (13-35%*not performed) -↓bw gain GD 0-20 (21-32%**) -swelling of mammary (13 vs. 0) -spleen congestion, discolored, hemosiderosis -liver hypertrophy	**F_1 Adult LOAEL HDT** Adult HDT: -↓ adult bw gain pre-mating (12% *not performed) -↓bw gain GD 0-20 (21-41%**) -swelling of mammary (15 vs. 0) **F_1 Offspring LOAEL HDT**: -↓ pup wt LD 4-21 (11-25%**) **F_1 Repro LOAEL HDT**: -↓ litter size Day 0 (12.4 ns vs. 13.8)	**F_2 Offspring LOAEL MDT** MDT: -↓ pup wt LD14 (10%**); LD21 (12%**) HDT: -↓ pup wt LD 4-21 (10-29%**) **F_2 Repro LOAEL HDT** -↓ litter size Day 0 (11.1** vs. 14)	No Trigger P LOAEL < F_2 LOAEL No C&L concern
6. Paclobutrazol	**No P LOAEL** But Mid (12.5 MK): -Chromodacryorrhea (2 vs. 1) -thickened eyelids (2 vs. 1) -dental malocclusion (0 vs. 1) HDT (62.5 MKD): -Chromodacryorrhea (2 vs. 1) -thickened eyelids (1 vs. 1) -dental malocclusion (1 vs. 1) -↓bw gain pre-mating (4-5%*) -↓food (4-6%*)	**No Repro LOAEL** But HDT: -↓litter size (9-17%**) **F_1 Offspring LOAEL HDT:** -↑liver wt. (19-20%*); histo -pups with chromodacryorrhea (17 vs 4), thickened eyelids (18 vs 4), dental malocclusion (9 vs 0) F_1 Adults Mid (12.5 MK): -Chromodacryorrhea (5 vs. 0) -thickened eyelids (4 vs. 2) -dental malocclusion (2 vs. 0) Adults HDT (62.5 MKD): -Chromodacryorrhea (10 vs. 0) -thickened eyelids (9 vs. 2) -dental malocclusion (1 vs. 0) *These adult effects were stated to be within historical values, but historical control #s not provided. -↓bw gain pre-mating (4-	**No F_2 Repro LOAEL** **F_2 Offspring LOAEL MDT** Mid: -pups with chromodacryorrhea (7 vs 0), thickened eyelids (4 vs 0), dental malocclusion (0 vs 0) HDT: -↓pup wt. gain (10-12%*) -↑liver wt. (11-12%*) & histo -pups with chromodacryorrhea (8 vs 0), thickened eyelids (8 vs 0), dental malocclusion (5 vs 0)	No Trigger P LOAEL = F_2 LOAEL No C&L concern

Table 3. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL > F_2 LOAELs

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
		9%*) -↓food (5-11%*)		
7. Prometon	**P LOAEL @ MDT** (35/39 MKD) Mid: ↓bw 7%** males week 9-14 HDT: -↓bw 6-16%** weeks 3-14 -↑rel testes wt (18%*)	**No Repro LOAEL** But LELs: HDT: -↑rel testes wt (14%*) -↑rel ovary wt (33%**) -↓bw12-18%** weeks3-14 **F_1 Offspring LOAEL @ HDT** HDT: -↓pup wt. 5-10%** PND0-21	**No F_2 Repro LOAEL** **F_2 Offspring LOAEL MDT** Mid: -↓pup wt. 3-8%** PND0-21 HDT: -↓pup wt. 7-14%** PND0-21	No Trigger P LOAEL = F_2 LOAEL No C&L concern
8. Metribuzin	**P LOAEL @ MDT** (7.5 MKD) MDT: -↓bw gain lactation -↑liver hypertrophy HDT: -↓bw gain (11*%-35%**) -↓food consumption -liver hypertrophy	**No Repro LOAEL** **F_1 Offspring LOAEL HDT** (37.5 MKD) HDT: -↓pup wt. PND21 (8%*) -↓ bw gain (9%*-22%**) -↓bw gain gestation (11%) -↑gamma glutamyl transferase (females only, 98%*) Mid Adult: -↓bw gain gestation (14%**) -↑liver hypertrophy -↑gamma glutamyl transferase (females only, 70%*)	**No F_2 Repro LOAEL** **F_2 Offspring LOAEL @ MDT** Mid: -↓pup wt. PND14 (4.7%*) -↓pup wt. PND 21 (5.9%*) HDT: -↓pup wt. PND14 (5.4%*) -↓pup wt. PND 21 (10.5%*)	No trigger P LOAEL = F_2 LOAEL No C&L concern No F_2 unique
F_1 LOAEL > F_2 LOAEL with trigger				
9. Biternanol	**P LOAEL MDT (females)** MDT (5 MKD): -↓bw females (8-9% weeks 9-14) -↓bw gain females (13%** weeks 9-11) HDT (25 MKD): -↓bw gain males (11-55%**; 2-3 weeks pre-mating) -↓bw gain females (19-56%** premating) -↓fertility index (30%) considered unrelated to treatment	**No Repro LOAEL but F_1 Repro LEL:** HDT: -↓**litter size** (LD0 9%*, LD5, pre-cull 27%*) **F_1 Offspring LEL:** HDT: -↓pup wt. LDo-28 (13-25%**) -↓pup wt. gain (LD0-28 22%-30%) -↓**viability index** (82.5 vs 90.7*) **F_1 Adult** MDT females: -↓bw week 5-14 (7-9%*) HDT: -↓bw gain males (17-	**No F_2 Repro** **F_2 Offspring MDT** MDT: -↓pup wt. gain (LD0-5: 40%*; LD0-28: 10%) -↓pup wt. (LD0-28: 7-22%*) HDT: -↓pup wt. gain (LD0-28: 20%) -↓pup wt. (LD0-28: 14-22%*) -↓viability index (62.8* vs 91.6) -↓lactation index (90.3 vs 97.9*)	Trigger P LOAEL = F_2 LOAEL No C&L concern

Table 3. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL > F_2 LOAELs

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
		19%** pre-mating) -↓bw gain females (25%** premating)		
10. Boscalid	**P LOAEL HDT** HDT (1035/1062 MKD): -liver degeneration -↓bw gain GD0-20 (11%*) -↓ **implantations** (15.2* vs 17.4) -**post-implantation loss** (18.6%^{ns} vs 12.8%)	**F_1 Repro LEL:** HDT: -↓litter size (12.5 vs 13.8) -post-implantation loss (15.4%* vs 7.0%) **F_1 Offspring LEL:** HDT: -↓pup wt. (7%*) Day 21 -↓pup wt. gain Day1-21 (7-11%**) -↑ rel. brain wt. (7%**) **F_1 adult LOAEL HDT** HDT: -↓bw (males 6-8%*) -↓bw gain (males 9%*) -liver degeneration	**No F_2 Repro LEL** **F_2 Offspring LOAEL MDT** (101/107 MKD) MDT: -↓pup wt. gain Day 14-21 (8%*) -↓ab. spleen wt. (12%*) -↑ rel. brain wt. (7%*) HDT: -↑pup wt. Day 14-21 (11-13%**) -↓pup wt. gain Day 4-21 (12-17%**) -↑viability (86%** vs 93%) -↑rel. brain wt. (13%**) -↓rel spleen wt.(13%**) -↓ab. thymus wt (15%**)	Triggers P LOAEL > F_2 LOAEL No C&L concern
11. Cycloate	**P LOAEL MDT** (20 MKD) MDT: -↓bw gain (females: Days 0-56 21%, Days 0-119 23%) -spinal cord degeneration -↓ food HDT (50 MKD): -↓bw gain (males: Days 0-56 12%, Days 0-119 14%; females: Days 0-56 32%, Days 0-119 22%) -↓ RBC ChE (15% males, 18% females)	**F_1 Repro LEL HDT** HDT: -↓**litter size** (17-22%**) **F_1 Offspring LELs HDT** HDT: -↓pup wt. (Day 4-21, 11%-19%**) -↓pup wt. gain (males: Days 0-56 22%, Days 0-119 22%; females: Days 1-56 30%, Days 1-119 20%) -↓**viability** (84.5% vs 96.4%) But further review at MDT: -↓pup wt. (Day 21, 2-6%^{ns}) -↓pup wt. gain (females: Days 26-56 10%, Days 26-119 10%)	**F_2 Repro LEL HDT** HDT: -↓litter size (32-41%**) **F_2 Offspring LOAEL MDT** MDT: -↓pup wt. (Day 4-21, 17-29%**) HDT: -↓pup wt. (Day 4-21, 29-40%**) -↓viability (38.3% vs 96.9%**) -↓lactation (Day 4-21 56%-58%**)	Triggers P LOAEL = F_2 LOAEL No C&L concern ToxRefDB F_1> F_2 Current policy F_1= F_2
12. Cyfluthrin	HDT: -splayed hindlimbs; F during lactation (15/30** vs 0/30 controls)	**F_1 Repro LOAEL MDT** (9/10 MKD) MDT: -↓**litter weight** (6-7%*) HDT: -↓**litter weight** (9-20%**) **Offspring LOAEL MDT** MDT: -tremors (16%; 4/25) LD 5-17 -↓pup wt (6-14%) PND4-21 HDT:	**F_2 Repro LOAEL MDT** but LEL @ LDT (3/4MKD): -↓litter weight (5-10%*) not considered adverse, w/in historical controls MDT: -↓litter weight (8-14%**) HDT: -↓litter weight (6-26%**) **F_2 Offspring LOAEL @MDT** MDT: -tremors (73%; 19/26) LD 5-	Trigger P LOAEL = F_2 LOAEL No C&L concern Current policy F_1= F_2

Table 3. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL > F_2 LOAELs

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
		-tremors (54%; 15/28) LD5-17 **P LOAEL @ MDT** Adult MDT: -↓bw (6-8%*) -↓food consumption (9-11%**) -splayed hindlimbs F during lactation (9/30** vs 0/30 controls)	17 -↓pup wt (6-14%) PND4-21 HDT: -tremors (36%; 9/25) LD5-17	
13. Diacamba	**P LOAEL HDT** HDT (419/450 MKD): -↑rel. liver wt (13%**)	**F_1 Repro LEL** HDT: -↓PPS (45.6 vs 43.7) **F_1 Offspring LEL HDT**: -↓ **pup wt.** (Day 0 6%*; Day 21 24%**) **F_1 LOAEL HDT** HDT (419/450 MKD): -↑rel. liver wt (11%*) -↓righting ability/stiffness	**No F_2 Repro** **F_2 Offspring LOAEL MDT (122/136 MKD)** Mid Dose: -↓pup wt. (Day 21 only 10%*) HDT: -↓pup wt. (Day 0 7.5%*, Day 21 26%**)	Trigger (pup wt.) P LOAEL > F_2 LOAEL No C&L concern
14. Dicofol	**P LOAEL 2nd Dose** 2nd Dose (2 MKD): Liver vacuolization (10 vs 2) Liver hypertrophy (34 vs 0) 3rd Dose (10 MKD): Liver vacuolization (14 vs2) Liver hypertrophy (46 vs 0) -↓food 4th Dose (20 MKD): Liver vacuolization (10 vs 2) Liver hypertrophy (49 vs 0) -↓food -↓bw -↓bw gain -↑ovary vacuolization (20 vs 1*)	**F_1 Repro LOAEL 2nd Dose** 2nd Dose: -↑ovary vacuolization (6/25 vs 0) 3rd Dose: -↑ovary vacuolization (2/25 vs 0/25) 4th Dose: -↑ovary vacuolization (20/25* vs 0/25) **F_1 Offspring LEL** 3rd Dose: -↓pup wt. (3-6%ns) 4th Dose: **Dead fetuses** (Day 0: 15* vs 0) -↓ pup wt. (6-13%*) -↓viability (91% vs 97%) **F_1 Adult LELs:** 2nd Dose: -liver hypertrophy (23/25 vs 0) 3rd Dose: -liver/adrenal hypertrophy (48/50 vs 0) 4th Dose: -liver/adrenal hypertrophy (50/50 vs 0)	**No F_2 Repro LEL** **F_2 Offspring LOAEL 3rd Dose** 3rd Dose: -↓viability (76%* vs 100%) 4th Dose: -↓viability (78%* vs 100%) -↓pup wt. (16-48%*) -dead fetuses (54 vs 7)	Trigger P LOAEL < F_2 LOAEL No C&L concern

Table 3. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL > F_2 LOAELs

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
15. Dicrotophos	**P LOAEL MDT (0.5 MKD)** Mid: -↓bw (2-3%*) -↓food efficiency (2-4%*) HDT (1.15/1.25 MKD): -↓bw (4-16%**) -↓food efficiency (4-8%) **-↓ Fertility** (12/36** vs 21/30 successful matings)	**F_1 Repro LEL HDT** HDT: -↓litter size **-litter loss** (8* vs 4 control) **Offspring LEL HDT** HDT: -↓live birth (111* vs 237) -**↓lactation** (57** vs 88) **F_1 Adult** Mid: -↓bw (11%*, Week1) -↓food efficiency (4%*) HDT: -↓bw (12%* Week1) -↓food efficiency (7%**)	**Repro LOAEL F_2 MDT** Mid: -litter size HDT: -Litter loss (6* vs 0) **F_2 Offspring LOAEL MDT** Mid: -↓lactation (84* vs 97) HDT: -↓lactation (76** vs 97)	Trigger P LOAEL= F_2 LOAEL No C&L concerns
16. Iodomethane	**P LOAEL HDT** HDT (50 ppm): -↓ bw/gain -adrenal -testis -cauda epididymis -thymus	**F_1 Repro LOAEL MDT** MDT (20 ppm): -delay VO (2 days) -↓ **litter size** HDT: -↓litter size (12%) -delay VO (3 days) **F_1 Offspring LOAEL HDT**: -↓pup wt (PND14, 6-10%) -↓**live birth index (90.5% vs 97%)** -↓**viability** -no milk in stomach (37/77 vs 8/30) -↓thymus (13-17%) -↓brain, spleen, thymus (8-27%)	**F_2 Repro LOAEL HDT** HDT: -↑primordial follicles (19%) -estrous cyclicity/corpora lutea (20-23%) -↓litter size (23%) **F_2 Offspring LOAEL MDT** MDT: -↓pup wt. (PND14 10-14%) -↓thymus (19-25%) HDT: -↓viability (8601% vs 95.7%) -↓pup wt gain (PND7 8-20%) -↓live birth (82.6% vs 98%) -no milk in stomach (23/32 vs 9/15) -↓thymus (24-28%) -↓spleen (23-28%), brain (6%M)	Triggers P LOAEL > F_2 Offspring No C&L concerns
17. Monosodium methane arsenate	**P LOAEL MDT** MDT (17/22.5MKD): -↓bw gain (M: 10%) -↑food (4-6%*) HDT: -↓bw gain (M: 10%) -↑food (12-16%**) **P Repro LOAEL MDT** MDT: -↓mating (14%) -**↓fertility** (4%) HDT: -**↓fertility (8-14%)** -↓mating (7-14%) -↑testes wt. (8%**) (rel. testes only 3% less than control)	**F_1 Repro LOAEL MDT** MDT: -↓fertility index (10-11%) HDT: -↓fertility index (15-22%*) -total litter loss (2 vs 1) -↓prostate (ab. 19%*, rel. 13%) **F_1 Offspring LOEL HDT** HDT: -**↓lactation** (8%: 92.4 vs 100) historical control F_1 pups: 98.7 range: 93.7-100 Also: -#pups dead Day0-21: 35 vs 8 -viability day0-4: 89.9 vs 96.4	**F_2 Repro LOAEL MDT** MDT: -total litter loss (4 vs 1) HDT: -total litter loss (2 vs 1) **F_2 Offspring LOAEL MDT** MDT: -↓lactation (88.1 vs 97.4) Historical control: mean 97.8, range: 92.9-100% -↓litter survival (86.4 vs 96) -#pups dead days 0-21: 35 vs 15 HDT: -↓lactation (91.3 vs 97.4) -↓litter survival (90.9 vs 96%) Also: #pups dead days 0-21: 32 vs 15	Triggers (lactation index, P fertility) P LOAEL = F_2 LOAEL No C&L concerns

Table 3. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL > F_2 LOAELs

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
		F_1 Adult LOAEL MDT MDT: -↑food consumption HDT: -↓bw (M:10%) -↓bw gain (M: 10%) -↑food		
18. Mesotrione	**P LOELs:** 3rd Dose (12MKD): -cloudy/opaque eyes (9/26 vs 0/26) -↓food females lactation (11-17%**) -↑liver wts. (8-13%**) 4th Dose (288/311 MKD): -cloudy/opaque eyes (24/52 vs 0/52) -↓food females lactation (17-28%**) -↑liver wts. (8-17%**)	**F_1 Repro LOEL 4th Dose** 4th Dose: -↓litter size (20-32%*) **F_1 Offspring LOELs:** 3rd Dose: -↓pup wt. (7-10%* PND22-29) -↓litter wt. (13-19%** Day15-29) 4th Dose: -↓live PND22 (33%, 169 vs 251 controls) -↓viability (16%, Day22) -↓litter size Day-29 (20-32%**) -↓ litter wt (19-36%**, Day1-29) **F_1 Adult LOELs:** 3rd Dose: -cloudy/opaque eyes (39/52 vs 0/52) -↓food females lactation (16-37%**) -↑liver wts. (11-18%**) 4th Dose: -cloudy/opaque eyes (51/52 vs 0/52) -↑liver wts. (11%**)	**F_2 Repro LOAEL 2nd Dose** 2nd Dose (1.1 MKD): -↓litter size (23%*, PND22-29) 3rd Dose: -↓litter size (20%*, PND22-29 -↓litter wt. (17-22%*, Day 15-29) 4th Dose: -↓litter size (27-44%**, Day 1-29) -↓litter wt. (32-43%**, Day 1-29) **F_2 Offspring LOEL** 1st Dose (0.3 MKD): -↑tyrosine male pups 4th Dose: -↓live birth (6%**) -↓viability (16%**, Day22) -↓ live pups PND22 (63%, 75 vs 205 controls) -Litter Loss (7/20 vs 1/21 controls) **Systemic LOAEL 1st dose based on F_2 adults** 1st Dose: -↑tyrosine (Males: 569-2478%; Females 289-285%) -↑liver wts. (14%**) 2nd Dose: -↑liver wts. (7-29%**) 3rd Dose: -↑liver wts. (24%**) 4th Dose: -↑liver wts. (27%**)	Triggers P LEL = F_2 & F_3 No C&L concern
19. Pyrasulfotole	**P LOAEL @ MDT** (27/33 MKD) MDT: -↑Spleen wt (F; 9%*) -↑Hematopoietic spleen -↑Liver hypertrophy (2 vs. 0) /cytoplasmic alteration (5 vs. 0) -↑Pituitary eosinophilic inclusions (21 vs. 1) -↑Kidney wt (5-7%*) -↑Kidney nephropathy (8 vs. 1)	**F_1 Repro LOAEL @ MDT** MDT: -↓rearing index 8.1% (87% vs. 95%, outside historical controls) -PPS (44.2 vs. 41**) HDT: -↓insemination index 4.03% (88% vs. 92%, outside historical controls) -↓rearing index 12% (83% vs. 95 %, outside h.) -PPS (46.3 vs. 41**)	**F_2 Repro LOAEL @ LDT** Total litter loss @ all doses **F_2 Offspring LOAEL @ LDT** LDT: -↓viability index (9%) MDT: -↓pup wt. PND 7(8%) -↓viability index (15%)	Triggers (P fertility, pup wt.) P LOAEL > F_2 LOAEL No C&L concern

Table 3. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL > F_2 LOAELs

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
	(Thyroid and eye changes no considered applicable to humans: thyroid wt thyroid colloid, pigmentation, hypertrophy, eye opacity, neovacularization, keratitis, and hyperplasia) HDT (272/346 MKD): **-↓fertility index 8.3% (88% vs. 96%)** -↑Spleen wt (F; 9%) -↑Kidney wt (12*%) -↑Kidney nephropathy (9 vs. 1) -↑ liver wt (4-13%**) -↑Liver hypertrophy (17 vs. 0) /cytoplasmic alteration (17 vs. 0) -↑Pituitary eosinophilic inclusions (15 vs. 1)	-VO (34.2 vs. 33*) **F_1 Offspring LOAEL @ LDT** LDT (2.5/3 MKD): -↓pup wt. (3-4%) MDT: -↓rearing -↓pup wt. (3-6%) HDT: -↓rearing **-↓pup wt. PND28 (6-8%**)** **F_1 Adults** MDT: -↓ prostate (10%) -↑ uterus (11%) -↑Liver hypertrophy (9 vs. 0) /cytoplasmic alteration (15 vs. 0) -↑Kidney nephropathy -↑Pituitary eosinophilic inclusions (12 vs. 2) HDT: -↓epididymis (left) 11.2%** -↓prostate (19.4%**) -↑uterus (11%) -↑Liver hypertrophy (15 vs. 0) /cytoplasmic alteration (19 vs. 0) -↑Kidney nephropathy -↑Pituitary eosinophilic inclusions (15 vs. 2)	HDT: -↓pup wt. PND28 (9-13%**) -No milk in stomach (10% vs. 5%) -autolysis -↓viability index (21%)	
20. Triclopyr	**P LOAEL MDT** MDT (25 MKD): -kidney degeneration HDT (250 MKD): -↓bw (7-15%* pre-mating) -↓bw gain (F:18%*) -kidney degeneration	**No F_1 Repro LOAEL but** HDT: -↓**litter size** Day 21 (5.7* vs 7.9) **F_1 Offspring LOAEL HDT:** -↓pup wt. (Day1-21: 9%-35%*) -↓**viability** (pup deaths Day1-4: 23 vs 12) -viability day21,post-cull: 76.3%* vs 99% -↓**lactation** (pup deaths Days 7-21: 32 vs 0) **F_1 Adult LOAEL MDT** MDT:	**No F_2 Repro LOAEL but** HDT: -↓litter size (Day 21:4.0* vs 7.2) (Day 7:5.4* vs 7.2) (Day 14: 4.4* vs 7.2) **F_1 Offspring LOAEL MDT:** -excencephaly (6 vs 0) -eye ablepharia (2 vs 0) HDT: -↓viability (pup deaths days 1-4:34 vs 16) -↓viability Day 21, post-cull: 69.6** vs 96% -excencephaly (1 vs 0) -eye ablepharia (2 vs 0) -↓lactation (pup deaths days 7-21: 23 vs 0) -↓pup wt. (Day 1-21: 18%*-41%*)	**Triggers** (viability, lactation, litter size) P LOAEL = F_2 LOAEL No C&L concerns

Table 3. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL > F_2 LOAELs

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
		Kidney degeneration HDT: -↓bw pre-mating (M:21-26%*; F:11-15%*) -↓bw GD0-21: 11-14%* -↓bw LD0: 11%* -↓bw gain ( ) -kidney degeneration	(Note: it is assumed no occurrence of malformations in control F_2 pups, control data not provided; total pups examined also was not provided; historical control data was not provided)	
21. Vinclozolin (EPA)	**P LOAEL 2nd Dose (30 MKD)** 2nd Dose: -↑liver wt (F; 110%**) -↓epididymal wts. -3rd Dose (101 MKD): -↑adrenal wts (M; 125%**; F, 130%**) -liver necrosis (3/24) -adrenal lipidosis (19/24) -↑adrenal wt (25%**) -↓epididymal wts. (10%**) -↑ testes/ovary hyperplasia -↑ testes wts (10%**) 4th Dose (290 MKD): -adrenal lipidosis (M&F, 24/24) -↑adrenal wt (111%**) -pituitary vacuole. Males -liver necrosis (24/24) -↓ **Fertility** (71%* vs 96% males; 74%* vs 96% females) -ovary/testes hyperplasia -testes atrophy -↑ testes wts (15%**) -↓epididymal wts. (28%**)	**F_1 Repro LEL 3rd Dose** 2nd Dose: -Fertility (83% vs 96% M&F) 3rd Dose: -hypospadias/hypoplasia -hermaphrodism -penis paraphimosis -ovary hyperplasia/lipidosis -↓ Fertility (0%* vs 96% males; 0* vs 96% females) 4th Dose: -↓epididymal wts. -testes hypoplasia -hypospadias -↓prostate size -↓abs. testes wt. -hermaphroditism -↓coagulating size -penis paraphimosis -ovary hyperplasia/lipidosis -↓ Fertility (0%* vs 96% M&F) **F_1 Offspring LEL 3rd Dose** 3rd Dose: -↓pup wt. Day 1-21(19*%* males; 18%** females) 4th Dose: -↓pup wt. Day 1-21 (31%** males, 29%** females) -↓**live birth** (171** vs 315) -↓**viability** (47%** vs 92%) -↓pinna unfolding -kidney wt. -↓grip strength -delayed ear **F_1 Adult LOAEL 2nd Dose** 2nd Dose: -↓ Na -↓RBC -↓Cl 3rd Dose: -↓ Na	**F_2 Repro LOAEL 2nd Dose** 2nd Dose: -↓epididymal wts. -↑testes wt. **F_2 Offspring LOAEL 2nd Dose** 2nd Dose: -↑adrenal wts.	Trigger P LOAEL = F_2 LOAEL No C&L concern

Table 3. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL > F_2 LOAELs

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
		-↓Cl -↓RBC, HCT, HGB -↑ adrenal wt. 4th Dose: -↓RBC, HCT, HGB -liver necrosis -kidney dilatation -↑adrenal wt./lipidosis -mortality (males) -eye degeneration -↑creatinine -↑cholesterol		

3. F_1 = F_2 LOEL/LOAEL but with unique F_2 effect or C&L concerns

The next evaluation in the retrospective analysis was examination of those studies that had a LOAEL/LOEL at the same dose in the F_1 and the F_2 generation. For this comparison, then, the effects in the F_2 generation would have been protected quantitatively in the risk assessment. There is a concern, however, that a new, different, or "unique" effect may occur in the second generation that would be important for risk assessment or for classification and labeling. Therefore, a qualitative comparison of effects in the F_1 vs. the F_2 was used to determine F_2 unique effects.

A total of 185 of the 341 (54%) pesticide studies had a LOAEL or LOEL at the same dose in the first generation as in the second generation, or F_1=F_2, for either a reproductive or offspring effect. The next step in the analysis was to determine how many of the 185 studies had a different or new effect in the second generation not observed in the first generation (e.g., F_2 unique). Forty-three of the 185 studies (23%) were identified with a unique effect in the second generation. Unique F_2 effects include: pup wt (acephate, fluazifop-butyl, flusilazole, fluvalinate, thiodicarb, tolyfluanid, and topramezone), delay in development (benomyl, clodinafop-propyl, dichlorprop, fipronil, fluazinam, lindane, mecoprop, tepraloxydim, and thiobencarb), viability (aldicarb, chlorfenapyr, lambda-cyhalot, spinosad, and sulfluramid), lactation (benfluralin, cyhexatin), live birth (isoxaben), pallor/weakness (azafenidin, and tetramethrin), organ effects (bispyribac-sodium, dinotefuran, fentin, fenoxaprop-ethyl, propanil, and spiromesifen), litter size (bisphenol A, imazalil, molinate, propetamphos, and triticonazole) as well as more than one unique F_2 effects (multiple effects: acetamiprid, diflufenzopyr, epoxiconazole, imiprothrin, methomyl, and tetraconazole). These 43 studies were further evaluated to determine if triggers were present in the P or F_1 generation to suggest the mating of F_1 animals to produce a second generation. Triggers that would have resulted in the mating of the F1 generation were identified either in the P or F1 generation for 31 of the 43 studies with a unique effect (72%). The remaining 12 studies (with a F_2 unique effect) would not have been missed quantitatively for risk assessment but may have had information important for classification and labeling. Therefore, these 12 studies were also re-reviewed. Upon re-evaluation of these 12 studies, 3 studies were

found to have potential triggers in the F_1 generation (fluazinam, aldicarb, and isoxaben). Another study, bispyribac-sodium, did not evaluate the F_2 unique effect (liver parameters) in the F_1 offspring. A comparison of the F_2 effect to the F_1 generation could therefore not be made. Another study, tolyfluanid, had decreased pup weight in the F_1 that would under current policy be considered in a LOAEL, therefore, the F_2 pup weight would not be unique. The remaining 7 studies (clodinafop-propyl, tepraloxydim, diflufenzopyr, imiprothrin, sulfluramid, tetramethrin, and propanil) are also presented below to determine if classification and labeling concerns exist for these studies. It should be noted that upon further review of sulfluramid a statistically significant decrease in pup weight (6-7%*) was noted at the mid dose of the F_2 during PND7-14. This would suggest an F_1 LOAEL > F_2 LOAEL by current policy. Although statistically significant, the toxicological relevance of this decrease is equivocal given that it is limited to this brief period of time.

Table 4 presents the studies identified with a unique F_2 effect. However, due to time constraints, the magnitude of changes for each study could not be provided. Therefore, the magnitude of change in each generation is provided for those studies identified without a trigger. The effects in each generation without magnitude of change is included for the remaining studies

Table 4. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL = F_2 LOAELs but with a unique F_2 effect

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
F_2 unique effect = pup wt.				
1. Acephate	**P LOAEL HDT** HDT: -alopecia -↓ bw	**No F1 Repro** **F1 Offspring LOAEL HDT** HDT: -↓viability	**No F2 Repro** **F2 Offspring LOAEL HDT** HDT: -↓**Pup wt.** -↓viability	Trigger (viability)
2. Fluazifop-butyl	**P LOELs** LDT: -↓rel/ab epididymis MDT: -↓rel/ab epididymis -↓rel/ab testes HDT: -↓rel/ab epididymis -↓rel/ab testes -↓ implantations -↑ gestational interval	**F1 Offspring HDT** HDT: -↓live birth index -↓viability **F1 Adults LOAEL MDT** LDT -kidney nephrocalcinosis -liver hyperplasia MDT: -↓rel/ab epididymis -↓rel/ab testes -↓spleen -↓pituitary -↑ gestational interval HDT: -↓rel/ab epididymis -↓rel/ab testes -kidney nephrocalcinosis -liver hyperplasia -↓spleen -↓pituitary -↓fertility -↑ovary wt. -↓uterus wt.	**F2 Offspring HDT** HDT: -↓**Pup wt.** -↓viability	Trigger (live birth index, viability, P Gestational interval, implantations)

Table 4. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL = F_2 LOAELs but with a unique F_2 effect

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
		-↑ gestational interval		
3. Flusilazole	**P LOAEL MDT** MDT: -liver hypertrophy -liver cytoplasmic alteration HDT: -liver hypertrophy -mortality (F) -liver eosinophilic focus, cytoplasmic alteration -↑gestational interval	**F1 Repro LOAEL HDT** HDT: -↓litter size -↑gestational interval **F1 Offspring LOAEL HDT** HDT: -↓viability index -↓live birth index	**F2 Repro HDT** HDT: -↓litter size **F2 Offspring LOAEL HDT** -↓**Pup wt.** -↓viability index -↓live birth index	Trigger (P gestation interval)
4. Fluvalinate	**P LOAEL HDT** HDT: -ulcer	**F1 Repro LOEL** HDT: -↓litter weight (4-9%^{ns}; PND12: 8%; PND21: 9%) **F1 Offspring LOAEL HDT** HDT: -tremors Also upon review: -↓pup wt (3.8%^{ns})	**F2 Repro LOAEL HDT** HDT: -↓litter weight (PND21: 16%) **F2 Offspring LOAEL HDT** HDT: -↓p**up wt.** **-tremors**	Trigger (litter wt.)
5. Thiodicarb	**P LOAEL MDT** MDT: -↓bw ↓bw gain -↓food HDT: -↓bw -↓bw gain -↓food	**No F1 Repro** **F1 Offspring LOAEL LDT** LDT: -↓viability index MDT: -↓pup wt. -↓viability HDT: -↓pup wt. -↓viability	**No F2 Repro** **F2 Offspring LOAEL LDT** LDT: -↓**Pup wt.** MDT: -↓pup wt. -↓viability HDT: -↓pup wt. -↓viability	Trigger (viability)
6. Tolyfluanid	**P LOAEL MDT** MDT (57/75 MKD): -↓bw (5-7%*) -↓bw gain (11-14%) -↓ab. liver wt. (9%*) -↓rel. liver wt. (4%*) HDT (449/567 MKD): -↓bw (5-12%*) -↓bw gain -↓ab. liver wt. (16%*) -↓rel. liver wt. (7%*) -↓ab. spleen (9-11%*) -↑rel. kidney (5-6%**)	**No F_1 Repro LOAEL** **F_1 Offspring LOAEL MDT** MDT: -labored breathing (41 vs 11) -discolored skin (41 vs21) **Also**, ↓**pup wt** (5-10%* LD0-4) HDT: -↓pup wt. (11-13%* PND7-21) -labored breathing (60 vs11) -discolored skin (60 vs 21) -cold to touch (48 vs 29) -emaciation (37 vs 29) **No F_1 Adult LOAEL**	**No F_2 Repro LOAEL/LOEL** **F_2 Offspring LOAEL MDT** MDT: -↓pup wt. (Day0-4: 5-10%*) -labored breathing (103 vs 9) -discolored skin (74 vs 56) -cold to touch (90 vs 85) -emaciation (90 vs 92) HDT: -↓pup wt. (Day 0-21: 8%-21%**) -labored breathing (80 vs 9) -discolored skin (58 vs 56) -cold to touch (68 vs 85) -emaciation (88 vs 92) Also, ↓viability (87.8%** vs 95.3% Day 4)	No Trigger P LOAEL = F_2 LOAEL **No F_2 unique since F_1 Pup wt** No C&L concerns

Table 4. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL = F_2 LOAELs but with a unique F_2 effect

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
7. Topramezone	**P LOAEL 2nd Dose** 2nd Dose: -↑ kidney -↑liver -↑thyroid -↑eye opacity 3rd Dose: -cannibalism -↑ kidney -↑liver -↑thyroid -↑eye opacity 4th Dose: -cannibalism -↑ kidney -↑liver -↑thyroid -↑eye opacity	**F1 Repro 2nd Dose** 2nd Dose: -PPS 3rd Dose: -↓ litter wt. -PPS 4th Dose: -↓ litter wt. -PPS **F1 Offspring LOELS** 2nd Dose: -↓spleen 3rd Dose: -↓ pup wt. -↓spleen -↓viability index 4th Dose: -↓ pup wt. -↓spleen -↓viability index -empty stomach -discolored intestine	**F2 Repro LOAEL 2nd Dose** -↓litter weight 3rd Dose: -↓litter weight 4th Dose: -↓litter weight **F2 Offspring 2nd Dose** 2nd Dose: -↓spleen -↓**pup wt.** 3rd Dose: -cannibalism -↓pup wt. -↓spleen -↓viability index 4th Dose: -↓pup wt. -↓spleen -↓viability index -distended intestine -kidney dilatation	Triggers (viability, cannibalism, PPS, litter wt)
F_2 unique effect = delay development (i.e., eye opening, pinna unfolding etc.)				
8. Clodinafop-propyl	**P LOAEL 3rd Dose** 3rd Dose (32/38 MKD): -↓bw gain (5-8%) -↓food (8%) -↑ liver 4th Dose (64/74 MKD): -↓bw gain -↓ bw (5-9%) -↓food (5-9%) -↑ ab. liver wt. -↑ liver hypertrophy -↑ kidney pigmentation	**No F_1 Repro LOAEL but** 4th Dose: -↓ ab. testes wt. (12%*) **F_1 Offspring LOAEL 3rd** 3rd Dose: -↓pup wt. (PND14: 16%*; PND21: 12%*) -↓lactation (85.2% vs 96.1%) 4th Dose: -↓ pup wt. (PND14: 27%*; PND21: 28%*) -↓lactation (76.7% vs 96.1%) -eye opening delayed days 17-21 (data not given); but not considered biologically significant since day 21 % was comparable among groups (93.1% vs 100%) **F_1 Adults LOAEL 3rd** 3rd Dose: -↑ ab. liver wt. -↑ liver hypertrophy -↑ kidney pigmentation/dilatation -↓food 4th Dose: -mortality (1 male) -kidney degeneration/atrophy -liver hypertrophy	**No F_2 Repro LOAEL/LOEL** **F_2 Offspring LOAEL 3rd** 3rd Dose: -↓pup wt. (PND7-21: 9-11%*) **-delay incisor** (delayed days 9-13, comparable day 16) -kidney dilatation 4th Dose: -↓pup wt. (PND1-21: 9-23%*) **-delay eye opening** (delayed days 17-20, comparable day 21) -kidney **-delay pinna unfolding** (delayed Days 4-7, comparable at 10) **-delay incisor** (delayed days 9-13, comparable day 16) -kidney dilatation	No Trigger P LOAEL = F_2 LOAEL F_2 unique delay effects No C&L concern

Table 4. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL = F_2 LOAELs but with a unique F_2 effect

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
		-↓ bw (5-6%) -↓food		
9. Benomyl	**P LOELS** 3rd Dose: -↑epididymis hypospermia -testes atrophy -testes degeneration 4th Dose: -↑epididymis hypospermia -testes atrophy -testes degeneration -↓ bw/bw gain	**F1 Offspring LOEL** 3rd Dose: -↓pup wt. 4th Dose: -reduced body size -↓pup wt. **F1 Adult** 3rd Dose: -↑epididymis hypospermia -↓ bw/bw gain 4th Dose: -↑epididymis hypospermia -↓ bw/bw gain	**F2 Offspring LOAEL 3rd Dose** 3rd Dose: -↓pup wt. 4th Dose: **-Delay eye open** -↓pup wt.	Trigger (pup wt.)
10. Dichlor prop	**P LOAEL HDT** HDT: -↓food -↓bw/bw gain -↑kidney wt. ↑water -anemia -↑urogilinogen -↑crystalluria -↓globulins, triglycerides -mortality (F) -kidney hyperplasia -poor maternal care -kidney basophilia -↑ Alkaline phosphatase -↑gestational interval	**F1 Repro HDT** HDT: -↓litter size -↓fertility -↓mating -↑gestational interval **F1 Offspring HDT** HDT: -↓live birth index -↓put wt. -↓lactation index -↓viability -kidney	**F2 Repro LOAEL HDT** HDT: -↓litter size **F2 Offspring LOAEL HDT** HDT: **-Delay eye open** -↓live birth index -↓viability kidney	Trigger (P gestational index, F1 lactation index)
11. Fipronil	**P LOAEL MDT** MDT (2.5/2.7 MKD): -↓pituitary -↑thyroid -↑liver HDT (26.3/28.4 MKD): -↓pituitary -↓food (week1: 29%-32%***) -↓bw (GD0-20: 18%***)c -↓bw gain Week 0-19 (482g*** vs 530g) -↑thyroid -↑liver -↑thyroid hypertrophy	**F_1 Repro LOAEL HDT** HDT: -↓**litter size** (LD1: 12.1*** vs 14.8; LD4 (pre-cull): 9.6*** vs 14.0) -↓mating (83%* vs 100%) -↓fertility (80%ns vs 90%) -↓ovary -↓epididymis -↑post-implantation loss (81%* vs 90% post-implantation survival) **F_1 Offspring LOAEL HDT**: -↓**live birth** (83%** vs 98%) -↓incisor eruption (10.4* vs 9.7) -↓pup wt. (KD1-45: 6%*-22%***) -↓viability (89%* vs 97%) **F_1 Adult LOAEL MDT**	**F_2 Repro LOAEL HDT** HDT: -↓litter size **F_2 Offspring LOAEL HDT** HDT: -↓live birth (78%*** vs 100%) -↓viability (73%*** vs 98%) -↓**pinna unfolding** (3.8ns vs 3.3) -↓pup wt (LD1-25:M 21%*** F 22%***)	Triggers P LOAEL < F_2 LOAEL No C&L concern, labeling from F_1 litter size, epididymis/ovary effects

Table 4. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL = F_2 LOAELs but with a unique F_2 effect

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
		MDT: -↑thyroid -↑liver -↓ pituitary -↓bw gain (F: HDT: -↓pituitary -↓food (Males, Week1: 8%^{ns}) -↓bw (Week0-1: 17%-22%***) -↓bw gain (Males Week0-10: 11%***; Week0-19: 15%***) -↑thyroid -↑liver -↑thyroid hypertrophy		
12. Fluazinam	**P LOAEL MDT** MDT (10 MKD): -fatty liver (F_1) HDT (47/54 MKD): -fatty liver -↑rel. liver (8%-12%**) -↓ bw gain (**F_1** 8%**):	**F_1 Repro LOAEL HDT** HDT: -↓implantations (12.2* vs 15.3) Also: -↓litter size (before cull: 9.8* vs 12.4) **F_1 Offspring LOAEL HDT** HDT: -↓pup wt. gain (Day1-21 10%***) -delay eye open (13.8** vs 14.7) Also, -↓viability index (87% vs 94%^{ns}) **F_1 Adult LOAEL MDT** MDT: -fatty liver HDT: -↓bw gain (12%-15%**) -↑rel. liver (Male: 8%**)	**F_2 Repro LOAEL HDT** HDT: -↓**litter size** **F_2 Offspring LOAEL HDT** HDT: -↓pup wt. gain (Day1-21 13%**) -early eye open (14.1** vs 14.8) -delay **pinna unfolding** (3.2** vs 3.9)	Potential Triggers No Trigger in ToxRefDB; however further analysis reveals F_1 litter size, viability as potential triggers P LOAEL < F_2 LOAEL No C&L concern (F_1 implantations) if pup wt., delay eye, and viability are used
13. Lindane	**P LOAEL MDT** MDT: -↑ alpha-2 globulin -↑kidney regeneration -↑kidney hyaline droplet -↑kidney inflammation -↑rel.kidney wt. -↑kidney necrosis -↑kidney casts HDT: -↓bw -↑ alpha-2 globulin -↑kidney regeneration -↑kidney hyaline droplet -↑kidney inflammation -↑rel.kidney wt. -↑kidney necrosis	**No F1 Repro** **F1 Offspring HDT** HDT: -↓pup wt. -↓viability	**No F2 Repro** **F1 Offspring LOAEL HDT** HDT: -↓pup wt. -↓viability -↓**Delay incisor eruption** **-↓ delayed hair growth**	Trigger (viability)

Table 4. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL = F_2 LOAELs but with a unique F_2 effect

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
	-↑kidney casts			
14. Mecoprop	**No P LOAEL** **P LOELs:** MDT (9.3/10.3 MKD): -↑rel. kidney (M: 7%*) HDT (47/51 MKD): -↑ab. kidney wt (M&F: 9%*) -↑ rel. kidney wt (M: 13%* &F: 6%*) -no histopathology changes present at either dose	**No F_1 Repro LOAEL** **F_1 Offspring LOAEL HDT** HDT: -↑pups dead/**cannibalized** (Days 0-4: 23 (6.78%*) vs 8 (2.29%) -↓pup wt. gain (LD7-14: 6%*) Also MDT: -↑pups dead/**cannibalized** (Days 0-4: 15 (4.42%) vs 8 (2.29%) **F_1 Adult LOELs:** MDT: -↑ab. kidney (M:8%*) -↑ rel. kidney (M:11%* &F: 4%*) HDT: -↑ab. kidney (M: 16%* &F: 8%*) -↑rel.kidney wt (M: 16%* &F: 9%*) -no histopathology changes present at either dose	**No F_2 Repro LOAEL** **F_2 Offspring LOAEL HDT** HDT: -↑pups dead/cannibalized (32 (9.25%*) vs 17 (5.28%)) -↓pup wt. (Day 14: 8%*M, 9%* F; Day 21: 7%* M, 8%* F) -↓pup wt. gain (LD4-21: 7-8%*) **-delay auditory canal opening** (171/183** (93%) vs 191/193 (99%)) & historical control: 94-99%	Trigger P LOEL > F_2 LOAEL F_2 unique effect No C&L concerns
15. Tepraloxydim	**P LOAEL HDT** HDT (253/274 MKD): -↓bw (5-6%*) -↓bw gain (5-8%) -↓food -↓bw gestation & lactation (7-12%*)	**NO F_1 Repro LOEL** **F_1 Offspring LOAEL HDT** HDT: -↓pup wt Day 0: 7%** GD20: 11%** LD1: 10%** LD21: 9%** -↓ pup wt gain LD4-21:10%** **F_1 Adult LOAEL HDT** HDT: -↓bw (7-13%*) -↓bw gestation & lactation (7-12%*) -↓bw gain (8-24%) -↓ food	**No F_2 Repro LOEL** **F_2 Offspring LOAEL HDT** HDT: -↓pup wt. LD14: 10%**, LD21: 11%** -↓pup wt. gain LD4-21: 13%** **-delay eye opening** (82.5%* vs 97.8%)	No Trigger P LOAEL = F_2 LOAEL F_2 unique (delay eye) No C&L concern if F_1 pup wt. used
16. Thiobencarb	**P LOAEL LDT** LDT: -liver hypertrophy -kidney degeneration MDT: -liver	**F1 Repro MDT** MDT: -↑rel. testes wt. HDT: -↑rel. testes wt.	**No F2 Repro** **F2 Offspring HDT** HDT:	Trigger (viability)

Table 4. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL = F_2 LOAELs but with a unique F_2 effect

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
	HDT: -liver -pituitary -kidney -↓bw	**F1 Offspring HDT** HDT: -↓viability index	**-Delay eye open**	
F_2 unique effect = litter size				
17. Bisphenol A	**P LOEL 5th Dose:** 5th Dose: -↓bw/bw gain 6th Dose: -↓bw/bw gain	**F1 Repro LOEL 6th Dose** 6th Dose: -PPS/VO -↓epididymal sperm -↓rel. ovary wt. -↓implantations **F1 Offspring LOEL 6th Dose** 6th Dose: -↓pup wt.	**F2 Repro LOEL 6th Dose** 6ht Dose: -↓**Litter size** -VO/PPS -↓ab. ovary wt. **F2 Offspring LOEL 6th Dose** 6th Dose: -↓pup wt.	Trigger (PPS/VO)
18. Imazalil	**P LOAEL HDT** HDT: -↓bw -dystocia (6/24 vs 3/24 controls) -↑gestational interval (24.5*** vs 23.5)	**F_1 Repro HDT** HDT: -↓implantations -↑gestational interval Also further review found -↓**litter size** (46%**)	**F_2 Repro HDT** HDT: **-↓litter size (49%**)**	Triggers (P gestational interval, viability index, lactation index, live birth index No C&L concern
19. Molinate	**P systemic LOEL @ MDT** MDT: -↓bw(9% wk 11 premating)/bwg (18%)/fc (females); -↓bw (8-13%/↓bwg (13-23%) during gestation;(females) -↓bw (5-11%/↓bwg (26-97%) during lactation (females) -↓FC (f) **P Repro LOEL @ MDT (male)** MDT: -↓rt cauda wt (6%**) (P) -↑abnormal sperm morphology (P) HDT: ↓rt cauda wt (13.5%**) -↓epididymis wt (9%**) -↑abnormal sperm morphology **P Repro LOEL @ MDT (female)** MDT: -lesions in ovary (vacuolation, hypertrophy)	**F_1 Repro LOEL @ MDT** MDT: -lesions in ovary (vacuolation, hypertrophy) -abnormal sperm morphology -↓testes (11%)* (no pathology) F_1 pups HDT: **-↓litter size 39-41%** days 1-22** -↓prostate (15%)**(no pathology) -↓testes (22%)(no pathology) F_1 pups -↓ovary (21%)(no pathology) F_1 pups -abnormal sperm morphology -delayed VO (9%)** (34 days, control vs 36.9 days)[34, 34.1, 34.6, 36.9** days, control to HDT] -↑gestational interval (1%)^{ns} **F_1 Offspring LOAEL @**	**F_2 Repro LOEL @ MDT** **MDT: ↓litter size (F_{2A}) 16%** (day 1)** HDT: **-↓litter size** (32-36%**) days 1-22 -↓testes (19%) (no pathology) F_{2A} pups -↓ovary (17%) (no pathology) F_{2A} pups **F_2 Offspring LOAEL @ MDT** -↓spleen, thymus HDT: -↓pup bw (11-19%)** (F_{2A})	Trigger (live birth) Only unique (litter size seen at F_1 HDT and F_2 MDT and HDT) No C&L issues

Table 4. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL = F_2 LOAELs but with a unique F_2 effect

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
	HDT: -↓uterus wt (12%*) -↑gestational interval (1%)**	**MDT** MDT: ↓spleen, thymus HDT: ↓**live birth** (3%)* -↓pup bw (10-17%)** F1		
20. Propetamphos	P LOAEL MDT MDT (2.1/2.8 MKD): -ChE HDT (5.5/7.1 MKD): -ChE -tremors -Resorptions	F1 Offspring MDT: -runts HDT: -runts -↓ live birth index -↓ lactation index F1 Adults HDT -resorptions -↓mating -↓ implantations	**F2 Offspring** LDT (0.3/0.4 MKD): -↓**Litter size** **-no milk in stomach** MDT: -↓ litter size -no milk in stomach HDT: -↓litter size -cannibalism -↓ lactation index -no milk in stomach	Triggers (lactation index, P resorptions)
21. Triticonazole	**P LOAEL 4th Dose** 4th Dose: -mortality (F) -↑ adrenal pigmentation, degeneration, giant cell, collagen -↑adrenal vacuolization -↑gestational interval .↑liver	**F1 Repro 4th Dose** 4th Dose: -↓fertility -↑gestational interval **F1 Offspring 4th Dose** -↓ live birth index -↓viability -↓pup wt.	**F2 Repro 4th Dose** 4th Dose: -↓**litter size** -↑gestational interval **F2 Offspring 4th Dose** -↓ live birth index -↓viability -↓litter viability -↓pup wt.	Trigger (P gestational interval) No C&L concern since trigger caught F_1 fertility
F_2 unique effect = multiple effects				
22. Acetamiprid	**P LOAEL HDT** HDT: -↓bw/bw gain -↓food	**F1 Repro HDT** MDT: -PPS HDT: -↓litter size -VO/PPS -↓litter weight **F1 Offspring HDT** HDT: -↓pup wt.	**F2 Repro HDT** HDT: -↓litter weight **F2 Offspring HDT** HDT: -↓**Viability** **-delay eye open** **-↓ lactation index** -↓pup wt.	Trigger (litter size, PPS at MDT)
23. Diflufenzopyr	**P LOAEL MDT** MDT (113/169 MKD): -↓bw gain (8-16%*) -↑food (5-9%*) HDT (466/670 MKD): -↓bw gain (14-21%**) -↑food (5-16%*) -↑ rel. seminal vesicle (15-24%**)	**F_1 Repro LOAEL MDT** MDT: -↑ rel.(12-16%**) seminal -↑ab. (12%*) seminal vesicle HDT: -↑ rel. seminal vesicle (16-31%**) -↑ab. seminal vesicle (13%*) **F_1 Offspring LOAEL** HDT: -↓pup wt. (LD 21:12-	**No F_2 Repro LELs** **F_2 Offspring LOAEL HDT** HDT: -↓**live birth index** (93%* vs 98%) -↓**viability** (28** deaths vs 6; 88ns vs 98) **-no milk in stomach** (8.4%** vs 1.2%)	No Trigger P LOAEL < F_2 LEL F_2 unique effects No C&L concern, if F_1 effects used

Table 4. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL = F_2 LOAELs but with a unique F_2 effect

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
		14%**) -runts (8%** vs 0.4%) **F_1 Adult LOAEL MDT** MDT: -↑food (8-9%**) HDT: -↑food (5-16%*) -↓bw gain (14-21%**)		
24. Epoxiconazole	**P LOAEL HDT** HDT (22/32 MKD): -↓**fertility** (88% vs 96%) -mortality (2F vs 0) -↑**precoital interval** (3.6 vs 2.2) -↑vaginal hemorrhage (6 vs 0) -↑**gestational interval** (22.8** vs 22.0) -↓gestation index (73% vs 100%)	**F_1 Repro LOAEL HDT** **HDT**: -↓fertility (84% vs 100%) -↓# litters 20 vs 24 -↑**precoital interval** (3.6 vs 2.6) **F_1 Offspring LOAEL HDT**: -↓Females with live born (16** vs 24) -females with all stillborn (4 vs 0) **F_1 Adult LOAEL HDT** HDT: -↓food -↓bw/gain -↓ adrenal wt.	**No F_2 Repro LEL** **F_2 Offspring LOAEL HDT** HDT: -↓females with live born (20 vs 25) -↓viability (82%** vs 98%) -↓**lactation** (94%** vs 99%) -↓**pup wt.** (Day7-21: 12%-14%**) -dead fetuses (stillborn 14 vs 10) -females with all stillborn (14** vs 5)	Triggers P LOAEL = F_2 LOAEL No C&L concern, labeling from P dams
25. Imiprothrin	**P LOAEL MDT Females** MDT (143.5 MKD): -spleen hemosiderosis **P LOAEL HDT Males** HDT (369 MKD): -↓bw gain (10-18%**) -↓food (9-10%**) -↑ liver -spleen hemosiderosis	**No F_1 Repro LOAEL but** HDT: -↓ab. ovary wt. (20-23%**) -↓rel./brain ovary wt. (19-20%**) **F_1 Offspring LOAEL HDT** HDT: -↓pup wt. (LD1-21: 9%-20%**) -F1 pups not subjected to skeletal evaluation, not in DER **F_1 Adult LOAEL F** MDT: -spleen hemosiderosis F1 Adult LOAEL M HDT: -↓bw gain (10-13%**) -↓food (12-13%**) -↑ liver wt. -spleen hemosiderosis	**No F_2 Repro LOAEL/LOEL** **F_2 Offspring LOAEL HDT** HDT: -↓pup wt. (LD4-21: 7%-20%**) Day 4 skeletal variations (selected data): -14th rib (88/175** (50%) pups vs 46/171 (27%) -Thoracic Vertebrae (5/175 (2.8%) vs 0/171 (0%) historical control 0-2.1% Day 21 skeletal variations (selected data): -14th rib: 27/198 pups (50%**) vs 1/182 (0.6%) Day 4 ossification sites (naturally delivered): -ribs pairs: 13.75* (4%) vs 13.21 Day 21 ossification sites (naturally delivered) -rib pairs: 13.12** (0.09%) vs 13.00	No Trigger P LOAEL < F_2 LOAEL F_2 unique cannot be compared to F_1 since no skeletal evaluation in F_1 pups No C&L concerns

Table 4. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL = F_2 LOAELs but with a unique F_2 effect

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
26. Methomyl	**P LOAEL MDT** MDT: -↓bw -↓food -↓HGB -↓RBC -↑ spleen -↑alopecia -↑brain wt. HDT: -↓bw -↓food -↓HGB -↓RBC -↑ spleen -↑alopecia -↑brain wt. -↑ rel. testes wt.	**F1 Repro LOEL** MDT: -↑rel. testes wt. -↓ ab. ovary wt. HDT: -↑rel. testes wt. -↓ ab. ovary wt. -↓uterus wt. **F1 Offspring LOAEL MDT** LDT: -↓pup wt. MDT: -↓pup wt. HDT: -↓pup wt. -↓viability index -↓lactation index	**F2 Repro HDT** HDT: -↓testes wt, **F2 Offspring LOAEL MDT** LDT: -adrenal MDT: -↓pup wt. -↓ viability index HDT: -↓pup wt. -**Adrenal** -spleen -↓viability index -↓lactation index	Trigger (lactation index)
27. Tetraconazole	**P LOAEL MDT** MDT: -↑ gestational interval HDT: -↑ gestational interval -liver -↓food -mortality (F) -kidney -lung -stomach -breathing rate	**F1 Repro HDT** HDT: -↓litter size -↑ dystocia **F1 Offspring HDT** HDT: -↓live birth index -↓viability -↑liver wt. -↓pup wt.	**F2 Offspring HDT** HDT: -↓pup wt. -↓**Righting ability** -↓ **kidney wt.** -↑liver wt.	Trigger (P gestational interval)
F_2 unique effect = viability				
28. Aldicarb	**P LOAEL 3rd Dose** 3rd Dose (0.7/0.9 MKD): -↓bw gain -↓ RBC ChE 4th Dose (1.4/1.7 MKD): -↓RBC ChE -↓bw gain (M: 37%* week 1; F: 13%* GD0-20 & 31%* GD7-14) -↓plasma ChE	**No F_1 Repro LOAEL/LOEL** **F_1 Offspring LOEL 4th** 4th Dose: -↓pup wt. (F: 10%* Day 14; 10%** Day 21) Also: pup death LD0-4 (75 vs 14) -↓viability (78% vs 96%) **No F_1 Adult LOAEL/LOEL**	**No F_2 Repro LOAEL/LOEL** **F_2 Offspring LOAEL 4th** 4th Dose: -↓pup wt. (LD0:7.3%**; LD21:14-15%**) -↓viability (only in F_{2B}) Also: -↓live born (28%**)	Potential triggers upon review (pup death, viability) No Trigger in ToxRefDB P LOAEL < F_2 LOAEL No C&L concerns Viability not unique since F_1 viability present with further review
29. Chlorfenapyr	**P LOAEL MDT** MDT: -↓bw HDT: -↓bw gain -↑gestational interval	**No F1 Repro** **F1 Offspring LOAEL MDT** MDT: -↓pup wt. HDT: -↓pup wt.	**No F2 Repro** **F2 Offspring LOAEL MDT** MDT: -↓pup wt. HDT: -↓pup wt. -↓**Viability index**	Triggers (pup wt., P gestational interval)

Table 4. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL = F_2 LOAELs but with a unique F_2 effect

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
30. Lambda-cyhalot	**P LOAEL HDT** HDT: -↓bw gain	**No F1 Repro** **F1 Offspring LOAEL HDT** MDT: -↓pup wt gain HDT: -↓pup wt gain	**No F2 Repro** **F2 Offspring LOAEL HDT** MDT: -↓**live birth index** HDT: -↓**Viability index**	Trigger (pup wt.)
31. Spinosad	**P LOAEL HDT** HDT: -↓bw -↑heart -↑spleen -↑thyroid necrosis -↑lung inflammation -↑kidney -lymph node histiocytosis -lung alveolar macrophages -stomach dilatation -↑prostate inflammation -vaginal hemorrhagic perineal	**F1 Repro HDT** HDT: -↓litter size -dystocia -vaginal hemorrhagic perineal **F1 Offspring HDT** HDT: -↓pup wt. -↓live birth index	**No F2 Repro** **F2 Offspring HDT** HDT: -↓**Viability index** -↓pup wt. -↓live birth index	Triggers (F1 live birth index, litter size)
32. Sulflura mid	**P LOAEL HDT** HDT (1.3/1.6 MKD): -↓bw gain (5-11%^{ns}; GD0-4: 17%; LD 0-4: 48%) -↑rel. liver wt.	**F_1 Repro LOAEL HDT** HDT: -↓PPS (day 56 vs 53) -↓ VO (day 40 vs. 35) LEL: ↓ab. testes wt (11%*) **F_1 Offspring LOAEL HDT** HDT: -↓ab. kidney -↓rel. brain -↓pup wt. (7-10%**) **F_1 Adult LOAEL HDT** HDT: -↓ ab. adrenal -↓rel. pituitary -↓bw (6-12%) -↓bw gain (6-13%; GD0-20: 10-13%; LD0-21: 7-12%)	**No F_2 Repro LOAEL/LOEL** **F_2 Offspring LOAEL HDT** HDT: -↓**viability** (22 died vs 11 controls); considered incidental in DER -↓pup wt. (10-15%**) Also MDT (0.45/0.5 MKD): -↓pup wt (PND7-14: 6-7%*)	No Trigger P LOAEL = F_2 LOAEL Current policy: P LOAEL > F_2 LOEL F_2 unique viability No C&L concern
F_2 unique effect = ↓lactation index				
33. Benfluralin	**P LOAEL MDT** MDT: -↑kidney wt. -kidney hyaline droplet -↑liver wt. -↓bw -↓food HDT: -↑kidney wt. -kidney hyaline droplet -↑liver wt. -liver hypertrophy -↓bw	**F1 Repro HDT** HDT: -↓litter size -↑gestational interval **F1 Offspring MDT** MDT: -↓pup wt. HDT: -↓pup wt. -↓viability	**No F2 Repro** **F1 Offspring MDT** MDT: -↓pup wt. HDT: -↓pup wt. -↓**Lactation index** -↓viability	Trigger (F1 litter size, viability index)

Table 4. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL = F_2 LOAELs but with a unique F_2 effect

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
	-↓food			
34. Cyhexatin	**P LOAEL MDT** MDT: -↓ bw HDT: -↓bw/bw gain -↓ implantations -↑ rel epididymis wt. -↑ rel testes wt.	**F1 Repro LOAEL HDT** HDT: -↓ litter size -↓ ab. testes wt. -↑ rel ovary wt. -↑ rel testes wt. **F1 Offspring LOAEL** MDT: -↓ pup wt. -delay eye opening	**F2 Offspring** HDT: -↓**Lactation index** -↓ pup wt. -delay eye opening **F2 Repro LOAEL** HDT: -↓litter size	Triggers (P implantations, litter size)
F_2 unique effect = ↓live birth index				
35. Isoxaben	**P LOAEL MDT** MDT (200 MKD): -↑rel. liver wt. HDT (1000 MKD): -↑rel. liver wt.	**No F_1 Repro LOAEL/LOEL** **F_1 Offspring LOAEL HDT** HDT: -↓**pup wt.** (PND21: 13%*) **F_1 Adult LOAEL MDT** MDT: -↑rel. liver wt. HDT: -↑rel. liver wt. -↓bw (M:10%* Day 0, F: 12%* Day 70) -↓bw gain (F: 15%* Day0-70; GD0-20 12%*) -↓food	**No F_2 Repro LOAEL/LOEL** **F_2 Offspring LOAEL HDT** HDT: -↓pup wt. (19%* PND14; 25%* PND21) -↓ **live birth index** (9.6^{ns} vs 11.0) Also, MDT: -↓pup wt. (9%* PND14)	Potential trigger (pup wt.) No Trigger in ToxRefDB P LOAEL < F_2 LOAEL F_2 live birth unique No C&L concern if use F_1 pup wt.
F_2 unique effect = pallor/weakness/lethargy				
36. Azafenidin	**P LOELS** 3rd Dose: -↑methhemoglobin -↓HGB, HCT 4th Dose: -↑methhemoglobin -↓HGB, HCT -↓mean corpuscular -↓food -↑mortality (F) -↓fertility -↓implantations	**F1 Repro LOAEL 3rd** 3rd Dose: -↓testes wt. -↓mating -↑epididymis oligospermia -↑testes degeneration -↓implantations -↑gestational interval **F1 Offspring 3rd Dose** 3rd Dose: -↓live birth index -↓lactation index -↓viability index -↓size of pups -skin discoloration	**No F2 Repro** **F2 Offspring 3rd Dose** 3rd Dose: -↓live birth index **-Pallor** -skin discoloration -↓lactation index -↓pup wt. -↓pup size	Triggers (P fertility, P implantations, live birth index, lactation index, viability index, pup wt)

Table 4. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL = F_2 LOAELs but with a unique F_2 effect

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
37. Tetramethrin	**P LOAEL HDT** HDT (150 MKD): -↓bw (F: 8%*) -↓food (F:7%)	**No F_1 Repro LOAEL/LOEL** **F_1 Offspring LOAEL HDT** HDT: -↓pup wt.(10-13%*) **F_1 Adults LOAEL HDT** HDT: -liver hyperplasia -↓bw (F:10%*)	**No F_2 Repro LOAEL/LOEL** **F_2 Offspring LOAEL HDT** HDT: -↓pup wt. (15-18%*) -**lethargy** (data not provided)	No Trigger P LOAEL = F_2 LOAEL F_2 unique No C&L concern if use F_1 pup wt.
F_2 unique effect = organ effect				
38. Bispyribac-Na	**P LOAEL MDT** MDT (76/86 MKD): -liver hyperplasia HDT (759/874 MKD): -liver hyperplasia -liver inflammation -↓bw (LD0:7%**) -↑ab. liver wt. (22%*)	**No F_1 Repro LOAEL/LOEL** **F_1 Offspring LOAEL HDT** HDT: -↓pup wt. (11-18%**) -↓pup wt. gain (16-17%) -liver wts not evaluated **F_1 Adult LOAEL MDT** MDT: -liver hyperplasia HDT (759/874 MKD): -liver hyperplasia -liver inflammation -↓bw (10-15%**) -↑ab. liver wt.	**No F_2 Repro LOAEL/LOEL** **F_2 Offspring LOAEL HDT** HDT: -↓pup wt. (14-19%*) -↓pup wt. gain (16-17%) -↑ab. liver wt. (24-25%**) -↓rel. liver wt (8-9%*)	No Trigger P LOAEL < F_2 LOAEL F_2 unique not evaluated in F_1 for comparison No C&L concerns
39. Dinotefuran	**P LOAEL 4th Dose** 4th Dose: -↓spleen -↓bw gain -↓food -↓uterus -↓estrous length -↓testicular spermatid -↑abnormal sperm -↑vaginal degeneration -↑vaginal atrophy -↑uterus atrophy	**F1 Repro 4th Dose** 4th Dose: -↑abnormal sperm -↓sperm motility -↓estrous periodicity -↑ovary depletion F1 Offspring 4th Dose 4th Dose: -↓spleen wt. -↓pup wt. gain -↓pup wt. -	**No F2 Repro** **F2 Offspring 4th Dose** 4th Dose: -↓pup wt. -↓pup wt. gain^{ns} -↓**Thymus NOS** -↓spleen wt.	Triggers (P estrous length, F_1 estrous periodicity)
40. Fentin	**No P Effects**	**F1 Repro HDT** HDT: -↓litter size -↓ovary wt. -↓testes wt. **F1 Offspring MDT** MDT: -↓slpeen HDT:	**F2 Repro HDT** HDT: -↓litter size -↓ovary wt. -↓testes wt. **F2 Offspring MDT** MDT: -↓spleen -↓**Liver wt.**	Trigger (litter size)

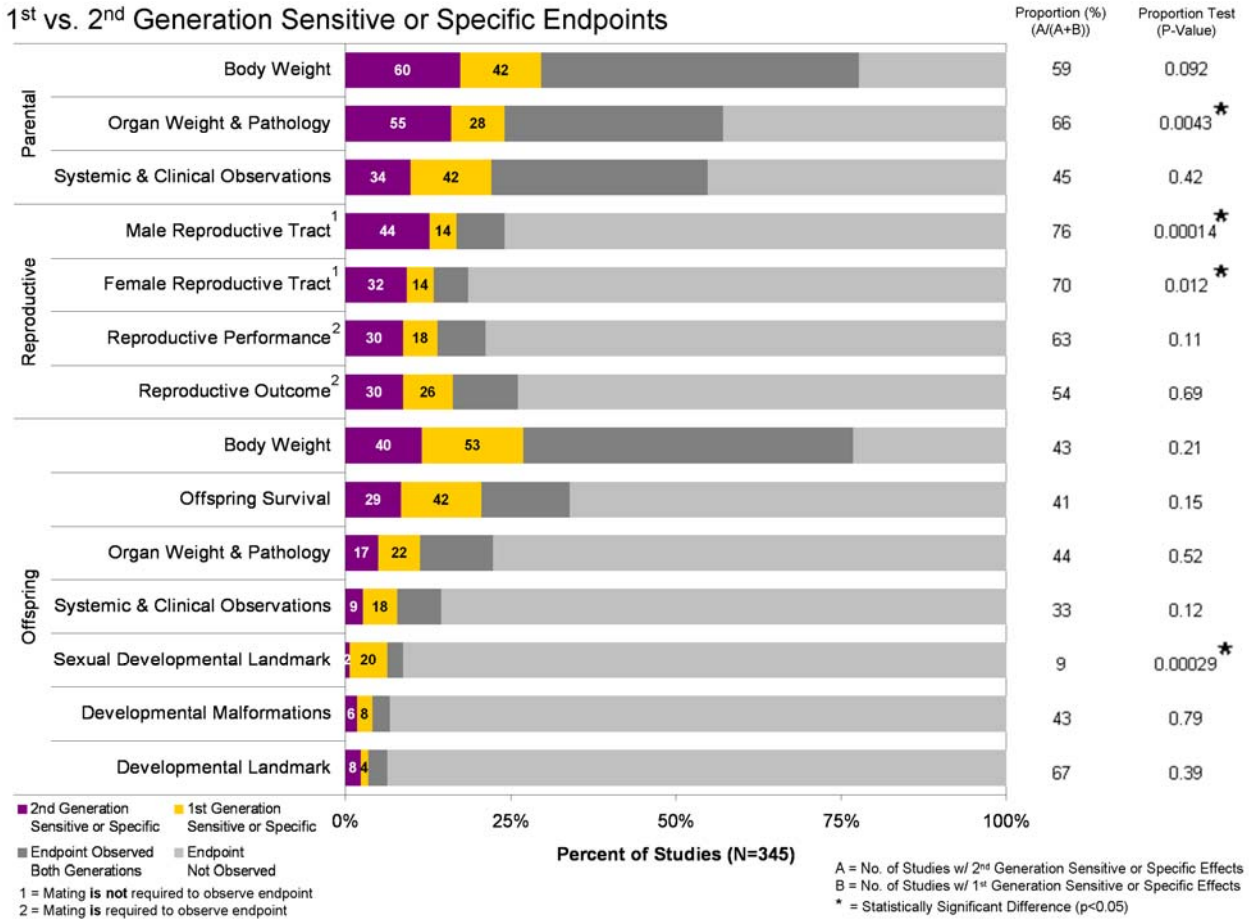
Table 4. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive F_1 LOAEL = F_2 LOAELs but with a unique F_2 effect

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
		-↓thymus -↓pup wt. -↓spleen -↓liver	HDT: -↓liver, -↓spleen -↓pup wt. -↓thymus wt.	
41. Fenoxaprop-ethyl	**P LOEL HDT** HDT: -↑liver wt.	**No F1 Repro** **F1 Offspring LOAEL MDT** MDT: -↓pup wt. -↑alkaline phosphatase HDT: -↓pup wt. -↓lung NOS -↓spleen NOS -↑alkaline phosphatase -↑liver NOS -↑kidney NOS	**No F2 Repro** **F2 Offspring MDT** MDT: -↑**alkaline phosphatase** HDT: -↑alkaline phosphatase -↑kidney NOS -↑liver NOS -↓lung NOS -↓spleen NOS -↓pup wt.	Trigger (pup wt.) F2 alkaline phosphatase not unique upon further review
42. Propanil	**P LOAEL @ HDT** HDT: -↓bw (7.9%, females, premating), also↓bw during gestation and lactation (no values given) -↓food consumption -↑spleen wt (P and F_1 adults) -↑spleen pathology (P and F_1 adults) (increased incidence and severity of pigmented macrophages)	**F1 Offspring LOAEL @ HDT** HDT: -↓pup wt (6%)* lactation days 7 and 21 **F1 Repro LOAEL @ HDT** HDT: -delayed PPS (8 days)** -delayed VO (7 days) -↓testicular sperm count (19%)* -↓testicular sperm production rate (18%)* Other effects at HDT not included in LOAEL: -↑testes wt (6%) F_1 weanlings	**F_2 Offspring LOAEL @ HDT** HDT: -↓pup wt (7-10%)** lactation days 1-21 -↑spleen wt -↓pituitary wt -↓liver and kidney wt **F_2 Repro LOEL HDT** HDT: -↑**ovary wt (20%)****	No Trigger P LOAEL = F_2 LOAEL F_2 unique effects No C& L issues
43. Spiromesifen	**P LOAEL MDT** MDT: -↓spleen HDT: -↓spleen -↓bw -adrenal atrophy -↑thyroid	**F1 Repro LOEL HDT** HDT: -↑PPS/VO -↑abnormal estrous cycle **F1 Offspring MDT** MDT: -↓pup wt. HDT: -↓pup wt.	**F2 Repro LOEL HDT** HDT: -↓**Uterus wt.** **F2 Offspring MDT** MDT: -↓pup wt. HDT: -↓pup wt. -↓spleen -↓thymus	Trigger (F_1 estrous)

6. Occurrence of 1st and 2nd Generation Sensitive or Unique Effects

In order to assess statistically the prevalence of second generation sensitive or unique (specific) effects, we compared the proportion of endpoints with either second generation sensitive or specific, or first generation sensitive or specific effects (Figure 3). For this analysis, a sensitive effect is defined as having a two-fold or greater difference in the lowest effect level across the endpoint class. This two-fold threshold accounted for generational, life-stage and gender differences in food consumption within studies. The proportion of second versus first generation sensitive or specific effects was then used in a proportion test to derive a p-value. P-values less than 0.05 were considered significant. Only Parental Organ Weight and Pathology, and Male and Female Reproductive Tract had statistically significant proportion of second generation sensitive or specific effects. Sexual Developmental Landmark was biased towards the first generation, since these measurements are typically only taken in the F_1. No endpoint class that would require mating of the F_1 demonstrated significance, and therefore occurred no greater than would be expected by chance. So F_2 pup weight, for example, was not significant and therefore did not occur greater than would be expected by chance.

Figure 3. 1st vs. 2nd Generation Sensitive or Specific Endpoints



7. C&L Concerns but No Quantitative Concerns

Five additional studies were evaluated for potential C&L concerns due to F_1 mating effects (*i.e.,* effects that would not have been detected if the F_1 generation was not mated). Three of the 5 studies did not have a trigger identified. However, all 3 of these studies had observed effects either in the P generation or early in the F_1 generation that could have been used for classification and labeling. The details of these three studies are in Table 5 below. The remaining 2 studies had triggers and would not have been missed. If assuming no triggers, then the remaining 2 studies (with triggers) were further examined for classification and labeling. These 2 studies also had observed effects either in the P or F_1 generation that could have been used for classification and labeling. Therefore, none of the 5 studies with F_2 less sensitive but identified solely for C&L concerns would have been missed, regardless if triggers were applied to produce the second generation.

Table 5. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive LOAEL/LOELs less sensitive than F_2 but with C&L concerns

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
F_2 less sensitive, C&L concerns identified and no trigger				
1. 3-(3,5-dichlorp) (Procymidone)	**P LOAEL MDT** MDT (12.5 MKD): -↓bw (2%) -↑ ab/rel liver wt. -↓testes wt. (3%*) HDT: -↑testes wt. (7%**) -↓food (4-6%*) -↓bw gain (7%*) -↓bw GD1-22(8%*) -↑ ab/rel liver wt.	**F_1 Repro LOAEL MDT** MDT: -↑testes wt. (8%**) -↓ epididymides -↓ prostate HDT: -↓fertility (10males** vs 22 males[potentially due to penis malformation?]) -penis malformation (8/30 vs 0; weeks 6-45; 4 additional males post-mortem) -↑testes wt. (8%**) **No F_1 Offspring LOAEL** **F_1 Adult LOAEL MDT** MDT: -↑ ab/rel liver wt. -↓bw pre-mating (4%*) HDT: -↓food (6%*) -↓bw gain (12%*) -↓bw GD1-22 (13%**) -↑ ab/rel liver wt.	**F_2 Repro LOAEL HDT** **HDT**: -penis malformation (6/30 vs 0; 2 additional males post-mortem) -↑testes wt. (7%**) **No F_2 Offspring LOAEL**	No Trigger P LOAEL < F_2 LOAEL No C&L concerns (since ample effects in P and F_1 to suggest C&L)
2. Bis(tributyltin)oxide	**P LOAEL HDT** HDT (2.95/3.43 MKD): -↓ thymus (8%^{ns} ab 8% rel^{ns}) -↓bw change (F: -11g** vs +2gl; LD0-4)	**No Repro LOAEL but HDT:** -↓gestational length (22.1* vs 22.6) **F_1 Offspring LOAEL HDT** HDT: -↓pup wt. (Day 14-21: 14-17%**)	**No F_2 Repro LEL** **F_2 Offspring LOAEL HDT** HDT: -↓pup wt. (Day14-21: 17%-20%**) -pups not evaluated for thymus	No Trigger P LOAEL= F_2 Offspring No C&L concern, if pup wt. used in F_1 Current policy consider F_1= F_2

Table 5. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive LOAEL/LOELs less sensitive than F_2 but with C&L concerns

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
		-pups not evaluated for thymus F_1 Adult LOAEL HDT HDT: -↓ab. thymus (M:38%**, F: 27%**) -↓rel. thymus (M:31%**, F: 26%**) -↓bw gain (35.6g vs 41.3g; 14%^{ns})		
3. Folpet	**P LOAEL HDT** HDT (150 MKD): -↓bw (M/F; 5%*/4%*) -↓ bw M Day1-106 8%** -↓food (7-25%*)	**F_1 Repro LOAEL HDT** HDT: -↓fertility (M: 64.3%* vs 88%) Also, MDT (40 MKD): -↓fertility (M: 69%^{ns} vs 88%) **F_1 Offspring LOAEL HDT** HDT: -↓pup wt. (LD21 13%**) **F_1 Adult LELs**: HDT: -↓bw GD0 10%** -↓bw LD14 6%*	**No F_2 Repro LOAEL** **F_2 Offspring LOAEL HDT** HDT: -↓pup wt. (LD21 16%) Also MDT: -↓pup wt (LD21 14%**)	No Trigger P LOAEL = F_2 LOAEL No C&L concern (F_1 fertility), if F_1 pup wt. used
F_2 less sensitive, C&L concerns identified and with trigger				
4. Dichlobenil	**P LOAEL HDT** HDT (100 MKD): -↓food Week1-10: M:31%***-11%***; F: 28%***-18%***) -↓bw gain Weeks0-10 (M: 11%-64%*; F: 14%-57%*) -↓bw gain GD0-20 (F:13%)	**No F_2 Repro LOAEL** but HDT: -↓implantations (12.3^{ns} vs 14.6) **F_1 Offspring LOAEL MDT** (17.5 MKD): -↓**pup wt.** (Day 1-21; 6-16%***) HDT: -↓pup wt. (Day1-21; 8%-27%***) **F_1 Adult LOAEL HDT** HDT: -↓food Weel0-10: M: 18%***-17%***; F: 17%***-19%***) -↓bw gain (GD0-20: 24%) -↓bw gain Week0-10: 18-25%	**No F_2 Repro LEL** **F_2 Offspring LOAEL MDT** MDT: -↓pup wt. (Day14-21; 9-10%*) HDT: -↓pup wt. (Day 14-21; 19%-22%***) -↓live born (11*** vs 13.7)	Trigger P LOAEL > F_2 LOAEL No C&L concern (F_1 implantations) if pup wt used for trigger or labeling
5. Emamectin benzoate	**No P LOAEL but** HDT (3.6 MKD): -spinal cord degeneration -nerve degeneration -brain degeneration -↓bw (6%*)	**F_1 Repro LOAEL HDT** HDT (1.8 MKD): -↓# pregnant Females (12 vs 20) -↓fertility (48%* vs 80%) -↓fecundity (52%* vs	**No F_2 Repro LEL**	Trigger (P fertility) F LOAEL = F_2 LOAEL No C&L concerns since labeling from P females

Table 5. Study details for the Parental Generation (P), the F_1 generation (F_1), and the F_2 generation (F_2) for those studies with offspring & reproductive LOAEL/LOELs less sensitive than F_2 but with C&L concerns

Chemical	P effects	F_1 effects	F_2 effects (unique)*	F_2 triggered?
	-↑bw gain (4%*) **Also,** -↓**fertility** (67% vs 91%) -↓fecundity (71% vs 91%)	80%) **F_1 Offspring LOAEL HDT (1.8 MKD)** HDT: -tremors (20/22 vs 0/30) -splayed leg (21/22 vs 0/30, hindlimb (21/22 vs 0/33) -↓pup wt. (Day21 29%* males; 33%* females) -altered limb development **Also,** -↓live pups Day 0 (287 vs 424 -↓mean# live pups Day 0 (13.0 vs 14.1*) -live birth index similar (96% vs 94%) **F_1 Adult LOAEL HDT** HDT: -↑bw (3-7%*) -↓bw gain (5%*)	**F_2 Offspring LEL HDT** HDT (1.8 MKD): -↓pup wt. (Day 21 7-9%*)	

8. Triggers Analysis

The number of times an appropriate endpoint was used as a trigger was also investigated in this retrospective analysis. Offspring and reproductive triggers, therefore, were evaluated to determine if a specific endpoint was falsely producing a second generation, a false positive trigger. A false positive trigger was defined as any appropriate endpoint (see Tables 6 and 7) in which a second generation was produced but not needed for risk assessment or classification and labeling because the second generation did not result in a lower NOAEL/LOAEL or identify a unique effect.

Some of the critical reproductive tract effects assessed in the analysis of the parental and F_1 generation are considered sensitive indicators that may signal adverse changes in the reproductive performance of the F_1 animals. These effects were selected to serve as triggers for mating the F_1 animals to produce an F_2 generation by Cooper *et al.* (2006). As described in Cooper *et al.* (2006), the use of a science and risk based approach to determine the need for an F_2 evaluation allows for a tailored approach to testing, reduces the numbers of animals (1200 animals per to generate an F_2), and the resources needed to manage, review, and document the study. Examples of reproductive triggers identified in Cooper *et al.* (2006) include:

- an adverse effect on fertility or fecundity of the parental generation,
- indications of abnormal sexual development of the F_1 pups,
- deaths or evidence of toxicity to the F_1 pups preweaning,
- equivocal effects on F_1 parameters or unusual control data compared to historical background may also trigger a second generation

Please refer to Cooper *et al.* (2006) and the draft OECD guideline for further clarification and discussion of the potential triggers proposed in the Extended One-Generation Reproductive Toxicity Test Guideline.

Table 6: List of potential reproductive endpoints considered for triggering an F_2 generation*

Reproductive Endpoint	Endpoint available in time to determine F_2?	Endpoint evaluated in F_2?	Decision
Fertility Endpoints			
P_1 Estrous Cycle Evaluation	Yes	No	Trigger[1]
P_1 Fertility (# implantations, pregnancy rate, gestational interval)	Yes	No	Trigger[2]
F_1 Litter parameters (litter size, litter weight, sex ratio)	Yes	No	Trigger[3]
F_1 Developmental landmarks (AGD, nipple retention, puberty onset, PPS, VO)	Yes	No	Trigger[4]
F_1 Estrous Cycle Evaluation	Yes	No	Trigger
Developmental Endpoints			
P_1 Reproductive Organ Weights	Yes	Yes	No
P_1 Reproductive Organ Histopathology	Yes	Yes	No
P_1 Andrology (sperm parameters)	Yes	No	No
P_1 Qualitative Ovarian Assessment	Yes	No	No
F_1 Endpoints			
F_1 Reproductive Organ Weights	No	Yes	No
F_1 Reproductive Organ Histopathology	No	Yes	No
F_1 Andrology (sperm parameters)	No	No	No
F_1 Qualitative Ovarian Assessment	No	No	No

*Criteria for establishing triggers taken from Cooper *et al.* 2006 and draft OECD Extended One-Generation Reproduction Toxicity Test Guideline (6-18-2008)
[1]If biologically relevant, dose-related changes in estrous cycle length without overt toxicity in the dams.
[2]In the absence of corresponding, treatment-related reproductive organ histopathology.
[3]If significant, treatment-related decreases in litter size/pup survival are seen in the absence of severe maternal toxicity or lethality.
[4]Dose-related effects; in the absence of body weight-mediated changes in these parameters.

In addition to evaluating the possible impact of chemical exposure on the reproductive system, reproductive toxicity studies also assess effects on offspring development that may occur as a

result of pre- and postnatal exposure. Some of the critical offspring effects observed in the F_1 generation may serve as sensitive indicators of effects that may impact the development of the F_2 offspring. During a meeting of US reproduction and developmental expert toxicologists held on November 2006, a subset of these effects were selected to serve as triggers for mating the F_1 animals to produce an F_2 generation. One of the basis for selecting an offspring effect to serve as a trigger is the potential to detect an enhanced sensitivity (quantitative or qualitative) in the F_2 generation. For instance, a decrease in pup weight (≥5% decrease in absolute or body weight gain) in the absence of maternal body weight decrements suggests that the offspring are more sensitive to the toxic effects of the test compound. Hence, it is plausible that the toxicant may cause a decrease in F_2 pup weights at doses lower than those eliciting decreased body weights in the F_1 animals (i.e., parents of the F_2 pups). In this example, an F_2 generation would be produced to determine if a lower offspring NOAEL could be identified in the F_2 generation (*i.e.,* F_1 NOAEL > F_2 NOAEL). Another criterion for inclusion of an F_1 offspring effect in the set of selected triggers is if the offspring effect is likely to occur due to chemical exposure during a critical time of pre- or postnatal development (*e.g.,* malformations). Some examples of offspring triggers include:

- Decreased F_1 pup weight (≥5% decrease in absolute or body weight gain) in the absence of maternal body weight decreases
- F_1 pup malformations (*e.g.,* hypospadia, cleft palate, hydrocephaly, limb malformations)
- Increased F_1 pup mortality
- Decreased live birth, lactation or viability indices

Please refer to Cooper *et al* (2006) and the draft guideline for further clarification and discussion of the potential triggers proposed in the Extended One-Generation Reproduction Toxicity Test Guideline.

Table 7: List of potential offspring endpoints considered for triggering an F_2 generation*

Endpoint	F_1 or P Endpoint available in time to determine F_2?	Will the endpoint be evaluated in F_2?	Decision
Offspring Endpoints			
↓Maternal (P) bodyweight same dose as ↓F_1 pup bodyweight	Yes	Yes	Not a Trigger[1]
↓ lactation index (PND4-21)	Yes	Yes	Trigger
F_1 pup mortality	Yes	Yes	Trigger[2]
F_1 pup malformations (*e.g.*., hypospadia, cryptocordysm, one eye, large head)	Yes	Yes	Trigger[2]
↓F_1 pup viability index (PND0-4)	Yes	Yes	Trigger[2]

↓F_1 live birth index	Yes	Yes	Trigger[2]
↓F_1 pup bodyweight only	Yes	Yes	Trigger[3]

*Criteria for establishing triggers taken from Cooper *et al.* 2006 and draft OECD Extended One-Generation Reproduction Toxicity Test Guideline (6-18-2008).
[1] Unless F_1 pup body weight decrease at a lower dose than the dose at which maternal body weight decreased.
[2] In the absence of severe maternal toxicity.
[3] If the pup bodyweight decrease is significant and in absence of maternal body weight decrement.

8. Results of Trigger Analysis

The offspring triggers had the highest incidence as well as the highest false positive rate compared to the reproductive triggers. For example, pup weight alone occurred most frequently of all offspring triggers (16%) as well as the highest false positive rate (80%) (Table 7). Lactation and pup viability triggers were the next most common triggers with only a 5-6% false positive rate. Of the reproductive triggers (Table 6), litter size occurred most frequently (6%) and with the highest false positive rate (55%). The other endpoints that were appropriate for use as triggers typically occurred less than 1% of the time.

The number of animals saved by not producing a second generation was also evaluated. There were 137 studies in which a trigger was not identified and the second generation did not contribute endpoints essential to classification and labeling or quantitatively in risk assessment. It can be assumed that approximately 1,200 animals would be saved for each reproductive study in which a second generation is not produced. Therefore, approximately 165,000 animals would have been saved if these 341 pesticides had been evaluated using the Extended One-Generation Guideline with the appropriate use of the proposed triggers. .

Table 8. The incident of specific triggers and false positive rate from the 341 pesticide studies in the retrospective analysis

TYPE OF TRIGGER	INCIDENT OF TRIGGER (%)	FALSE POSITIVE INCIDENT OF TRIGGER (%)
Offspring Triggers		
Lactation/lactation index	18/341 (5%)	10/18 (56%)
Pup weight/pup weight gain	55/341 (16%)	44/55 (80%)
Viability/viability index	19/341 (6%)	13/19 (68%)
Reproductive Triggers		
Anogenital Distance	2/341 (<1%)	1/2 (50%)
Litter size/litter weight	22/341 (6%)	12/22 (55%)
P gestational index	3/341 (<1%)	3/3 (100%)
P gestational interval	10/341 (3%)	4/10 (40%)

P mating	2/341 (<1%)	2/2 (100%)
P precoital interval	1/341 (<1%)	1/1 (100%)
P implantation	1/341 (<1%)	1/1 (100%)
P implantation + litter size	2/341 (<1%)	0/2
PPS	1/341 (<1%)	1/1 (100%)
VO	2/341 (<1%)	1/2 (50%)
VO/PPS	1/341 (<1%)	0/1

9. Conclusions from this Retrospective Analysis

The current retrospective analysis utilized the newest relational database, ToxRefDB, to focus on the potential sensitivities of the second generation of multi-generational reproduction studies. Unlike the retrospective analysis in 2008, the US EPA evaluated 350 studies which consisted of 341 pesticides; 21 from PMRA and 320 OPP as well as the same 9 industrial compounds. This retrospective analysis considered not only the quantitative and qualitative nature of the effects in the F_1 and F_2 generation, but also considered endpoints that would be critical for classification and labeling by the EU.

This revised retrospective analysis supports the proposed Extended One-Generation Reproductive Toxicity Test Guideline. None of the F_2 effects from the 9 industrial compounds would have been missed for risk assessment or classification and labeling. For pesticides, application of the proposed reproductive and offspring triggers is protective in all but two of the studies in this analysis. Furthermore, 13 studies were identified with F_2 only reproductive or offspring effects that may have been important for classification labeling. However, 6 of these 13 studies were pup weight only in the F_2.

The most common reproductive triggers were litter parameters while the most common offspring trigger was decreased F_1 pup weight in the absence of parental effects. Furthermore, the number of studies that would not have required an F_2 generation would result in animal savings of almost 165,000 animals.

Finally, results of this revised retrospective analysis suggest that the Extended One-Generation Reproductive Toxicity Test Guideline would adequately protect for not only potential sensitive effects in the second generation, but also for endpoints essential to classification and labeling. In fact, this retrospective analysis would suggest that the proposed triggers would conservatively produce a second generation. Further examination into the proposed list of triggers by Cooper et al. demonstrated the propensity to trigger a false positive F_2 generation. This false positive rate further supports the projection that F_2 effects will not be missed by the proposed triggers in either the P or F_1 generation.

Acknowledgements

We thank Gregory Akerman, Lisa Austin, Karlyn Bailey, Gerome Burke, Daniel Corum, Tameka Edwards, Nancy McCarroll, Rob Mitkus, Whang Phang, Daniel Rotroff, and Linda Taylor for their invaluable contribution of data entry into ToxRefDB as well as Aldert Piersma, Lauri Stachiw, and Deborah Ramsingh for contribution of data to this retrospective analysis.

References

Cooper et al. (2006). A tiered approach to life stages testing for agricultural chemical safety assessment. Critical Reviews in Toxicology. 36:69-98.

Martin et al. (2009a) "Profiling Chemicals Based on Chronic Toxicity Results from the U.S. EPA ToxRefDB Database" Environmental Health Perspectives; 117:392-399.

Martin et al. (2009b) "Profiling the Reproductive Toxicity of Chemicals from Multigeneration Studies in the Toxicity Reference Database (ToxRefDB)" Toxicological Sciences 110:181–190.

National Research Council (NRC) (2007). "Toxicity Testing in the 21st Century. A Vision and a Strategy." The National Academies Press, Washington, DC. 196 pp.

"Test No. 416: Two-Generation Reproduction Toxicity Study" (2001) *OECD Guidelines for the Testing of Chemicals* 1(4)**:**1-13.

US Expert Group Meeting in November 15-17th 2006. US EPA Research Triangle Park Raleigh/Durham, NC.