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REPORT OF THE THIRD OECD BIOPESTICIDES STEERING GROUP SEMINAR ON CHARACTERISATION AND ANALYSES OF BOTANICALS FOR THE USE IN PLANT PROTECTION PRODUCTS

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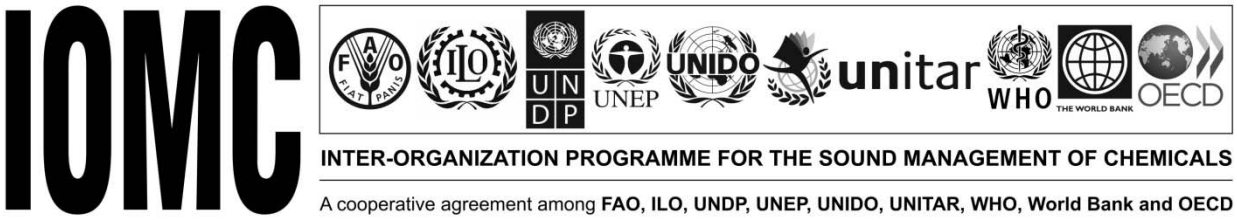
Report of the Third OECD BioPesticides Steering Group Seminar on Characterisation and Analyses of Botanicals for the Use in Plant Protection Products



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Paris 2012

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OECD Guidance for Country Data Review Reports on Plant Protection Products and their Active Substances-Monograph Guidance (1998, revised 2001, 2005, 2006)
OECD Guidance for Industry Data Submissions on Plant Protection Products and their Active Substances-Dossier Guidance (1998, revised 2001, 2005)
Report of the Pesticide Aquatic Risk Indicators Expert Group (2000)
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Report of the 2nd OECD Workshop on Pesticide Risk Indicators (1999)
Guidelines for the Collection of Pesticide Usage Statistics Within Agriculture and Horticulture (1999)
Report of the [1st] OECD Workshop on Pesticide Risk Indicators (1997)
Report of the OECD/FAO Workshop on Pesticide Risk Reduction (1995)

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FOREWORD

This report presents the outcomes of an OECD Biopesticide Seminar on issues related to the characterisation and analyses of botanicals for the use in plant protection products, which took place on 30 March 2011 at OECD, in Paris, France. This Seminar was held back-to-back with the annual meeting of the BioPesticides Steering Group (BPSG), a sub-group of the OECD Working Group on Pesticides (WGP). The Seminar was the third one of a series of BPSG seminars that focus on Biopesticide-related issues of interest to OECD member countries' governments.

The Seminar was chaired by Jeroen Meeussen (European Commission), Chairman of the BPSG. Thirty eight experts from 11 OECD countries, the European Commission, the European Food Safety Authority (EFSA), IBMA (International Biocontrol Manufacturers Association) and research institutes participated in the Seminar. The list of participants is in Annex 2.

Botanicals are also known as plant extracts and they are used as e.g. insecticide, fungicide, repellent, plant strengthener. They cover a wide variety of very different types of substances, with very different properties and biological activity.

The objectives of the Seminar were to:

(i) identify key issues and challenges in the area of 'botanicals';

(ii) provide updates of national and international activities and initiatives in the area of 'botanicals';

(iii) exchange information on OECD countries' current activities in the area of 'botanicals';

(iv) exchange information and needs between scientists and stakeholders;

(v) suggest and discuss options of further steps for OECD countries and key stakeholders in OECD and non-OECD countries to address the identified issues; and,

(vi) recommend possible further steps for OECD.

The Seminar was organised in a way that there was a short discussion after each (set of) presentation(s). The presentations addressed the experience and perspectives of research institutes, governments and other stakeholders (such as industry). The Seminar participants' conclusions, observations and recommendations are included in the first part of this report. The Seminar programme is presented in Annex 1. The abstracts of presentations are compiled in Annex 3, while presentations are provided in Annex 4.

The draft Seminar report was approved out-of-session by the Working Group on Pesticides by written procedure finishing on 27 August 2012.

This document is being published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology, which has agreed that it be unclassified and made available to the public.

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INTRODUCTION

This report presents the results and recommendations of an OECD Seminar on issues related to the characterisation and analyses of botanicals for the use in plant protection products. This one-day Seminar, held on 30 March 2011, was chaired by Jeroen Meeussen (European Commission), Chairman of the OECD BioPesticides Steering Group (BPSG), and took place at OECD, Paris, France.

This Seminar was the third in a series of Seminars on biopesticides organised by the OECD BioPesticides Steering Group (BPSG). The BPSG is a sub-group of the OECD Working Group on Pesticides (WGP).

BPSG Seminars focus on key issues on biopesticides of interest to OECD governments. "*Characterisation and Analyses of Botanicals for the use in Plant protection Products*" was selected as the topic of this Seminar considering its significance for the registration of biopesticides and to take the first steps to resolve sciences issues associated with registering botanicals. Botanicals are also known as "plant extracts" and include a wide variety of substances with different properties and biological activity. The Seminar focused on issues like description of plant material; extraction methods; identification and analytical methods; methods of manufacture; quality control. On the longer term the aim is to develop a comprehensive guidance document on botanicals.

PARTICIPANTS

People attending the OECD Seminar included:

- members of the OECD Working Group on Pesticides and BioPesticides Steering Group;
- invited experts from key stakeholder groups such as industry (IBMA) and manufacturers of micro-organisms;
- invited experts from research institutes (academia); and,
- regulators and evaluators from governmental bodies.

A participant list is provided in Annex 2.

PURPOSE AND SCOPE OF THE SEMINAR

The main objectives of the Seminar included:

- to identify key issues and challenges in the area of 'botanicals';
- to provide updates of national and international activities and initiatives in the area of 'botanicals';
- to exchange information on OECD countries' current activities in the area of 'botanicals';
- to exchange information and needs between scientists and stakeholders.
- to suggest and discuss options of further steps for OECD countries and key stakeholders in OECD and non-OECD countries to address the identified issues; and,
- to recommend possible further steps for OECD.

In particular the following issues were discussed during the Seminar:

- For which substances should identification and analytical methods be required? E.g. for the active substance(s), and/or for those substances which are mainly responsible for the effects on the target pest and/or of those which are known to be of concern or potentially critical.
- Which information should be included in the description of the method of manufacture (e.g. information on the plant material of origin, such as the plant parts used, the physiological ages, harvesting times, growing conditions (like nutrient, water or light availability), regions and variety/genotypes/chemotype (if known), the range of materials used, processing).
- The EU Draft Working Document SANCO/10472/2003 covers only water and ethanol extracts, and a limited number of plant parts. The possibility to broaden its scope to cover other or even all extraction methods and all plants and plant parts should be discussed. As a result of the development of a broadened scope, a tiered system will be needed.
- If a plant extract has been used in plant protection or for other purposes without evidence of adverse effects, its history of safe use should be adequately taken into account. This includes the use of information from the literature and from other public sources.

STRUCTURE OF THE SEMINAR

The Seminar programme is provided in Annex 1. Invited speakers included:

- International experts in this field;
- Government representatives;
- Representatives from industry (IBMA); and
- Representatives from research institutes.

Due to the diversity of issues addressed by the speakers, short discussions were held after each (set of) presentation(s).

SUMMARY OF PRESENTATIONS AND DISCUSSIONS

All abstracts and slides of presentations are presented in Annexes 3 and 4.

Introduction to the Seminar by the Chair, Jeroen Meeussen, European Commission [PPT1]

The Chair gave a presentation on the OECD, the work of OECD-BPSG and general introduction to the Seminar on 'botanicals'. He explained that the Seminar would focus on issues related to identification, manufacturing process –including quality control- and analytical methods of botanicals. However, it was not intended to discuss issues like mode of action and toxicology.

Research Institutes Experience and Perspectives

The potential of botanicals in plant protection, by Lucius Tamm (Research Institute of Organic Agriculture [FiBL], Frick; Switzerland) [PPT2a]

Lucius Tamm gave an overview of reported uses of botanicals. He explained that extracts of plants have been used to protect plants from organisms for many centuries. He gave examples of insecticides, repellents, fungicides, herbicides and adjuvants. It was indicated that it is difficult to get a precise and comprehensive overview of the status of what actives are used around the world and suggested that it would be useful to have a database of what active substances are approved; ideally this should also include actives in China and India.

The next generations of botanicals are currently being researched by public institutions. There is increasing interest of the industry in the development of novel botanicals. A number of IBMA member companies work in this area. However, it does not include any companies from India and China. Current trends include the 'rediscovery' of traditional uses, the screening of substance libraries to identify novel lead substances, the refined formulation to improve efficacy, and the systematic development of blends of active substances. Botanicals have a number of potential benefits such as in resistance management, IPM, low/no residues systems, organic agriculture, and subsistence/home-garden situations.

It was pointed out that limited efficacy can often be a problem due to a number of reasons such as activity, UV stability, rain fastness, limited plant uptake and limited shelf life. However, botanicals have great potential in sustainable, resilient production systems (i.e. IPM, organic, low input, low residues). It was suggested that the registration requirements need to be adapted to the properties of botanicals and it was highlighted that the quest for more botanicals was intensifying.

Experiences from the development and field testing of two botanicals by Annegret Schmitt (Institute for Biological Control -JKI, Darmstadt; Germany) [PPT2b]

The presentation provided an overview of experiences gained during the development and testing of two botanicals, Giant knotweed (*Fallopia sachalinensis*) and Sweetwood (liquorice) (*Glycyrrhiza glabra*).

It was explained that Giant knotweed (*Fallopia sachalinensis*) was screened by BBA/JKI and developed together with BASF. It is sold as a plant strengthener. In screening it was shown to be active against a number of plant diseases. The mode of action appears to be related to the stimulation of the plant's site-specific defence mechanisms. The active compounds identified in the mode of action are physcion, physcion-glycoside and other unidentified compounds.

It was indicated that Sweetwood (*Glycyrrhiza glabra*) has high efficacy in protected crops, but lower efficacy in open field. It was explained that this was an example of variable efficacy, so the factors that cause this variability – rain, UV stability, crop cultivar susceptibility, etc. – would need to be established.

In the discussion that followed the presentation it was highlighted that Giant knotweed is registered as a plant strengthener, but this was not an 'approval category' in the EU. The European Commission indicated that the plant strengthener category was created as a result of a grey area under the Directive 91/414/EEC. However, it was agreed that how these legal issues had arisen should not form part of the topic of this Seminar.

It was also highlighted that the US-EPA require product identification data, a standard toxicology '6-pack tox' and, as a protected use, there were no environmental requirements.

Natural extracts characterization and application of analytical methods for botanical quality determination by Cédric Bertrand (University of Perpignan; France) [PPT3]

Cédric Bertrand gave a presentation on natural extract characterization and provided an overview of analytical methods for botanical quality determination. He explained that plant extracts usually consist of a mixture of a wide range of chemical compounds. Natural extracts are very complex mixtures and can have huge variability. Therefore to characterise them, there is a need for: i) metabolomics approaches combined to bioassay leading to identification of biomarker or fingerprint, and ii) a bio-guided purification or semi-purification leading to the bioactive compounds identification.

The question was asked whether the bioactive compound could be identified from such techniques. It was indicated that it was possible to establish a biomarker, but it might not necessarily be the bioactive compound. It was suggested that the technique only gives a method for quality assurance and was not a measure of efficacy. It was pointed out that regulators ideally needed to have techniques that completely characterise the active so what is causing effects could be identified, not only in terms of efficacy but also regarding the effects on non-targets.

It was indicated that biomarkers have been used for some compounds. It was also suggested that there was a need for a harmonised approach. Therefore, regulators and industry need to discuss what is needed and what is feasible.

Government Experience and Perspectives

EU Draft Working Document on Plant Extracts SANCO/10472/2003 - key points and practical experiences by Thierry Mercier (French Agency for Food, Environmental and Occupational Health & Safety [ANSES], Paris; France) [PPT4]

Thierry Mercier gave a presentation providing an overview of the EU Draft Working Document on Plant Extracts (SANCO/10472/2003). He indicated that it was only a draft and not agreed, but it has been used as a basis for evaluating some of the List 4 Review plant extract compounds. It was first produced as a national level document in France and then presented into the EU arena.

The goal of the document was to propose on a weight-of-evidence basis a tiered approach to the data requirements for active substances of plant protection products made from plants or plant extracts.

The document provides definitions of what is considered to be a plant extract and also by what method they are extracted. The data requirements are then divided into two categories depending on the type of extract that is being considered. See document SANCO/10472/2003 and slides for details.

The EU Fenugreek seed powder evaluation was used as an example of what was provided in the dossier. This was made up of a mix of data and public literature. The conclusion was that it was a useful document and that the amount of data (that needs to be) provided was proportional to the risk. However, it was suggested that the document was not applicable to all plant extracts. It was also highlighted that it needs to be updated.

Industry suggested that there is a need to broaden the range of extraction methods that are considered in the guidance. It was agreed that the document needed updating, and it was highlighted that a risk-proportional approach was needed. The European Commission suggested that the document could perhaps be used as a basis of expanding the guidance to cover wider compounds and incorporate input from other OECD countries.

EFSA's experiences in evaluating 'botanicals' by Herman Fontier (European Food Safety Authority [EFSA], Parma; Italy) [PPT5]

Herman Fontier gave a presentation providing an overview of EFSA's role in the EU process, legal context and experience with plant extracts so far. It was highlighted that there are no specific data requirements for botanicals and therefore the standard chemical requirements have to be considered. But it was stressed that data requirements could always be waived if appropriate rationale was provided for the non-submission of data.

EFSA's experience included the drafting of two examples of two conclusions (Fenugreek seed powder and azadirachtin), neither of these compounds however are covered by the SANCO/10472/2003 draft guidance as they are not listed in the document. Considerations of a number of other 4th List Review ('Green Track') plant extracts are also ongoing. There have also been examples of EFSA conclusions made on synthesised compounds that also occur naturally.

The word 'botanicals' can cover a wide variety of very different types of substances with very different properties (some commonly consumed and some being very toxic). It does seem that

some of the data requirements are not always appropriate. However, establishing specific sets of data requirements may be difficult as the chemicals can be very different. Guidance on waivers may be possible to provide as more experience is gained. Experience so far has highlighted that the non-availability of radio-labelled material can be an obstacle for risk assessment. Normal risk assessment methodology is also not always adequate and more guidance is needed. There are often higher levels of uncertainty. Therefore these factors often combine to result in a need for risk management measures.

Industry supported the need for a lesson-learned exercise after completion of the EU 4th List review process involving regulators, EFSA and IBMA/industry.

Clarification was given on whether the SANCO/10472/2003 draft document was applicable to new active substances and compounds not included in the list contained within the document. The European Commission indicated that the document remained only as a draft as it was not noted within the Standing Committee.

Government and Stakeholder Experiences and Perspectives

OECD-countries and industry presented their views:

Neem/Margosa extract -and its constituents – Experience in EU evaluation and registration - Hubertus Kleeberg (Trifolio-M; Germany) [PPT6]

It was explained that 5-10 candidate actives are usually considered from traditional literature to select one substance to take forward. In addition, it was indicated that it takes approximately 12 million Euro to develop and register a new plant extract. Background on the marker compounds, identification, product formulation, mode of action (which includes feeding repellency, fertility reduction) and available data contained within the "NeemAzal®" dossier were provided.

Some details on the development of "Quassin" extract were also provided. This product is included in US GRAS-List (Generally Considered As Safe). In Germany, it is used in beverages and cosmetics.

Margosa extracts – experiences in the evaluation under the Plant Protection Products Directive and the Biocides Directive by Vera Ritz (Federal Institute for Risk Assessment [BfR], Berlin; Germany) [PPT7]

Vera Ritz provided an overview of experiences of Germany as a RMS for Neem/Margosa extracts under PPP (91/414/EEC) and Biocides (98/8/EC) Directives. It was highlighted that there is an agreed guidance document for plant extract biocidal products (entitled *'How to deal with extracts and oils of plant or animal origin'* and endorsed at the 23rd CA meeting, November 2006) that covers the naming, identity and methods of extracts. In this document the active substance is considered as the whole mixture of all constituents.

A comparison of the approaches taken regarding the technical specification under the two directives was provided. It was concluded that there was a need for better harmonisation between the PPP and Biocide approaches. The probable explanation for the different approaches is the lack of guidance on PPPs. There has also been a switch from the 'lead substance' concept to the 'whole

extract' concept during the Biocide evaluation process. It was generally considered that both current approaches were not ideal and there would be a need for further discussions to establish an approach that meets the needs of both the regulator and industry.

Algae extracts and Laminarin – experience in EU evaluation and registration by Jean-Marie Joubert (Laboratoires Goëmar; France) [PPT8]

Dominique Ambrosi provided a comparison of the experiences gained in the EU process during the consideration of algae extract (extracted from *Ascophyllum nodosum*) and laminarin (extracted from *Laminaria digitata*).

It was highlighted that laminarin was included as a biostimulant Algae extract, which is also listed on Annex I, is included as a Plant Growth Regulator (PGR).

Characterisation is the key issue as there seems to be variability in the approaches been taken and without clear guidance industry cannot move forward. It was suggested that may be the biocide 'whole approach' is a pragmatic way forward. Regulators expressed reservations regarding this approach as it does not necessarily link the material with material that has been tested. However it was indicated that regulators are content to give some flexibility or alternatively for actives to be indicated in a range, as it is accepted that there will be some variation with plant materials.

It was also suggested that some of the variation may come from the method of manufacturing i.e. method of extraction could provide the explanation for the differences in the specification.

Updates to PMRA's regulatory proposal on non-conventional pesticide registration by Brian Belliveau (Health Canada Pest Management Regulatory Agency, Ottawa, Canada) [PPT9]

Brian Belliveau provided an update on the Canadian proposal to replace the 'Registration Guidelines for the registration of Low Risk Biochemicals and Other non-conventional pesticides' with updated guidelines, Regulatory Proposal 2010-06, 'Guidelines for the Registration of Non-Conventional Pest Control Products'.

The change was a result of stakeholder comments. It is hoped that the new guidelines provide more flexibility. Under the guideline products eligible for consideration under this proposal must meet some characteristics listed in the guideline. Substances eligible for review under this proposal could include: food items, extracts, preservatives or additives; plant extracts and oils; commodity chemicals that have a range of non-pesticidal uses; fertilizer or other plant growth supplements, commonly used in the agricultural sector; or inert materials.

The approach taken with respect to the technical specification is similar to those taken for conventional chemicals. The active must be identified and the active is considered as the component that is identified as being involved in the mode of action. Pre-submission consultation is encouraged and flexibility is provided on a case-by-case basis. For plant extracts a method is required to determine the composition of the product. Major and representative components in each extract/oil must be determined and quantified. Standard and literature methods are acceptable.

US experiences with the biochemical/botanical pesticide system, including the 'biochemical classification' system by William Schneider (Biopesticides and Pollution Prevention Division, EPA, Arlington; USA) [PPT10]

William Schneider provided an overview and update of the US experiences with biochemical/botanical pesticide system. He also outlined the tiered approach taken with respect to the data requirements. The presentation covered details of the 'biochemical classification' system and how the Biochemical Classification Committee takes decisions on classification. Information needed for a successful classification was described.

Chenopodium extract and its constituents – experience with US evaluation and registration and approach to EU by Nicholas Wright (AgraQuest Inc.; USA) [PPT11]

Nicholas Wright gave a presentation outlining AgraQuest's experience with *Chenopodium* extract in the US and EU. The source plant is used as a spice/flavouring and folklore medicine. The essential oil contains numerous terpenoid compounds. Composition varies greatly depending on plant variety, growing conditions and growth stage. Oil is extracted by steam distillation (further processing is required in the US to gain registration). In the US there were a number of rejected attempts to get the compound classified. It was rejected based on insufficient characterisation and presence of ascaridole (a purported toxic compound). Characterisation was based on marker compound approach. Achievable limits for each marker to account for plant variability had to be established. Analytical methods had to be refined and the manufacturing process needed to include ascaridole removal. AgraQuest subsequently developed a blended version of the compound. This is registered in the US as 'Terpene Constituents of Extract of Chenopodium ambrosiodes near ambrosiodes (ECANA) as synthetically manufactured.

AgraQuest are currently in the process of making a submission to the EU for the blended material. They have identified a number of similarities and differences in the registration process in the US and EU.

SUMMARY OF THE DISCUSSION, IDEAS FOR FOLLOW-UP, RECOMMENDATIONS FOR POSSIBLE FURTHER OECD WORK

A number of key points were summarised as follows:

- There is a definition problem of what the compounds should be called – biochemicals, botanicals, plant extracts etc.. This include possible legal implications depending on the terminology used (e.g. growth regulators, biostimulants, plant strengtheners).

- It is clear that the term 'botanical' covers a very diverse group of compounds therefore, depending on the characteristics of an active substance, flexibility and consideration on a case-by-case may be needed.

- It is also clear that the issue of specification for 'botanicals' is more complex than for conventional chemicals.

- There are problems of how to provide a technical specification. Plant extracts are complex mixtures of a wide range of chemical compounds and biological activities. Several approaches were discussed:
  - Biomarker approach in which the key compounds of the bioactive plant extract are determined. This approach can be used for quality assurance. Question remains how this is related to the efficacy of the substance/product;
  - Biocide 'whole extract' approach, but this may lead to 'variability issues';
  - Blending (technical mixture of active substances) may be an option.

- EU Draft Working Document on Plant Extracts SANCO/10472/2003 – clearly needs to be updated. In updating this document there is the possibility to take on board the guidance documents available from Canada, USA and the EU biocides area. The document should also cover other extraction methods than those using water/ethanol.

- History of use needs to be taken into account as well as natural occurrence, background levels, and other uses (herbal drugs, animal feed, human food).

- It is still unclear how to deal with synthesised analogues or mimics, which are nature identical but synthesised versions. Should they be treated as 'conventional chemicals'? In this respect it should also be mentioned that radio-labelling techniques are impossible to use for plant extracts. A more balanced approach is needed.

- The 'lead component' concept in which studies will be performed with the whole extract using a certain compound as analytical lead substance, needs to be further explored.

- The use of 'botanicals' can be promoted as part of IPM strategies.

ANNEX 1

BioPesticides Steering Group (BPSG)
Seminar on "Characterisation and Analyses of Botanicals for the Use in Plant Protection Products"

Wednesday 30 March 2011
OECD, Paris, France (2 rue André Pascal, 75016 Paris)
Conference Center Room 15

Seminar Programme

Chair: Jeroen Meeussen, European Commission

9.00 – 9.30 PPT1	**Introduction** • Purpose and structure of the seminar • Tour de table to introduce participants • Presentation on the OECD and the work of OECD-BPSG and general introduction to the seminar on 'botanicals' *by Jeroen Meeussen, BPSG Chair* (European Commission)
9.30 - 10.15 PPT2a PPT2b	**Research Institutes Experience and Perspectives** - Joint presentation: - The potential of botanicals in plant protection *Lucius Tamm* (Research Institute of Organic Agriculture [FiBL], Frick; Switzerland) - Experiences from the development and field testing of two botanicals *Annegret Schmitt* (Institute for Biological Control -JKI, Darmstadt; Germany)
10.15 – 10.45	**Coffee break**
10.45 – 11.15 PPT3	- Natural extracts characterization and application of analytical methods for botanical quality determination *Cédric Bertrand (*University of Perpignan; France)
11.15 - 12.15 PPT4	**Government Experience and Perspectives** - EU Draft Working Document on Plant Extracts SANCO/10472/2003 - key points and practical experiences. *Thierry Mercier* (French Agency for Food, Environmental and Occupational Health & Safety [ANSES], Paris; France)

PPT5	- EFSA's experiences in evaluating 'botanicals' *Herman Fontier* (European Food Safety Authority [EFSA], Parma; Italy)
12.15 – 13.45	**Lunch break**
13.45 – 15.15	**Government and Stakeholder Experiences and Perspectives** OECD-countries and industry will present their views:
PPT6	- Neem/*Margosa* extract¨-and its constituents – experience in EU evaluation and registration *Hubertus Kleeberg* (Trifolio-M; Germany)
PPT7	- *Margosa* extracts – experiences in the evaluation under the Plant Protection Products Directive and the Biocides Directive *Vera Ritz* (Federal Institute for Risk Assessment [BfR], Berlin; Germany)
PPT8	- Algae extracts and Laminarine – experience in EU evaluation and registration *Jean-Marie Joubert* (Laboratoires Goëmar; France)
15.15 – 15.45	**Coffee break**
15.45 – 17.15	- Updates to PMRA's regulatory proposal on non-conventional pesticide registration
PPT9	*Brian Belliveau* (Health Canada Pest Management Regulatory Agency, Ottawa, Canada)
PPT10	- US experiences with the biochemical/botanical pesticide system, including the "biochemical classification" system *William Schneider* (Biopesticides and Pollution Prevention Division, EPA, Arlington; USA)
PPT11	- Chenopodium extract and its constituents – experience with US evaluation and registration and approach to EU *Nicholas Wright* (AgraQuest Inc.; USA)

17.15 – 17.30	**Summary of the Discussion, Ideas for Follow-up, Recommendations for possible further OECD work** **Discussion** • For which substances should identification and analytical methods be required? E.g. for the active substance(s), and/or for those substances which are mainly responsible for the effects on the target pest and/or of those which are known to be of concern or potentially critical. • Which information should be included in the description of the method of manufacture (e.g. information on the plant material of origin, such as the plant parts used, the physiological ages, harvesting times, growing conditions (like nutrient, water or light availability), regions and variety/genotypes/chemotype (if known), the range of materials used, processing). • The EU Draft Working Document SANCO/10472/2003 covers only water and ethanol extracts, and a limited number of plant parts. The possibility to broaden its scope to cover other or even all extraction methods and all plants and plant parts should be discussed. As a result of the development of a broadened scope, a tiered system will be needed. • If a plant extract has been used in plant protection or for other purposes without evidence of adverse effects, its history of safe use should be adequately taken into account. This includes the use of information from the literature and from other public sources. *Instead of presentations in the morning and a round table discussion in the afternoon it is proposed to have a short discussion after each (set of) presentation(s) due to the diversity of issues.*
17.30	End of the Seminar

ANNEX 2

LIST OF PARTICIPANTS

BioPesticides Steering Group (BPSG)
Seminar on "Characterisation and Analyses of Botanicals for the Use in Plant Protection Products"

Wednesday 30 March 2011
OECD, Paris, France

Belgium/Belgique	M. Jérémy DENIS Service Pesticides & fertilizers FPS Public Health
Canada	Dr. Brian H. BELLIVEAU Head Microbial and Biochemical Evaluation Section Pest Management Regulatory Agency Health Canada Health Effects Division
Denmark/Danemark	Dr. Henrik Frolich BRODSGAARD Technical Administrator, Pesticides & Genetechnology Pesticides & Genetechnology Danish Environmental Protection Agency
France	Mme Anne DUVAL Chargée de mission affairs européennes et internationals Direction générale de l'alimentation (DGAL) Sous-direction de la qualité et de la protection des végétaux (SDQPV) Ministère chargé de l'agriculture (MAAPRAT)
	M. Thierry MERCIER Directeur Adjoint direction des produits réglemés ANSES-DPR
Germany/Allemagne	Mr. Bilgin KARAOGLAN Environmental Risk Assessment and Management of Plant Protection Products, EU Active Substances Program Federal Environment Agency(UBA)
	Dr. Eckhard KOCH Expert Julius Kuehn-Institute, Institute for Biological Control

	Dr. Vera RITZ Chemicals Safety Federal Institute for Risk Assessment (BfR)
	Dr. Annegret SCHMITT Expert Julius Kuehn-Institute, Institute for Biological Control
Hungary/Hongrie	Dr. János MOLNÁR Senior Counsellor Department of Plant Protection and Soil Conservation Ministry of Rural Development
Mexico/Mexique	Ms. Rocío ALATORRE EDEN WYNTER Risk Analysis Commissioner Ministry of Health Federal Commission for Protection from Sanitary Risks
	Mr. José Alberto ROSALES CASTILLO Executive Subdirector of Risk Factors Federal Commission for the Protection against Sanitary Risks
Netherlands/Pays-Bas	Mrs. Marion PENNEKAMP Scientific Assessor Board for the Authorisation of Plant Protection Products and Biocides
New Zealand/Nouvelle-Zélande	Mr. John REEVE Principal Adviser (Toxicology) New Zealand Ministry of Agriculture ond Forestry Science Information and Risk Directorate
United Kingdom/Royaume-Uni	Mr. John DALE Project Manager Chemicals Regulation Directorate Health and Safety Executive
	Dr. Darren MINGO Project Manager Chemicals Regulation Directorate Health and Safety Executive
United States/États-Unis	Dr. William SCHNEIDER Biopesticides and Pollution Prevention Division (7511) US Environmental Protection Agency
EU/UE	Mr. Jeroen MEEUSSEN DG SANCO Commission Européenne - SANCO - Santé et protection des consommateurs

	Mr. Wolfgang REINERT Administrator Health & Consumers Directorate-General European Commission
European Food Safety Authority (EFSA). /Autorité européenne de sécurité des aliments (AESA)	Mr. Herman FONTIER EFSA European Food Safety Authority
International Biocontrol Agent Manufacturers Association	Dominique AMBROSI Ambrosio Scientific Consulting
	Mr. David BERNAD Technical Dept. Daymsa Desarrollo Agricola y Minero SA
	Mr. Sergio FRANCESCHINI Regulatory Affairs Director International Biocontrol Manufacturers Association Intrachem Production S.r.l.
	Dr. Roma GWYNN Biocontrol Consultant Rationale Biopesticide Consultants
	M. Ulf HEILIG International Relations Regulatory Affairs International Biocontrol Manufacturers Association (IBMA)
	Ms. Maria HERRERO Director of Regulatory Affairs Manager Valent BioSciences Corporation/ Sumitomo Chemicals
	Hubertus KLEEBERG Trifolio-M
	Ms. Denise MUNDAY Vice-President IBMA SCAE-Valent BioSciences Sarl International Biocontrol Manufacturers Association (IBMA)
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Mme Sylvie PORET
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Other experts

Dr. Cédric BERTRAND
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Mr. Jean-Marie JOUBERT
Directeur R&D
Laboratoires Goëmar

Dr. Lucius TAMM
Crop Protection & Biodiversity
Research Institute of Organic Agriculture FiBL

ANNEX 3

ABSTRACTS OF PRESENTATIONS

Introduction: Presentation on the OECD and the work of OECD-BPSG and general introduction to the Seminar on 'botanicals'
By Jeroen Meeussen, BPSG Chair (European Commission)

The potential of botanicals in plant protection
By Lucius Tamm (Research Institute of Organic Agriculture [FiBL], Frick; Switzerland)

Experiences from the development and field testing of two botanicals
By Annegret Schmitt (Institute for Biological Control -JKI, Darmstadt; Germany)

Natural extracts characterization and application of analytical methods for botanical quality determination
By Cédric Bertrand (University of Perpignan; France)

EU Draft Working Document on Plant Extracts SANCO/10472/2003 - key points and practical experiences.
By Thierry Mercier (French Agency for Food, Environmental and Occupational Health & Safety [ANSES], Paris; France)

EFSA's experiences in evaluating 'botanicals'
By Herman Fontier (European Food Safety Authority [EFSA], Parma; Italy)

Neem/Margosa extract¨-and its constituents – experience in EU evaluation and registration
By Hubertus Kleeberg (Trifolio-M; Germany)

Margosa extracts – experiences in the evaluation under the Plant Protection Products Directive and the Biocides Directive
By Vera Ritz (Federal Institute for Risk Assessment [BfR], Berlin; Germany)

Algae extracts and Laminarine – experience in EU evaluation and registration
By Jean-Marie Joubert (Laboratoires Goëmar; France)

Updates to PMRA's regulatory proposal on non-conventional pesticide registration
By Brian Belliveau (Health Canada Pest Management Regulatory Agency, Ottawa, Canada)

US experiences with the biochemical/botanical pesticide system, including the "biochemical classification" system
By William Schneider (Biopesticides and Pollution Prevention Division, EPA; USA)

Chenopodium extract and its constituents – experience with US evaluation and registration and approach to EU
By Nicholas Wright (AgraQuest Inc.; USA)

Introduction
Presentation on the OECD and the work of the OECD-BioPesticides Steering Group (BPSG) and general introduction to the Seminar on 'botanicals'

By Jeroen Meeussen

(*European Commission, DG SANCO)*

[PPT 1]

In 1961 the Organisation for Economic Co-operation and Development (OECD) was established with a trans-Atlantic and then global reach. Today the OECD has 33 member countries. More than 70 developing and transition economies are engaged in working relationships with the OECD.

OECD is a forum in which governments work together to address the economic, social and environmental challenges of interdependence and globalisation. OECD is also a provider of comparative data, analysis and forecasts to underpin multilateral co-operation.

The OECD work on agricultural pesticides (i.e. chemical and biological pesticides) aims to help member countries improve the efficiency of pesticide control, share the work of pesticide registration and re-registration, minimise non-tariff trade barriers and reduce risks to human health and the environment resulting from their use. In support of these goals, the Pesticides Programme has undertaken work to:

(i) identify and overcome obstacles to work-sharing;
(ii) harmonise data requirements and test guidelines; and
(iii) harmonise hazard/risk assessment approaches.

The BioPesticides Steering Group (BPSG) was established by the WGP in 1999 to help member countries harmonise the biological pesticides assessment and improve the efficiency of control procedures. Biological pesticides involve: microbials, pheromones and other semiochemicals, plant extracts (botanicals) and invertebrates as biological control agents. The BPSG has been chaired by Canada since its inception and by The Netherlands from mid 2005 onward. The first tasks of the BPSG consisted of:

(i) reviewing regulatory data requirements for three categories of biopesticides (microbials, pheromones and invertebrates); and
(ii) developing formats for dossiers and monographs for microbials, and pheromones and other semio-chemicals.

This was achieved in 2004 and resulted in several OECD-publications in the Series of Pesticides (No. 12, 2001; No. 18, 2003 and No. 21, 2004).

The BPSG then decided to concentrate its efforts on science issues that remain as barriers to harmonisation and work-sharing. This resulted in the preparation of a "working document" which does not provide 'mandatory' guidance but being essentially a set of examples/case studies aimed at helping the regulatory authorities. The document is titled: *"Working Document on the Evaluation of Microbials for Pest Control"* and has been published in OECD Series on Pesticides No. 43, 2008.

The report of the *Workshop on the Regulation of Biopesticides: Registration and Communication issues, 15 – 17 April 2008, EPA, Arlington, USA*, has been published in the OECD Series on Pesticides (No. 44, 2009).

In 2009 the BPSG organised the first seminar on *Identity and Characterisation of micro-organisms*. The report of this seminar is the most recent publication of the work of the BPSG in the OECD Series on Pesticides (No. 53, 2010).

The 2nd seminar on *The fate in the environment of microbial control agents and their effect on non-target organisms* was held in May 2010. Publication of the report of this seminar is in preparation.

The 3rd seminar is titled: *Characterisation and Analyses of Botanicals for the use in Plant protection Products*. This topic was selected considering its significance for the registration of biopesticides.

In particular the following issues will be discussed during the Seminar:

- For which substances should identification and analytical methods be required?
- Which information should be included in the description of the method of manufacture?
- Discussion on the broadening of the scope of the EU Draft Working Document SANCO/10472/2003 which now covers only water and ethanol extracts, and a limited number of plant parts.
- In what way should the history of safe use be adequately taken into account if a plant extract has been used in plant protection or for other purposes without evidence of adverse effects?

The Seminar will focus on issues related to identification, manufacturing process –including quality control- and analytical methods. The Seminar is not intended to discuss issues like mode of action and toxicology.

The objectives, scope and structure of the seminar are described in detail in the 'Seminar outline'.

The potential of botanicals in plant protection

By Dr. Lucius Tamm

(Research Institute of Organic Farming (FiBL), Switzerland)

[PPT 2a]

Extracts of plants have been successfully used to protect plants from noxious organisms for centuries. Whereas the use of plants in ancient Greece and Rome is well documented, reports of uses in other civilizations (e.g. Asia, India, the Americas, Africa, Australia) are less easily accessible. Reports of traditional uses mainly address the protection against insect pests, whereas reports of uses against microbe-mediated diseases are rare before the 19th century.

Botanicals are currently used as (i) insecticides (e.g. pyrethrum, rotenone, rape seed oil, quassia extract, neem), (ii) repellents or antifeedants (e.g. neem), (iii) fungicides and inducers of resistance (e.g. laminarine, fennel oil, lecithin), (iv) herbicides (e.g. pine oil), (v) nematicides (e.g. neem), (vi) sprouting inhibitors (e.g. caraway seed oil), and (vii) adjuvants such as stickers and spreaders (e.g. pine oil). It is currently tedious to obtain a comprehensive and updated overview regarding registration status and uses of botanicals in OECD countries and worldwide, as information is scattered with no common key word access and major areas of usage are not easily accessible (India, China). A comprehensive tool to gain overview is therefore needed.

The next generations of botanicals are currently sought by world-wide activities by public research institutions, and there is increasing interest of the industry in the development of novel botanicals. Current trends include the 'rediscovery' of traditional uses, the screening of substance libraries to identify novel lead substances, the refined formulation to improve efficacy, and the systematic development of blends of active substances.

Botanicals have great potential in sustainable, resilient agricultural production systems currently favored by agricultural policy such as IPM schemes, low input/organic farming, and low/no residue production systems. Potential limitations of botanicals include lack of efficacy (as compared to chemicals), limitations in availability, uncertainties in registration requirements, and costs as compared to chemicals. Therefore, the quest for novel botanicals (and mixtures thereof) has to be intensified in order to develop innovative and safe tools for plant protection, and registration requirements need to be adapted to the properties of botanicals.

Experiences from the development and field testing of two botanicals

By Dr. Annegret Schmitt

(Institute for Biological Control -JKI, Darmstadt; Germany)

[PPT 2b]

The research required for development of a botanical comprises a multi-step process, in which the following aspects need particular attention: (i) identification of candidate extract plants (ii) identification of the spectrum of activity of selected candidates (crops/pathogens) (iii) for promising crops/pathogens: the determination of efficacy under (semi-)commercial conditions (iv) identification of bottlenecks (v) identification of active compounds (lead substances) and mode of action (vi) troubleshooting and optimization (e.g. extraction procedure, formulation, application timing, application equipment etc.) and (vii) identification of additional positive features. Finally, registration, production and marketing need to be taken-care of before a botanical can enter the market.

In the presentation, Milsana serves as example for a commercialized botanical, which is sold in Germany and the USA. The extract is made of above-ground parts of *Fallopia sachalinensis*, the giant knotweed. The second example is a plant extract from *Glycyrrhiza glabra*, liquorice or sweetwood. Rhizomes of liquorice are used for medicinal purposes and for production of sweets. For plant protection, the above-ground parts are used. Liquorice extract is not yet commercialized, but under development and in a pre-marketing experimental stage.

The presentation gives an insight in the different aspects and experiences gained during development and testing of these two botanicals along the above mentioned multi-step research process.

Natural extracts characterization and application of analytical methods for botanical quality determination

By Cédric Bertrand

(University of Perpignan, France)

[PPT 3]

Plant extracts consist of a mixture of wide range chemical compounds with biological activity. Moreover, genetic diversity of plants and different environmental conditions implies a huge variability in metabolites productions, implying that there is a need for chemical standardization for biological assays and quality assurance. To characterize complex plant extract two approaches are proposed,

i) a metabolomics approaches combined to bioassay leading to identification of marker or fingerprint,

ii) a bio-guided purification or semi-purification leading to the bioactive compounds identification.

Specific apparatus for sample preparation (SPE, SPME, ASE….) and efficient separation tools (HPLC/MS, GC/MS….) are required for analysis of biomarker in plant extract. Those techniques allowed the development of efficient analytical systems. These analytical systems can be used for batch validation, monitoring batch-to-batch variations, or even stability studies. This analytical system could be also used to environmental fate studies (biotic and abiotic degradation).

EU Draft Working Document on Plant Extracts SANCO/10472/2003
Key points and practical experiences.

By Thierry Mercier

(French Agency for Food, Environmental and Occupational Health & Safety [ANSES], Paris; France)

[PPT 4]

The draft working document, Sanco/10472/2003 –rev.5 (6.7.2004) "CONCERNING THE DATA REQUIREMENTS FOR ACTIVE SUBSTANCES OF PLANT PROTECTION

PRODUCTS MADE FROM PLANTS OR PLANT EXTRACTS" was intended to provide initial guidance for notifiers and Member States in the context of the 4th stage of the review programme of existing active substances and for applications for new active substances under Council Directive 91/414/EEC concerning the placing of plant protection products on the market. The aim of this document is to propose on a weight of evidence basis a tiered approach to the data requirements for active substances of plant protection products made from plants or plant extracts. This document has not been finalized in the Standing Committee on the Food Chain and Animal Health.

Nonetheless, the document still provides some recommendations, which might be helpful in maintaining harmonized assessment schemes and decision making in Member States. It is the responsibility of the applicant to provide the data and information required. Annexes II and III to Directive 91/414/EEC lay down the information and studies that have to be submitted as a minimum for active substances and plant protection products. However the introduction to the Annexes II and III provides that the applicant can provide a justification, which is acceptable to the competent authority, where particular data and information would not be necessary owing to the nature of the product or its proposed uses or where it is not scientifically necessary, or technically possible to supply information and data. The experience has shown that it has been used by the relevant Rapporteur Member States designated in the context of the 4th stage of the review programme, it might be updated as a result of this experience.

EFSA's experiences in evaluating 'botanicals'

By Herman Fontier

(European Food Safety Authority [EFSA], Parma; Italy)

[PPT 5]

It is highlighted that there are no specific data requirements for botanicals and therefore the standard chemical requirements have to be considered. But it is stressed that data requirements could always be waived if appropriate rationale is provided for the non-submission of data.

EFSA's experience include the drafting of two examples of two conclusions (Fenugreek seed powder and azadirachtin), neither of these compounds however are covered by the SANCO/10472/2003 draft guidance as they are not listed in the document.

The word 'botanicals' can cover a wide variety of very different types of substances with very different properties. Although it does seem that some of the data requirements are not always appropriate, establishing specific sets of data requirements may be difficult as the chemicals can be very different. As more experience is gained it may be possible to provide some guidance on how to prepare waivers. Experience so far has highlighted that the non-availability of radio-labelled material can be an obstacle for risk assessment. Normal risk assessment methodology is also not always adequate and more guidance is needed. There are often higher levels of uncertainty. Therefore these factors often combine to result in a need for risk management measures.

Neem/Margosa extract¨-and its constituents
Experience in EU evaluation and registration

By Hubertus Kleeberg

(Trifolio-M; Germany)

[PPT 6]

The same high quality and safety/risk criteria have to be guaranteed for Synthetics and Natural Products (Plant Extracts or Microbial Substances). However, these aims can usually not be fulfilled by the same registration procedure due to specific requirements of natural active ingredients. The development of one new marketable Plant Protection Product (PPP) is very expensive. The cost for the development of one synthetic product is stated (IVA) to be above 200 mio € and that of a biological (with active ingredients from plant extracts or of microbiological origin) Plant Protection Product (bPPP) amounts to about 5 to 15 mio €.

For the development of a bPPP the standardisation of the composition and hence the extraction process is a necessary prerequisite. For Azadirachtin (Neem Extract) (according to EU-plant protection legislation) or Margosa Extract (according to biocide regulations) we developed a standardised extraction process for "NeemAzal® technical" which yields a product containing about 52 to 55% (w/w) of total Azadirachtins. The Aflatoxins are below the limit for food. The Azadirachtins are the substances which contribute to the insecticidal activity of the product. Other limonoids – which may be present in NeemAzal® technical – (like Salannin or Nimbin) amount to a few percent only. It is important that the efficacy tests, but as well toxicological and ecotoxicological studies have been conducted always with this standardised composition, so that possible contributions to the intended activity and possible negative side effects are always traceable for the whole composition. The same is true for NeemAzal®-formulations. The toxicological and ecotoxicological properties of NeemAzal® and its formulations are very favourable.

On behalf of the effects of NeemAzal® and its formulations on insect development and the similarity of the molecular structure of the Azadirachtins and Ecdysons (which is in charge of the interruption chitinsynthesis) it can be concluded that azadirachtins interfere with the hormonal system of insects which triggers their development.

On behalf of the fast degradation of Azadirachtins (half life of a few days) the insecticidal effects quickly disappear, so that the regulatory authorities have decided for waiting periods (PHI-values) of zero to a few days.

In the framework of the process of the EU-Annex I inclusion (which has finally been voted for positively by the EU-commission on 11th March 2011) questions with respect to the degradation products and metabolites have been risen. However, since the polar side chains in which the Azadirachtin**s** differ structurally are cleaved easily, it can quite reasonably be assumed, that the main degradation products and metabolites of **all** the Azadirachtin**s** present in NeemAzal® are very similar or even identical. This means that it would be of highest interest to look at degradation products and metabolites of Azadirachtin **A** (the analytical lead substance). However, due to the complexity of the Azadirachtin**s** these molecules cannot be labeled radioactively and it is practically impossible to trace degradation products and/or metabolites. In order to answer these questions one possibility in addition to fundamental analytical research may be trials with aged substance/residues or mesocosmos investigations.

Another example of the difficulties involved in the registration of plant extracts is "Quassin". Quassin

is an insecticidally active substance extracted from bitterwood (Quassia amara). In the USA Quassin is included in the GRAS-List (Generally Regarded As Save) and in Germany according to Aroma Guidelines (2006) (Quassin $\leq$ 50 mg/kg is permitted in alcoholic beverages), in homoeopathic medicine (max: D2/C1), and cosmetics (INCI-List: skin conditioning, denaturating agent, body lotion). For our standardised Quassia-extract we carried out basic toxicological and eco-toxicological studies and applied for registration according to EU-SANCO 10472, however, EU-authorities asked for more, especially long term toxicological studies – although Quassia-extract is ingested by many people after each meal!

All this shows a few deficits and obscurities in the EU registration process for bPPP. Only a reasonable scientifically based approach may help in which the special properties of the respective extracts are taken into account and waivers for unnecessary requirements are possible. In the case that registrations should not be carried out totally in a "case to case" approach SANCO 10472 may serve as a good starting point for the harmonisation of procedures.

Margosa extracts – Experiences in the Evaluation under the Plant Protection Products Directive and the Biocides Directive

By Vera Ritz

(Federal Institute for Risk Assessment (BfR), Germany)

[PPT 7]

Several margosa extracts, also known as neem extracts, are produced from fruit, seeds or leaves of the tree *Azadirachta indica.* Extracts from seed kernels are used for biocidal/pesticidal purposes as they are known to have antifeedant, insecticidal, nematicidal, fungicidal and bactericidal properties. Biologically active compounds in the seed kernels are predominantly azadirachtins though other biologically active limonoids such as salannin, and nimbin were also identified.

Germany is Rapporteur Member State for margosa extracts under the framework of two different European Legislations: under the Plant Protection Products Directive (PPPD) 91/414/EEC and Biocidal Products Directive (BPD) 98/8/EC. One important difference was that the supported uses under BPD do not result in residues, whereas the uses under PPPD may lead to residues in food.

No special technical guidance applicable to the evaluation of margosa extracts (notified as azadirachtin) is in place under the PPPD, i.e. no guidance on the data requirements concerning identity, physico-chemical properties, and analytical methods. A draft of a guidance document from 2004 refers only to plants listed in an annex to the document and it was intended only for extracts prepared with water and/or ethanol. Hence, the usual data requirements were applied during the PPP procedure and where adapted to the specific conditions of the application where possible and necessary.

Under the BPD, there is a technical guidance document in place ("How to deal with extracts and oils of plant or animal origin?" Addendum to the Technical Notes for Guidance on data requirements for active substances, Nov. 2006) stating that the whole mixture of all constituents is considered the active substance in an extract or oil and that constituents ≥ 1 % (w/w) have to be identified (hazardous constituents down to 0.1 %).

Under the PPPD, Germany was evaluating three different extracts that were notified as azadirachtin. After evaluation and peer review within the EU it was concluded "that the nature of residues in plants had not been elucidated" and "on this basis a valid [dietary] risk assessment cannot be conducted." Another problem encountered was that azadirachtin A was proposed as lead substance initially, because it was the most abundant limonoid in the extracts. As this was not accepted during the peer review process, a data gap in the areas identity, physico-chemical and technical properties as well as methods of analysis was claimed for other biologically active compounds in the extract. However, two of the three extracts (called azadirachtin in this procedure) were finally approved within the EU in 2011.

Two extracts were notified under the BPD, one of them identical to one evaluated under the Plant Protection Products Directive. As a result of lessons learned from the review process under the Plant Protection Products Directive and the guidance in place, the evaluation was based on the whole mixture

instead of azadirachtin A as a lead substance which was accepted during the European peer review process. Since no residues in food or feed are expected from the uses notified and no data gaps were identified, the European peer review process for one of the two extracts is finalised, most likely resulting in an approval for in 2011, the approval for the other margosa extract is expected to follow in 2012.

Algae extracts and Laminarine
Experience in EU evaluation and registration

By Jean-Marie Joubert

(Laboratoires Goëmar; France)

[PPT 8]

Laboratoires Goëmar, a French company sells two ranges of products:

- Physio Activators. The seaweed extract is registered as Plant Growth Regulator according to the 91/414/EEC Directive,

- Natural Defense Stimulants. These later are registered as Plant Protection Products. The active substance is included in Annex I of the 91/414/EEC Directive.

The company compares its experience to register the two active ingredients:

- " laminarin" extracted from *Laminaria digitata*

- a seaweed extract from *Ascophyllum nodosum* another seaweed, non purified.

The presentation shows the difficulty to characterize a non purified natural product such as seaweed extract, and identify the impurities. A focus is done on the way to find waivers in order to explain the non relevance of the requested studies.

To finish, a table indicates the authorizations, the registration files submitted and the files to be submitted in the following years.

Updates to PMRA's Guidelines for the Registration of Non-Conventional Pest Control Products

By Brian Belliveau

(Microbial and Biochemical Evaluation Section, Health Evaluation Directorate, Pest Management Regulatory Agency, Health Canada, Ottawa, Canada)

[PPT 9]

Health Canada's Pest Management Regulatory Agency published a new regulatory proposal in October 2010 revising its registration guidelines for non-conventional pest control products and replacing Regulatory Proposal PRO2007-02, "Registration Guidelines for the Registration of Low Risk Biochemicals and Other Non-Conventional Pesticides" which was released in the fall of 2007 as a pilot programme. The updated guidelines, Regulatory Proposal 2010-06, "Guidelines for the Registration of Non-Conventional Pest Control Products" were released for public comment on 28 October 2010 and take a flexible approach for active ingredients that meet the criteria for reduced data requirements. The PMRA assesses the eligibility of products for review under this proposal on the basis of all the evidence available. Applicants are required to submit a detailed rationale explaining why they believe their product is eligible for review under this proposal and include details of the proposed use pattern and label claims, and as much scientific evidence as possible on the characterization of the components, toxicity, exposure and environmental fate.

The PMRA supports a flexible approach to setting data requirements for registration and recognizes that the information needed to make a regulatory decision should be commensurate with the level of anticipated risks. Products eligible for consideration under this proposal must have some, but not necessarily all, of the following characteristics:

1) low inherent toxicity to humans and other non-target organisms (N.B. Substances with chronic toxicity, carcinogenicity, genotoxicity, neurotoxicity, reproductive/developmental effects, or that metabolize into compounds of toxicological concern are not eligible for review under this proposal);

2) low potential for their use to result in significant human or environmental exposure;

3) not persistent in the environment;

4) already widely available to the public for other uses, with a history of safe use under conditions posing equivalent potential for exposure to humans and the environment;

5) pesticidal action that is not the result of toxicity to the target organism, e.g., products that work by attracting, repelling, desiccating or smothering pests; and

6) unlikely to select for pest resistance.

Substances eligible for review under this proposal could include: food items, extracts, preservatives or additives (e.g., crushed garlic, garlic powder, table salt, citric acid); plant extracts and oils (e.g., vegetable or mineral oils); commodity chemicals that have a range of non-pesticidal uses (e.g., acetic acid); fertilizer or other plant growth supplements, commonly used in the agricultural sector (e.g., mineral salts, such as sodium and potassium salts of phosphorus acid); or inert materials (e.g., diatomaceous earth).

US experiences with the biochemical/botanical pesticide system, including the "biochemical classification" system

By William Schneider

(Biopesticides and Pollution Prevention Division, EPA; USA)

[PPT 10]

The US created a class of pesticides in 1979, Biochemical Pesticides, that includes many botanical pesticides. The main advantage of such a class is that the substances often pose less risk than conventional pesticides and can be adequately evaluated using less data. Less data can be used since the natural occurrence of these substances can often provide information on their potential toxicity. Since there are other substances than just botanicals that can be evaluated this way, the US elected to include all naturally occurring substances (providing they have a history of exposure to humans and the environment demonstrating minimal toxicity) such as semiochemicals, growth regulators, acids (i.e. vinegar as an herbicide), oils and abrasive dusts. Some botanical substances are very toxic, e.g. ricin, or rotenone, and would not qualify for this reduced data set.

Over 200 biochemical pesticide active ingredients have been registered.

Chenopodium extract and its constituents
Experience with US evaluation and registration and approach to EU

By Nicholas Wright

(AgraQuest Inc.; USA)

[PPT 11]

AgraQuest, Inc. (Davis, California, US) is a company focused on discovering, developing, manufacturing and marketing highly effective biopesticides and yield enhancing products for agriculture, home and garden, and food safety markets. In 2006 AgraQuest acquired the rights to an insecticide technology based on an essential oil extracted from a specific variant of the flowering plant *Chenopodium ambrosioides*.

The focus of this presentation will be on AgraQuest's experience in the US leading up to and registering the plant extract-based active substance and the development and registration of an analogous "blended" active substance. A brief comparison of the US and EU approaches to the registration of the blended product will also be discussed.

Biopesticides are defined by the US EPA as pesticides derived from natural materials such as animals, plants, bacteria, and certain minerals. EPA further delineates biopesticides into three categories: microbial, consisting of microorganisms; biochemical, which are naturally occurring substances that control pests by non-toxic mechanisms, for example extracts and pheromones; and plant-incorporated protectants, which are substances that plants produce from genetic material added to the plant.

With respect to biochemical pesticides, since it is sometimes difficult to determine whether a substance meets the criteria for such a classification, EPA has a review committee to make such determinations. Because they are thought to pose fewer risks than conventional pesticides, EPA utilizes a tiered data requirement approach for biopesticides rather than the full battery needed to register conventional pesticides. Therefore, biopesticides generally require less data and time for registration. However, that does not mean that the data requirements are minimal, or that the review process is less rigorous.

A case in point would be the difficulties experienced by the original developers of the Chenopodium extract in getting it classified as a biochemical pesticide. The process began in 1999 and continued until January 2004 when the EPA accepted the product as a biochemical pesticide. Later that year the registration application and initial data package were submitted to the EPA. The secondary review of the registration dossier was completed in November of 2005 and the results of that review were obtained by AgraQuest in January 2006.

Registration of the active substance was granted in April 2008 following discussions with the EPA (BPPD - Biopesticides and Pollution Prevention Division) concerning issues such as the; characterization of the extract active substance, identification of the appropriate marker compounds, identifying potential impurities, establishing certified limits to account for the variability inherent in plant extracts. The registration was amended in December 2008 to allow use on food crops and to obtain an exemption for the requirement of a tolerance.

Due to the high cost of production, which included factors such as the growing, harvesting, and on-site processing of the plant to obtain the essential oil, additional downstream processing necessary to produce the extract, and the high batch to batch variability, AgraQuest began work on developing an

analogous active ingredient. This work began in 2007 and the resulting active ingredient is an optimized blend that in essence is a mimic of the original plant extract. The blend is optimized in the sense that, when formulated, it is functionally indistinguishable from the plant-based extract and compositionally, it is identical to the insecticidally active subset of the plant-produced extract.

The US registration process for this active ingredient began in the same manner as the plant extract – discussions and meetings with the EPA and a review to determine if the active substance could be classified as a biochemical pesticide. The outcome was anything but assured as the active substance was a mixture of three conventional chemicals, two of which are not registered active ingredients. However, unlike the classification of the plant extract which took five years to resolve, the arguments presented combined with an agreement to provide data to show that the toxicological, phys/chem., and performance profiles were essentially the same resulted in a relatively quick decision to (1) accept the concept of a plant extract mimic and (2) classify the blended active as a biochemical pesticide. The blended active ingredient registration application was submitted in December 2008 and registered June 2010 for use on food crops with an exemption from a requirement of a tolerance.

An Annex II and Annex III dossier for the blended active substance and plant protection product are under preparation for submission in 2011.

ANNEX 4

PRESENTATIONS (SLIDES)

Introduction: Presentation on the OECD and the work of OECD-BPSG and general introduction to the Seminar on 'botanicals'
By Jeroen Meeussen, BPSG Chair (European Commission)

The potential of botanicals in plant protection
By Lucius Tamm (Research Institute of Organic Agriculture [FiBL], Frick; Switzerland)

Experiences from the development and field testing of two botanicals
By Annegret Schmitt (Institute for Biological Control -JKI, Darmstadt; Germany)

Natural extracts characterization and application of analytical methods for botanical quality determination
By Cédric Bertrand (University of Perpignan; France)

EU Draft Working Document on Plant Extracts SANCO/10472/2003 - key points and practical experiences.
By Thierry Mercier (French Agency for Food, Environmental and Occupational Health & Safety [ANSES], Paris; France)

EFSA's experiences in evaluating 'botanicals'
By Herman Fontier (European Food Safety Authority [EFSA], Parma; Italy)

Neem/Margosa extract¨-and its constituents – experience in EU evaluation and registration
By Hubertus Kleeberg (Trifolio-M; Germany)

Margosa extracts – experiences in the evaluation under the Plant Protection Products Directive and the Biocides Directive
By Vera Ritz (Federal Institute for Risk Assessment [BfR], Berlin; Germany)

Algae extracts and Laminarine – experience in EU evaluation and registration
By Jean-Marie Joubert (Laboratoires Goëmar; France)

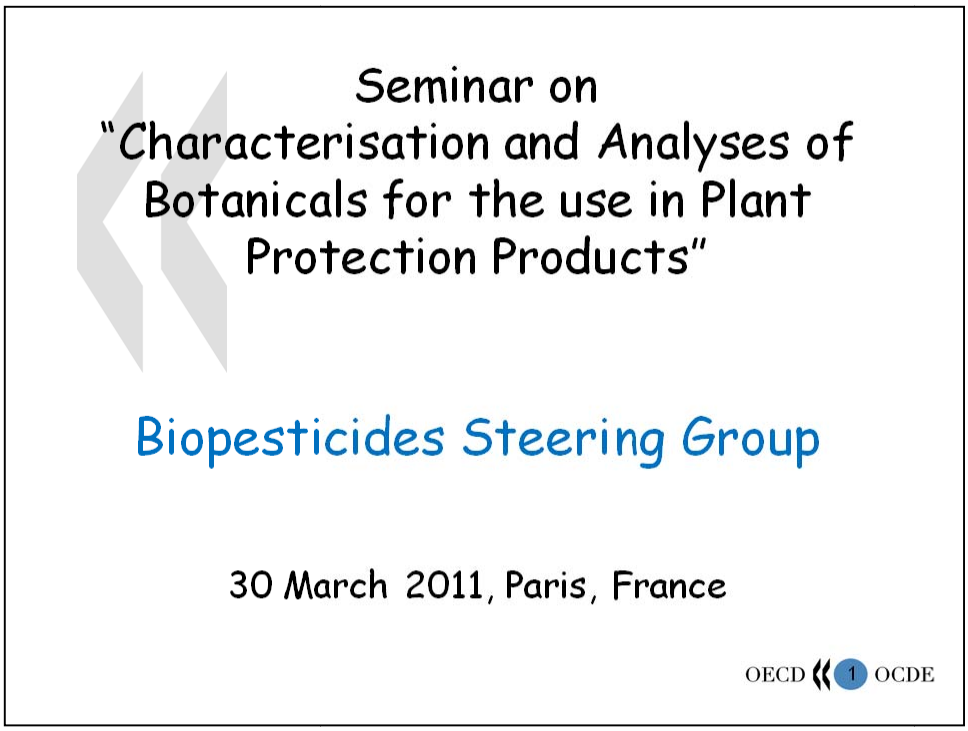
Updates to PMRA's regulatory proposal on non-conventional pesticide registration
By Brian Belliveau (Health Canada Pest Management Regulatory Agency, Ottawa, Canada)

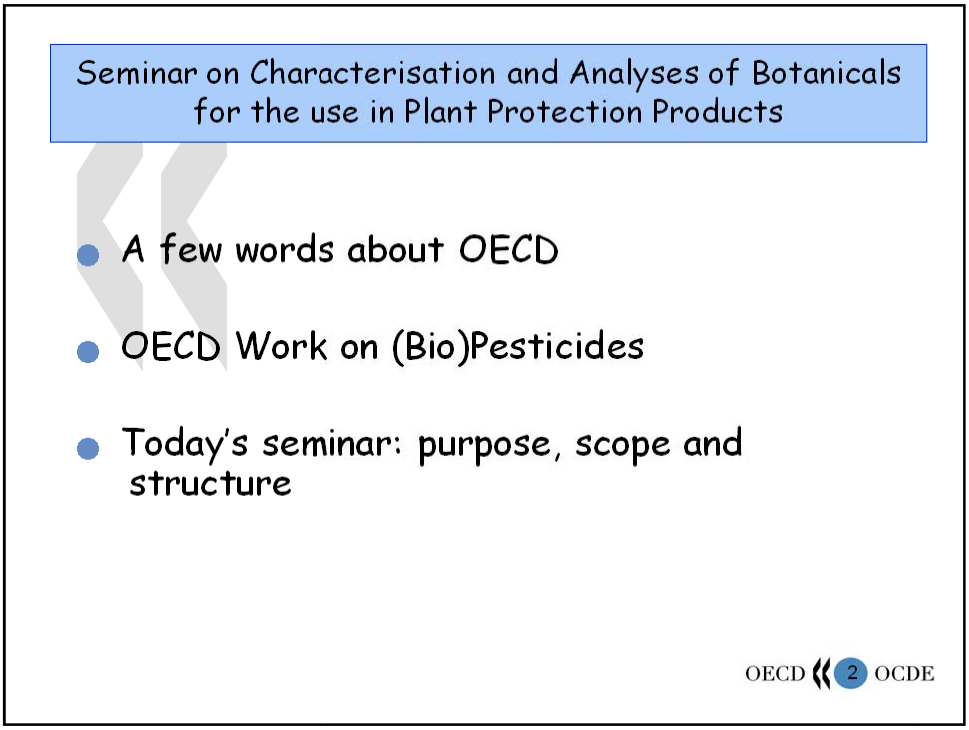
US experiences with the biochemical/botanical pesticide system, including the "biochemical classification" system
By William Schneider (Biopesticides and Pollution Prevention Division, EPA; USA)

Chenopodium extract and its constituents – experience with US evaluation and registration and approach to EU
By Nicholas Wright (AgraQuest Inc.; USA)

Presentation 1
Presentation on the OECD, the work of OECD-BPSG and general introduction to the seminar on 'botanicals'

By Jeroen Meeussen, BPSG Chair (European Commission)





OECD

OECD: The Organisation for Economic Co-operation and Development

OECD 3 OCDE

OECD

What is OECD?

- A forum in which governments work together to address the economic, social and environmental challenges of interdependence and globalisation.
- A provider of comparative data, analysis and forecasts to underpin multilateral co-operation with more than 250 publications per year.

OECD 4 OCDE

OECD's Mission

OECD brings together the governments of countries committed to democracy and the market economy from around the world to:

- Support sustainable economic growth;
- Boost employment;
- Raise living standards;
- Maintain financial stability;
- Assist other countries' economic development;
- Contribute to growth in world trade.


OECD 5 OCDE

A tool for governments

- Started after World War II;
- Transformed in 1961 into the Organisation for Economic Co-operation and Development with trans-Atlantic and then global reach;
- Today the OECD has 33 member countries;
- More than 70 developing and transition economies are engaged in working relationships with the OECD.

OECD 6 OCDE

OECD - Working Group on Pesticides

The OECD work on **agricultural pesticides** aims to help member countries:

- improve the efficiency of pesticide control;
- share the work of pesticide registration and re-registration;
- minimise non-tariff trade barriers;
- reduce risks to human health and the environment.



OECD 7 OCDE

OECD - Working Group on Pesticides

Working Group on Pesticides:

- Registration Steering Group
- Risk Reduction Steering Group
- BioPesticides Steering Group

OECD 8 OCDE

OECD-BPSG

The BioPesticides Steering Group (BPSG) was established by the WGP in 1999:

- to help member countries harmonise the methods and approaches used to assess biological pesticides and

- improve the efficiency of control procedures.

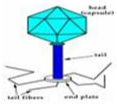
OECD 9 OCDE

OECD-BPSG

Biological Pesticides:

- Macro-organisms
- Microbial Biopesticides
- Semiochemicals
- Plant extracts/Botanicals





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OECD-BPSG

The first tasks of the BPSG consisted of:

(i) reviewing regulatory data requirements for three categories of biopesticides; and
(ii) developing formats for dossiers and monographs for microbials, and pheromones and other semio-chemicals.



OECD 11 OCDE

OECD-Publications

Registration requirements:

- for pheromones (Series on Pesticides, No. 12, 2001);
- for microbial pesticides (Series on Pesticides, No. 18, 2003);
- for invertebrate biocontrol agents/IBCAs (Series on Pesticides, No. 21, 2004).

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OECD-Publications

- OECD Guidance for **Industry Data Submissions** for Microbial Pest Control Products and their Microbial Pest Control Agents (Dossier Guidance for Microbials), August 2006.
- OECD Guidance for **Country Data Review Reports** on Microbial Pest Control Products and their Microbial Pest Control Agents (Monograph Guidance for Microbials), August 2006.

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OECD-Publications

- OECD Guidance for **Industry Data Submissions** for Pheromones and other Semiochemicals and their Active Substances (Dossier Guidance for Pheromones and other Semiochemicals), 2003.
- OECD Guidance for **Country Data Review Reports** for Pheromones and other Semiochemicals and their Active Substances (Monograph Guidance for Pheromones and other Semiochemicals), 2003.

OECD 14 OCDE

OECD-BPSG

The BPSG then decided to concentrate its efforts on **science issues** that remain as barriers to harmonisation and work-sharing.

OECD 15 OCDE

Working Document

"Working Document on the Evaluation of Microbials for Pest Control"

This document is essentially a set of examples/case studies aimed at helping the regulatory authorities to deal with these issues in the assessment of (microbial) biopesticides.

OECD Environment, Health and Safety Publications, Series on Pesticides No. 43, 2008

OECD 16 OCDE

Working Document - chapters

- Taxonomic identification of micro organisms in MPCP
- Genetic toxicity assessment of microbial pesticides
- Exposure (operators, bystanders, consumers)
- Microbial metabolite residues in food
- Efficacy evaluation of microbials

OECD 17 OCDE

Workshop on the Regulation of Biopesticides

- *"Workshop on the Regulation of Biopesticides: registration and Communication issues" 15-17 April 2008, EPA, Arlington, USA; OECD Environment, Health and Safety Publications, Series on Pesticides No. 44, 2009*

The objectives of the workshop were met:

- To collect input to resolve science issues;
- To improve communication and information exchange;
- To take forward some of the conclusions from REBECA.

OECD 18 OCDE

OECD-Seminars

- OECD, *Report of Seminar on Identity and Characterisation of micro-organisms*, OECD Environment, Health and Safety Publications, Series on Pesticides No. 53, 2010.
- OECD, *Report of Seminar on The fate in the environment of microbial control agents and their effect on non-target organisms* Publication in preparation

OECD 19 OCDE

Botanicals – Way forward?

OECD 20 OCDE

Botanicals - Workplan 2009-2012

- *Resolve **science issues** associated with registering botanicals.*
- *Develop a **comprehensive Guidance Document** on botanicals covering issues like extraction methods; identification and analytical methods; method of manufacture; low risk/concern; efficacy.*

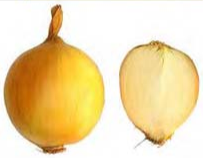
- ***Definition** in the framework of this seminar:*
  "Botanicals are plant substances resulting from simple processing e.g. pressing or from extraction. By extension the definition applies to a small number of compounds or even single ones extracted from plants and purified natural identical synthetic molecules and even analogues/mimics."

OECD 21 OCDE

Seminar on "*Characterisation and Analyses of Botanicals for the use in Plant protection Products.*"









OECD 22 OCDE

Botanicals - Documentation

- *Guidelines for the Registration of Non-Conventional Pest Control products (Health Canada).*
- *EU Working Document on plant extracts (SANCO/10472/2003).*
- *REBECA project (deliverables 16, 17, 18).*
- *EFSA opinion on plants/herbs as additives for use in animal production.*
- *EFSA-conclusions on fenugreek seed powder and Azadirachtin.*
- ....

OECD 23 OCDE

Seminar - Objectives

The objectives of the seminar are:

- identify key issues in the area of characterisation and analyses of botanicals for the use in plant protection products;
- exchange information on national and international activities in the area concerned; and
- make recommendations for further actions and/or possible activities for OECD.

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Seminar - Scope

- For which substances should identification and analytical methods be required?
- Which information should be included in the description of the method of manufacture?
- Discussion on the broadening of the scope of the EU Draft Working Document SANCO/10472/2003 which now covers only water and ethanol extracts, and a limited number of plant parts.
- In what way should the history of safe use be adequately taken into account if a plant extract has been used in plant protection or for other purposes without evidence of adverse effects?

OECD 25 OCDE

Seminar - Scope (cont'd)

- The seminar will focus on issues related to identification, manufacturing process -including quality control- and analytical methods.
- The seminar is not intended to discuss issues like mode of action and toxicology.

OECD 26 OCDE

Seminar - Structure

Presentations on:

- government, research and stakeholder experience and perspectives,

followed by discussion after each set of presentations.



OECD 27 OCDE

Seminar - Results

With the focus on characterisation and analyses of botanicals for the use in plant protection products, the goals of this seminar are

1. for participants to share information and to promote a dialogue on botanicals, and
2. to suggest future work/issue papers in the field of characterisation and analyses of botanicals for the use in plant protection products.

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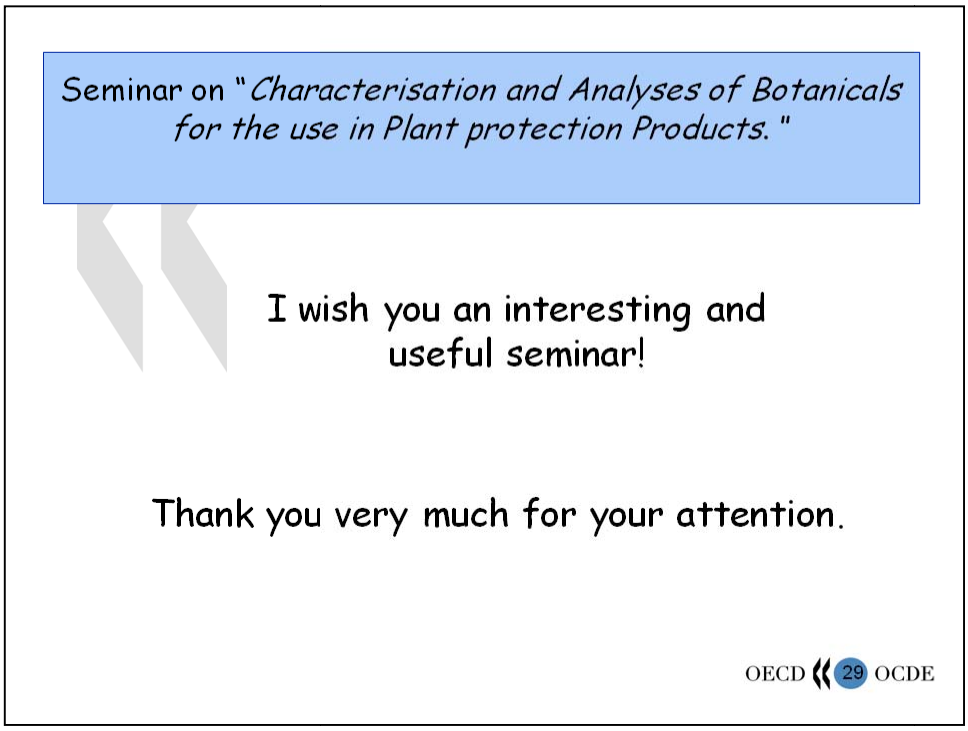

Seminar on "Characterisation and Analyses of Botanicals for the use in Plant protection Products."
I wish you an interesting and useful seminar!
Thank you very much for your attention.
OECD 29 OCDE

Presentation 2a
The potential of botanicals in plant protection
By Lucius Tamm (Research Institute of Organic Agriculture [FiBL], Frick; Switzerland)

FiBL
EXCELLENCE FOR SUSTAINABILITY

Research Institute of Organic Agriculture
Forschungsinstitut für biologischen Landbau
Institut de recherche de l'agriculture biologique

The potential of botanicals in plant protection

Lucius Tamm (lucius.tamm@fibl.org)

Content

- History of use of botanicals
- Current use of botanicals
- Types and properties of botanicals
- Potential uses and limits
- Future opportunities

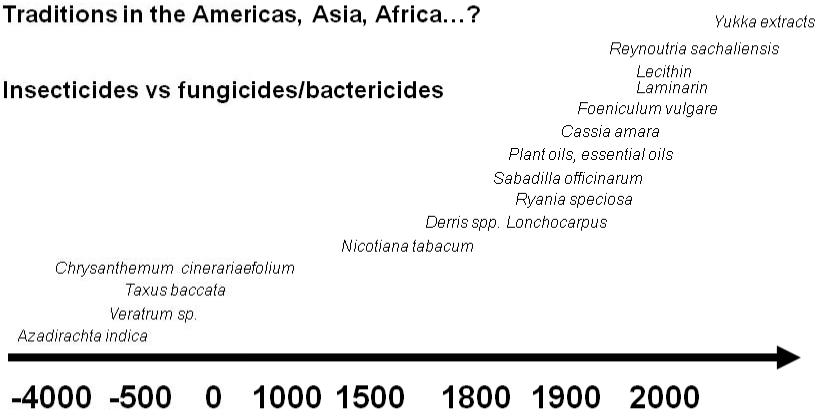
Some reported uses of botanicals

Indian tradition ?

Chinese tradition ?

Traditions in the Americas, Asia, Africa…?

Insecticides vs fungicides/bactericides



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Uses of botanicals

- insecticides (e.g. pyrethrum, rotenone, rape seed oil, quassia extract, neem),
- repellents or antifeedants (e.g. neem),
- fungicides and inducers of resistance (e.g. laminarine, fennel oil, lecithin),
- herbicides (e.g. pine oil),
- nematicides (e.g. neem),
- sprouting inhibitors (e.g. caraway seed oil),
- adjuvants such as stickers and spreaders (e.g. pine oil)

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Current status of botanicals in Annex I 91/414

included	pending	excluded
Azadirachtin	Ascorbic acid	Citrus extract
Carvone	Orange oil	Citrus extract/grapefruit extract
Extract from teatree		
Fatty acids C7 to C20	Tagetes oil	Citrus extract/grapefruit seed extract
Fatty acids C7-C18 and C[illegible] ated potassium salts	Thym[illegible]	Extract from Equisetum
Fatty acids C8-C1[illegible]		Extract from Menta piperita
Garlic extra[illegible]		Extract from Plant Red oak, Prickly pear cactus, Fragrant sumac, Red mangrove
Lami[illegible]		Fatty acids/ Isobutyric acid
L[illegible] 143-07-7)		Fatty acids/ Isovaleric acid
Pe[illegible]		Fatty acids/ Valeric acid
Plan[illegible]/ Citronella oil		Fatty acids: potassium salt- caprylic acid
Plant oils/ Clove oil		Fatty acids: potassium salt- tall oil fatty acid
Plant oils/ Spear mint oil		Garlic pulp
Plant oils/ Rape seed oil		Gentian violet
Pyrethrins		Lecithin
Sea-algae extract		Nicotine
		Onion extract
		Plant oils/ Bl[illegible]
		Plant oils/ [illegible]
		Plant[illegible], Daphne oil
		Pla[illegible] oil (Eugenol)
		Plant[illegible] calyptus oil
		Plant oil/ Gaiac Wood oil
		Plant oils/ Garlic oil
		Plant oils/ Lemongrass oil
		Plant oils/ Maize oil
		Plant oils/ Marjoram oil
		Plant oils/ Olive oil
		Plant oils/ Peanut oil, Pinus oil
		Plant oils/ Soya oil
		Plant oils/ Soybean oil, epoxylated
		Plant oils/ Sunflower oil
		Plant oils/ Thyme oil
		Plant oils/ Ylang-Ylang oil
		Quassia
		Rotenone
		Soybean extract
		Wheat gluten

Included: 16

pending: 4

excluded: 40

FiBL Source: EU pesticides database, March 2011

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US-EPA biopesticides

Allium sativum (Garlic)	Farnesol
Allyl isothiocyanate (Mustard, oil of)	Gamma aminobutyric acid (GABA)
Allyl isothiocyanate (AITC) (Oriental Mustard Seed)	Geraniol
Anise oil	Jojoba oil
Anthraquinone	Lavandin oil
Azadirachtin	Lemon Grass oil
Balsam Fir Oil	Maple Lactone
Bergamot oil	Mint Oil
Black Pepper oil	Mustard oil
Canola oil	Neem oil
Capsaicin	Neem oil, clarified hydrophobic
l-Carvone	Orange oil
Castor oil	Oriental Mustard Seed (Allyl isothiocyanate (AITC))
Catmint Oil	Plant Extract 620
Cedarwood oil	Plant Oils
Chenopodium ambrosioides near ambrosioides	Quillaja saponaria
Chenopodium quinoa, Saponins of	Red pepper
Cinnamaldehyde	Reynoutria sachalinensis
Citronella oil	Saponins of Chenopodium quinoa
Citronellol	Sesame stalks (128970)
Corn gluten meal	Soybean oil
Eucalyptus oil	Thyme (herb)
Eugenol	Thymol (5-methyl-2-isopropyl-1-phenol)
	Xanthine

Included: 47

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Current status of botanicals according to IUPAC database, 'biopesticides'

registered	active substance
yes	azadirachtin
yes	capsaicin
yes	citronella oil
yes	fatty acids
yes	FEN 560
	jasmone
	limonene
	nepetalactone
	nicotine
	olein
yes	piperidine
	p-menthane-3,8-diol
	precocene II
yes	pyrethrins
yes	rotenone
	sabadilla
yes	sucrose octanoate
	thymol
yes	zeatin

Source: IUPAC database, 'biopesticides'

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Conclusions regarding the registration status and uses of botanicals

- Very difficult to obtain a comprehensive overview regarding registration status and uses of botanicals
- Information is often scattered in various databases, with no common key word access (e.g. 'botanical', 'biopesticide')
- Major sources not easily accessible (China, India, others?)

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Origin of the next generations of botanicals?

Political Map of the World, April 2005

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Interest of the industry in botanicals?

www.action-pin.fr, www.agrauxine.fr, www.agrolevures.com, www.agron.co.il, www.agrotecnologia.net, www.algacan.com, www.alidad.eu, www.arystalifescience.com, www.atflda.pt, www.atlanticaagricola.com, www.barrier-biotech.com, www.beauvilliersflavors.com, www.belchim.com, www.biagro.es, www.biofa-farming.com, www.biogarten.ch, www.biogreenwisdom.com, www.biomor.com, www.bio-protect.de, www.biosphereconsulting.com, www.biotech-int.com, www.biotop.fr, www.biotus.fi, www.boyutft.com, www.brandtconsolidated.com, www.cabi.org, www.certiseurope.com , www.certisusa.com, www.chemcom.be, www.chemia.it, www.chipro.de, www.daymsa.com, www.decco-web.com, www.desangosse.com, www.dksh.com, www.ecoflora.com, www.fibl.org, www.fsagx.ac.be, www.futurecobioscience.com, www.fytofend.be, www.gab-consult.de, www.gba.com.ir, www.gowanintl.com, www.greenuniverseagriculture.com, www.iabiotec.com, www.ibioc.com, www.imp-impact.com, www.intrachem.com, www.jsci.co.uk, www.koppert.com, www.koppert.com, www.kwizda-agro.at, www.lagrotecnico.it, www.marronebioinnovations.com , www.massoagro.com; www.massopaqueteria.com, www.mbm-info.de, www.naturalti.it, www.neemnico.com, www.neudorff.com, www.novagrica.com, www.opennatur.com, www.pherobank.com, www.pireco.nl, www.planprotect.com, www.plantimpact.com, www.plodovizemlje.hr, www.quimicasmeristem.com, www.rivale.fr, www.sbm-formulation.com, www.seipasa.com, www.silvateam.com, www.sipcam.es, www.stc-nyorks.co.uk , www.technopole-bordeaux-montesquieu.com, www.tilco-biochemie.de, www.trifolio-m.de, www.tsgeurope.com, www.tstanes.com, www.valentbiosciences.com, www.vivagro.fr, www.westbridge.com

Source: http://www.ibma.ch/pg_natural_products.html

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Current trends in development of botanicals

- 'Rediscovery' of traditional uses (folk medicine)
- Screening of substance libraries to identify novel lead substances
- Refined formulation to improve efficacy
- Systematic development of blends of substances based on Chinese and Indian traditions

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Potential uses of botanicals

- Resistance management to protect chemicals
- As part of IPM production systems
- Development of low or no residue production systems
- Organic and low input farming systems
- Subsistence and home gardening

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Example: Growth potential of organic agriculture

Table 3: Organic agricultural land (including in-conversion areas) and shares of total agricultural land 2009

	Agr. land (hectares)	Share of total agr. land
Africa	1'026'632	0.10%
Asia	3'581'918	0.25%
Europe	9'259'934	1.87%
Latin America	8'558'910	1.37%
Oceania	12'152'108	2.82%
Northern America	2'652'624	0.68%
Total	37'232'127	0.85%

Source: FiBL/IFOAM Survey 2011
Shares of total agricultural land (including only the countries that are included in the survey).

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Willer, Helga and Lukas Kilcher (Eds.) (2011) The World of Organic Agriculture. Statistics and Emerging Trends 2011. FiBL-IFOAM Report.

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What if botanicals replace sulphur? A calculation exercise

Table 2.4.1: Top-10 chemical classes in the EU – fungicid

	Chemical classes	uantity (in tonnes)
1	INORGANIC SULPHUR	59053
2	DITHIOCARBAMATE FUNGICID	21149
3	CONAZOLE FUNGICIDES	3865
4	ORGANOPHOSPHO	66
5	COPPER CO	
6	PHTHAL	3004
7	ST	2255
8		2016
9		1418
	FU	1174
	top-1	100801
	Total	107823

Example 1: 1 kg lecithin equals app. 4 kg sulphur:
App. 15'000t lecithin needed to replace 59000t sulphur
Annual production (world): app. 10M tonnes

Example 2: 1 kg *oleum foeniculi* equals app. 5 kg sulphur:
App. 10'000t *oleum foeniculi* needed to replace 59000t sulphur
Annual production: ???

Source: Pesticide use in the EU; EUROSTAT, 2007

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Market potential for botanicals: is the current pesticide market a starting point for discussion?

Crop	Total (tonnes a.i.)	insecticides (%)	fungicides (%)	herbicides (%)	insecticides (tonnes a.i.)	fungicides (tonnes a.i.)	herbicides (tonnes a.i.)
Grapevine	110000	5	85	10	5'500	93'500	11'000
Cereals	45000	5	30	65	2'250	13'500	29'250
Maize	25000	4	0	96	1'000	0	24'000
Pomefruit	20000	20	75	5	4'000	15'000	1'000
Potato	10000	10	65	25	1'000	6'500	2'500
Vegetable	12000	20	75	5	2'400	9'000	600
Sugar beet	10000	10	20	70	1'000	2'000	7'000
Oil seeds	8000	5	20	75	400	1'600	6'000

Pesticide use in the EU: Source : EUROSTAT, 2007

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Potential limitations to botanicals

- Efficacy
- Availability
- Registration
- Costs

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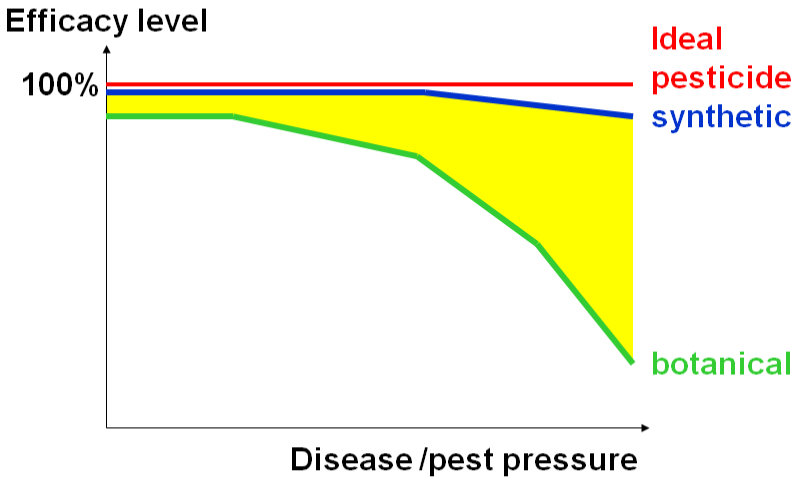
The efficacy gap of biopesticides: a prevalent phenomenon



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Reasons for limited efficacy

- Limited activity of a.i.
- UV stability
- Limited rain fastness
- Limited uptake into plant tissue
- Limited shelf life
- etc.

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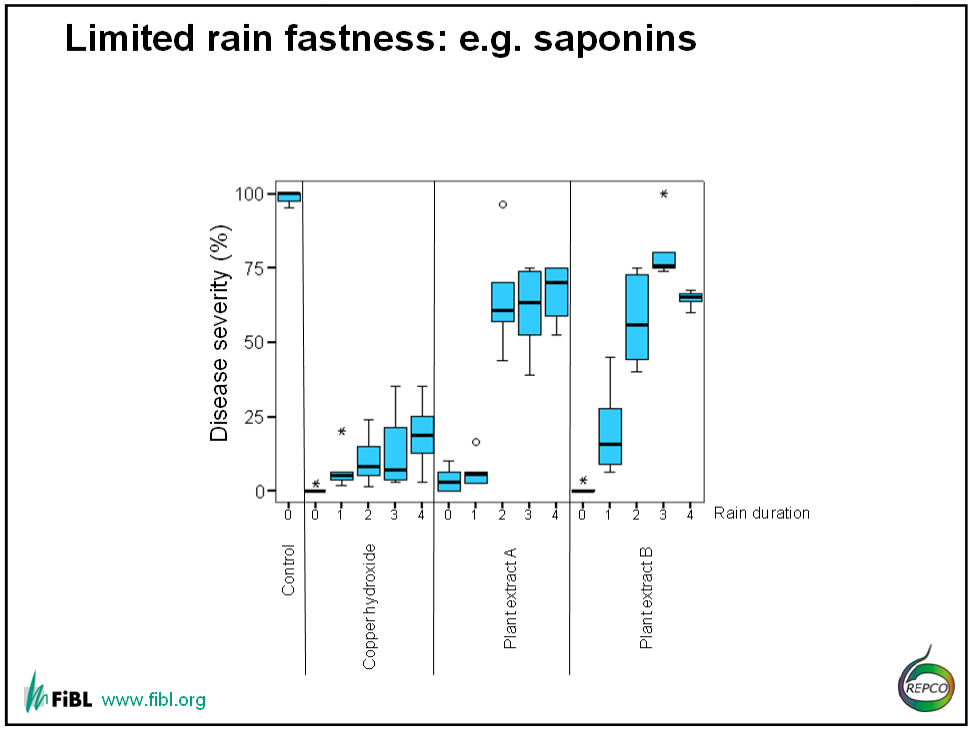
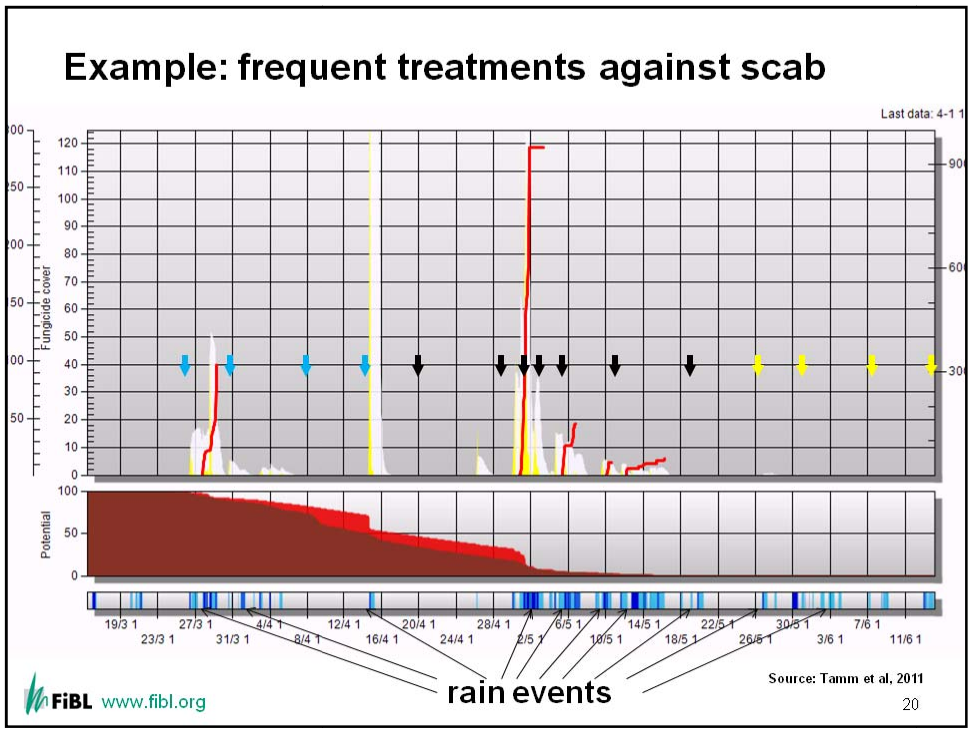

Limited rain fastness: e.g. saponins
Disease severity (%)
100
75
50
25
0
0 1 2 3 4
Rain duration
Control
Copper hydroxide
Plant extract A
Plant extract B
FiBL www.fibl.org
REPCO


Example: frequent treatments against scab
Last data: 4-1 1
Fungicide cover
Potential
19/3 1
23/3 1
27/3 1
31/3 1
4/4 1
8/4 1
12/4 1
16/4 1
20/4 1
24/4 1
28/4 1
2/5 1
6/5 1
10/5 1
14/5 1
18/5 1
22/5 1
26/5 1
30/5 1
3/6 1
7/6 1
11/6 1
rain events
Source: Tamm et al, 2011
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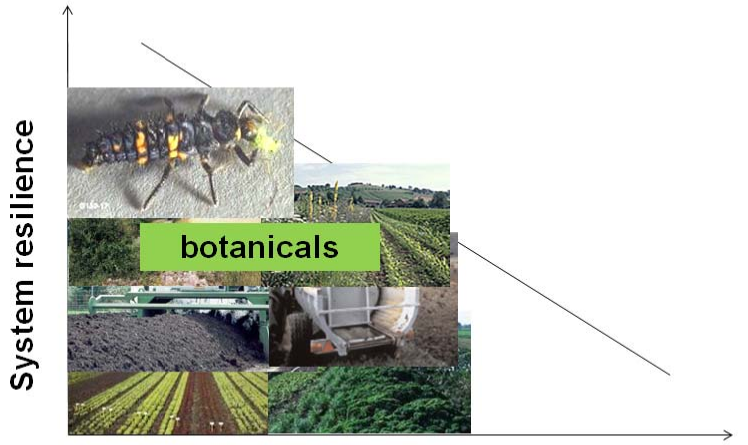
20

Impact of system resilience



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Source: Tamm, 2011 21

Conclusions

- Botanicals have great potential in sustainable, resilient production systems (i.e. IPM, organic, low input, low residue sytems)
- Registration requirements need to be adapted to the properties of botanicals (not the reverse)
- The quest for botanicals (and mixtures thereof) is intensifying: innovations are upcoming

Thank you for your attention!

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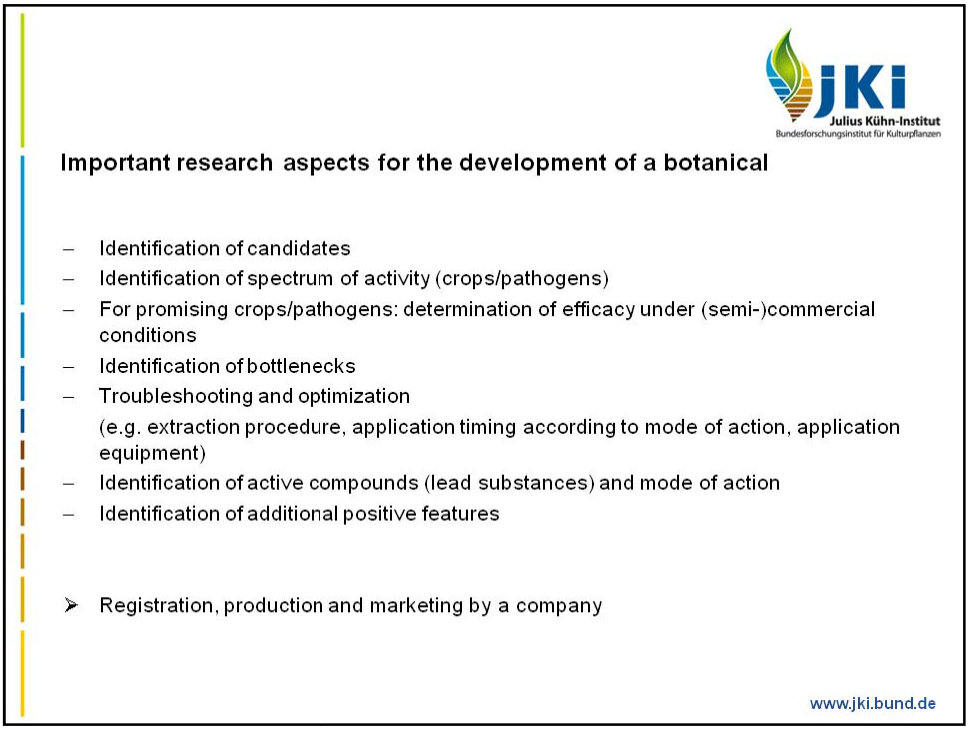
22

Presentation 2b
Experiences from the development and field testing of two botanicals
By Annegret Schmitt (Institute for Biological Control -JKI, Darmstadt; Germany)



Examples:
Giant knotweed (*Fallopia sachalinensis*) and Sweetwood (liquorice) (*Glycyrrhiza glabra*)

***F. sachalinensis*, Family Polygonaceae**
Young shoots are eaten like asparagus;
Plant was introduced as fodder plant and ornamental

Plant extract for plant protection is commercialized

***G. glabra*, Family Fabaceae**
Rhizomes are used for medicinal purposes and for production of sweets

Plant extract for plant protection is under development

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Giant knotweed (*Fallopia sachalinensis*)

Commercialized product:

Plant extract based on the above-ground parts of *Fallopia* (formerly *Reynoutria) sachalinensis*

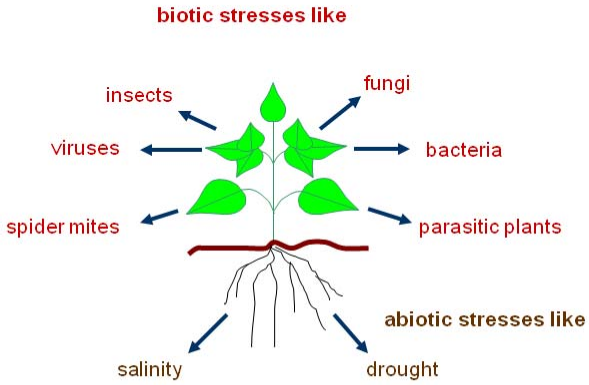
- extract found in a screening in BBA (now JKI) in 1988
- developed by BBA together with BASF Limburgerhof
- commercialised in Germany as plant strengthener under the name MILSANA® by BIOFA and
  in USA as biopesticide under the name REGALIA® by Marrone Organic Innovations
- mode of action is stimulation of the plant´s self defense mechanisms (plant strengthener)

www.jki.bund.de

Giant knotweed (*Fallopia sachalinensis*)

Plant strengtheners

- activate the plant´s natural defense mechanisms
- induce resistance/tolerance in plants against a variety of stresses



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Giant knotweed (*Fallopia sachalinensis*)

Important research aspects for the development of a botanical

- Identification of candidates
- Identification of spectrum of activity (crops/pathogens)
- For promising crops/pathogens: determination of efficacy under (semi-)commercial conditions
- Identification of bottlenecks
- Troubleshooting and optimization
  (e.g. extraction procedure, application timing according to mode of action, application equipment)
- Identification of active compounds (lead substances) and mode of action
- Identification of additional positive features

➢ Registration, production and marketing by a company

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Giant knotweed (*Fallopia sachalinensis*)

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Spectrum of activity against plant diseases

- **In the greenhouse**
  - Powdery mildew in cucumber, tomato, pepper, begonia, cereals (++)
  - Grey mould in begonia and cucumber flowers, young tomato and pepper plants, ornamentals (++)
  - Rust in carnation and beans (+)
  - Tobacco mosaic virus (+)
- **In open-field**
  - Powdery mildew in grape vine (++), roses (depending on cultivar), ornamental trees (+)
  - Grey mould in grape berries (+)

++ highly effective; + moderately effective

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Giant knotweed (*Fallopia sachalinensis*)

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Grey mould on sweet pepper (*Botrytis cinerea*)

1 % *F. sachalinensis*-extract control

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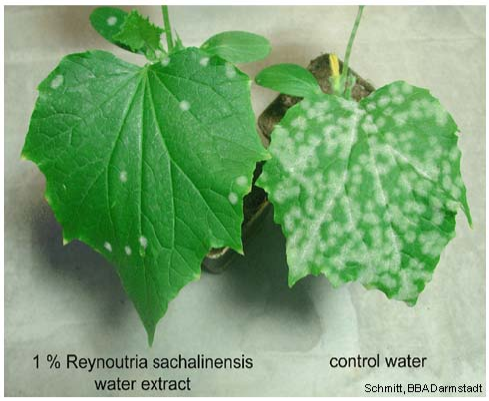
Giant knotweed (*Fallopia sachalinensis*)

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Powdery mildew on cucumber (*Podosphaera xanthii*)



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Giant knotweed (*Fallopia sachalinensis*)

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Powdery mildew on cucumber (*Podosphaera xanthii*)

Greenhouse trial

Water-treated control

Application of Milsana in 7-day intervals

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Giant knotweed (*Fallopia sachalinensis*)

Powdery mildew on begonia (*Golovinomyces cichoracearum*)

Greenhouse trial

control treatment with Milsana in 7-day intervals

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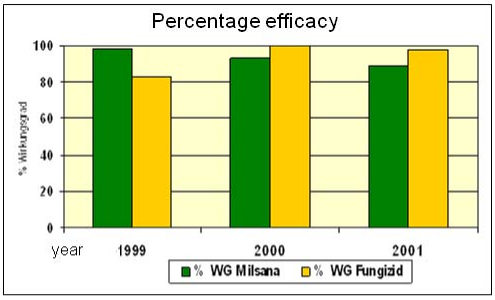
Giant knotweed (*Fallopia sachalinensis*)

Powdery mildew on tomato (*Oidium neolycopersicum*)

Greenhouse trial



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Giant knotweed (*Fallopia sachalinensis*)

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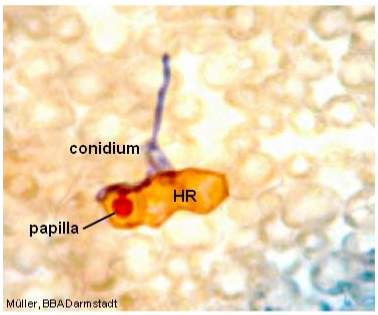
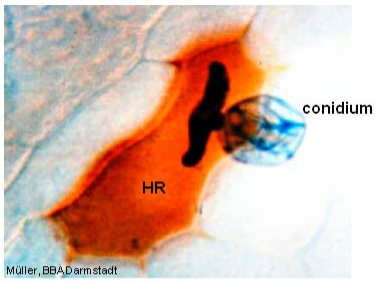
Site-specific defense mechanisms after treatment with Milsana (cucumber/powdery mildew)





Hypersensitive response (HR) at the infection site

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Giant knotweed (*Fallopia sachalinensis*)

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Site-specific production of flavonoid phytoalexins induced after Milsana treatment (cucumber /powdery mildew)

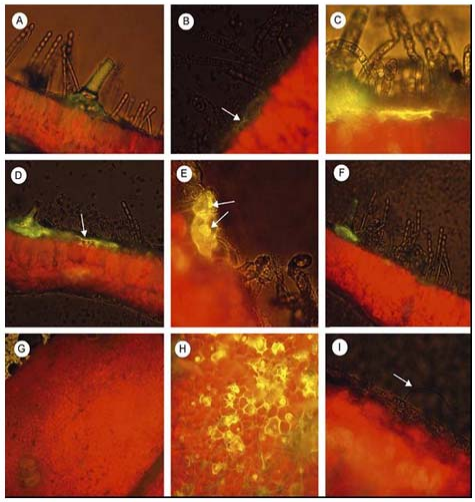

Fig. 3. Fluorescence microscopy analysis (488 nm) of fresh transverse-sectioned leaf tissues from inoculated (E - I +), and elicited/inoculated (E + I +) cucumber plants sampled at different times following the first Milsanaw treatment.
(A) E + I + leaf at time 0 h (200 X).
(B) An E + I + leaf 4 h after elicitation, the arrow indicates a faint yellow autofluorescence visible within the periphery of infected epidermal cells (400 X).
(C), An E + I + leaf 20 h after elicitation (400 X) and; (D) an E + I + leaf 30 h after elicitation, the arrow indicates yellow autofluorescence visible within the entire periphery of an infected epidermal cell and within the haustorial complex of this cell (100X).
(E) An E + I + leaf 48 h after elicitation, the arrows indicate yellow autofluorescence visible within the haustorial complexes of infected epidermal cells (400X).
(F) An E - I + leaf at 48 h (100 X) and;
(G), the surface view of an E - I + leaf at 48 h (100 X).
(H) The surface view of an E + I + leaf at 48 h (200 X). (I) An E + I + leaf 96 h after elicitation, the arrow indicates a collapsed conidial chain (200 X).

From McNally et al., Physiological and Molecular Plant Pathology 63 (2003) 293–303

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Giant knotweed (*Fallopia sachalinensis*)

General effects of *F. sachalinensis* extracts on the plant

- Increase in chlorophyll content
- Increase in photosynthetic activity
- Reduced side shoot development, enhanced main shoot development
- Influence on general habitus
- Reduced senescence
- Enhanced flower induction, flower size

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Giant knotweed (*Fallopia sachalinensis*)

Effects of *F. sachalinensis* extracts on flower size of Rex-begonia

control treatment with Milsana

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Giant knotweed (*Fallopia sachalinensis*)

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Greenhouse trials with Milsana in cucumber/powdery mildew

	Efficacy [%]	Yield increase (total weigth) over control [%]
Germany	85.8	24.7
Greece	98.3	21.6
The Netherlands		

Disease severity at the end of the trials: 89-100%

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Giant knotweed (*Fallopia sachalinensis*)

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Greenhouse trials with Milsana in cucumber/powdery mildew

	Efficacy [%]	Yield increase (total weigth) over control [%]
Germany	85.8	24.7
Greece	98.3	21.6
The Netherlands	28.5	29.5

Disease severity at the end of the trials: 89-100%

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Giant knotweed (*Fallopia sachalinensis*)

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Active compounds

- Physcion
- Physcionglycoside

And other compounds not yet identified

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Giant knotweed (*Fallopia sachalinensis*)

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

F. sachalinensis

- commercialized plant extract used in plant protection in Europe and USA
- high efficacy in protected and open-field crops against a variety of pathogens
- plant extract with broad spectrum of additional positive features
- plant extract with well defined mode of action (induced resistance)

F. sachalinensis **extract is a plant protection agent suitable for organic and integrated farming**
Due to its mode of action there is only a minimal risk for development of resistance in the pathogen

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Sweetwood (liquorice) (*Glycyrrhiza glabra*)

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

***G. glabra*, Family Fabaceae**
Rhizomes are used for medicinal purposes and for production of sweets

Extract for plant protection is based on above-ground parts of *G. glabra*
Extract is currently still under development and in pre-marketing experimental stage

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Sweetwood (liquorice) (*Glycyrrhiza glabra*)

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Important research aspects for the development of a botanical

- Identification of candidates
- Identification of spectrum of activity (crops/pathogens)
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- Identification of bottlenecks
- Troubleshooting and optimization
  (e.g. extraction procedure, application timing according to mode of action, application equipment)
- Identification of active compounds (lead substances) and mode of action
- Identification of additional positive features

➢ Registration, production and marketing by a company

www.jki.bund.de

Sweetwood (liquorice) (*Glycyrrhiza glabra*)

Spectrum of activity of *G. glabra* extract against Oomycetes on potted plants (climate chamber/greenhouse)

Pathogen	Crop	Efficacy (5% extract concentration)
Pseudoperonospora cubensis	cucumber	+++
Bremia lactucae	lettuce	+++
Peronospora destructor	onion	++ / +++
Phytophthora infestans	tomato	+++
P. infestans	potato	++

+++ = efficacy > 80 %
++ = efficacy 50-79 %

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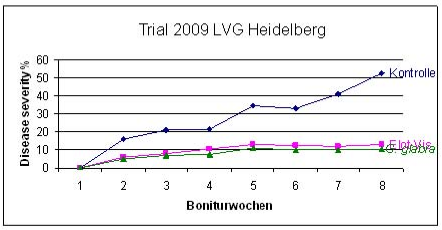
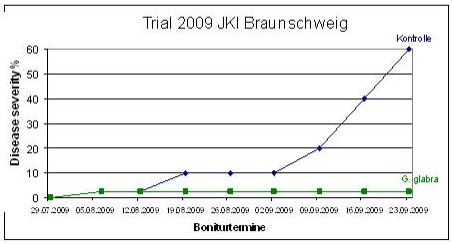
Sweetwood (liquorice) (*Glycyrrhiza glabra*)

Semi-commercial greenhouse trials in cucumber/downy mildew





***G. glabra* extract**
- extract concentration 3%
- application interval 7 days

Trial Site	Efficacy (based on AUDPC)
LVG Heidelberg	68%
JKI Braunschweig	83%

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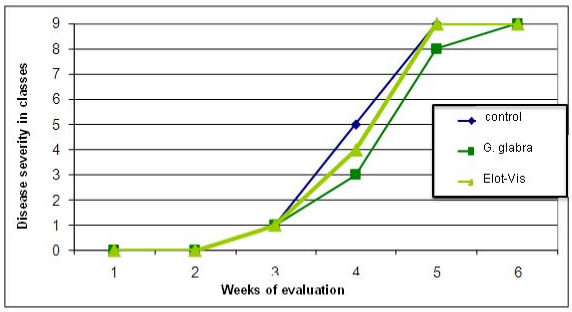
Sweetwood (liquorice) (*Glycyrrhiza glabra*)

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Open-field trial in cucumber/downy mildew

***G. glabra* extract**
- extract concentration 3%
- application interval 7 days



Disease severity in classes:
1 = 0 - 2,5 %
2 = 2,5 - 5 %
3 = 5 - 10 %
4 = 10 - 15 %
5 = 15 - 25 %
6 = 25 - 35 %
7 = 35 - 50 %
8 = 50 - 75 %
9 = 75 - 100 %

With *G. glabra* extract retardation of disease development over a period of approx. 3 weeks

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Sweetwood (liquorice) (*Glycyrrhiza glabra*)

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Comparison of results under greenhouse and open-field conditions in cucumber/downy mildew

G. glabra in greenhouse:
Efficacy (based on AUDPC):
- *G. glabra:* 68 % - 83 %

G. glabra in open-field
Retardation of disease development

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Sweetwood (liquorice) (*Glycyrrhiza glabra*)

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Bottleneck for *G. glabra* extract:

Good efficacy in protected crops but lower, no or variable efficacy in the field

Crops/diseases showing this bottleneck for *G. glabra* extract

cucumber/downy mildew

onion/downy mildew

lettuce/downy mildew

tomato/late blight

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Sweetwood (liquorice) (*Glycyrrhiza glabra*)

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Troubleshooting

Possible reasons for reduced efficacy in the field:

- High infection pressure (susceptibility of cultivars, weather)
- Sub-optimal application timing
- Uneven distribution of extract in the crop (application equipment)
- Low adhesion on or uneven coverage of leaves with extract
- Low UV- stability or rain fastness of extract

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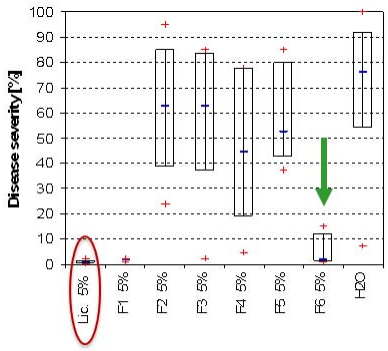
Sweetwood (liquorice) (*Glycyrrhiza glabra*)

"Hunt" for the active compounds of *G. glabra*

Fractionation of *G. glabra* extract by shake-out procedure

– Fractions were separated and tested for efficacy in cucumber/downy mildew (potted plants)



***G. glabra* extract-fraction 6 (F6, acidic) showed efficacy comparable to the efficacy of the full extract**

- Until now three polyphenols have been identified in F6
- Trials on efficacy with compounds from F6 are running
- Most likely more than one active compound
- Most likely different modes of action

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Sweetwood (liquorice) (*Glycyrrhiza glabra*)

***G. glabra* extract**

- high efficacy in protected crops against Oomycete plant pathogens
- variability and lower efficacy in the open-field (due to rain, UV light, infection pressure, cultivar susceptibility, mis-timing of application etc.)

G. glabra extract is a promising candidate for control of downy mildews and reduction of copper in organic vegetable production

But optimisation of extract with respect to effiacy is necessary

- extract formulation (stickers, UV- and rain stability, etc.)
- application method or technique
- clarification of mode of action
- harvesting times (highest level of active compounds)
- extraction procedure

Optimisation will be done together with Trifolio-M GmbH and other research partners

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Thanks to

Research on *F. sachalinensis*

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Presentation 3
Natural extracts characterization and application of analytical methods for botanical quality determination
By Cédric Bertrand (University of Perpignan; France)

UPVD Université de Perpignan Via Domitia

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Natural extracts characterization and application of analytical methods for botanical quality determination

Seminar on "Characterisation and Analyses of Botanicals for the Use in Plant Protection Products"
30 March 2011, Paris, France

Dr. Cédric BERTRAND
Laboratoire de Chimie des Biomolécules et de l'Environnement
EA 4215
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1

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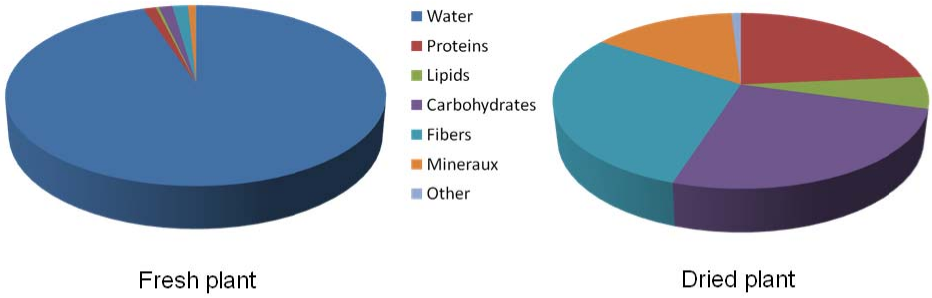
Scope : Chemical characterization and quality of Botanicals used for plants protection

Purpose : analytic methods applied to the analysis of complex naturals mixtures

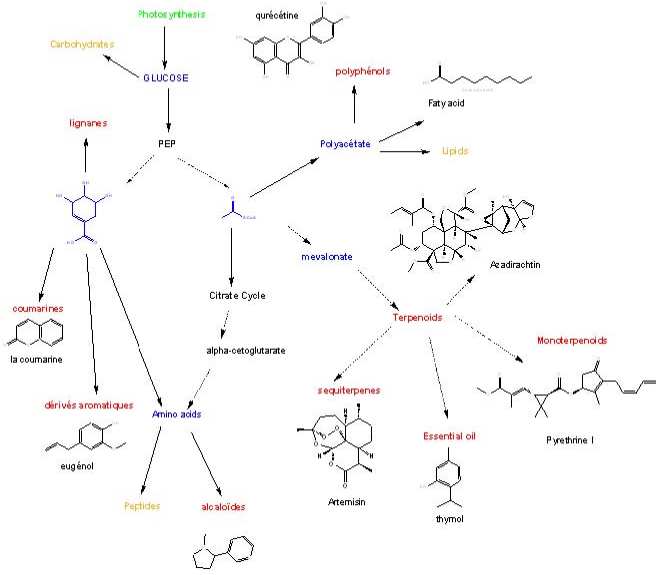
2

General chemical composition of plant



3

Botanicals : a wide structural diversity of compounds



4

Botanicals : a wide structural diversity of compounds

Some compounds

- Bioactive compounds mainly derived from the plant secondary metabolite pathway :
  - terpenoids
  - alcaloids
  - polyphenols
  - ….
- Carbohydrate : Laminarin is an original storage compound
- Lipid : Fatty acid
- Peptide

5

Natural extracts : a wide range of biological activities

- Antifungal (thymol)
- Aphicidal (nicotin)
- Herbicidal (nonanoic acid)
- Elicitor of plant defense (laminarin)
- Biocide, bactericide, antioxidative…

Plant extract = complex mixture of a wide range of chemical compounds and biological activities.

6

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Choice of biomarkers compounds

1) Biomarkers = Bioactive compounds
   a - plant active is known
   b - plant active is unknown

2) Biomarkers = keys compounds (of the bioactive plant extract)

Use of same analytical equipment

7

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Plant extract characterization

Goals : Characterization of the mixture diversity and / or determination of amount of biomarkers

Strategies :

1 Applying a metabolomic approach for the identification of characteristics biomarkers of bioactive extract
   - Fingerprinting (biomarker = fingerprint)
   - Amount of major compound
   - Amount of specific compound
   - …

2 Applying a bio-guided purification for the identification of bioactive compounds as biomaker

8

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What is the standard laboratory equipment required for high-perfomance botanicals analysis?

- Sample preparation
  - Extraction instruments
  - Instrumentation for concentrating

- Analytical instruments (HPLC-UV, LC/MS/MS,GC, NMR….)

http://www.sigmaaldrich.com/etc/medialib/sigmaldrich/migrationresource4/p001015.Par.0001.Image.341.gif
http://www.buchi.com/typo3temp/pics/product_overview_R-II_15_b151fd563a.jpg
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http://www.jeol.com/Portals/0/prodshots/AI/ecs.jpg

9

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Botanicals Quality

Some examples of analytical systems for :

A- characterization of biological extracts and

1. *Characterization of bioactive compounds*
2. *Identification of bioactive compounds*
3. *Characterization of specific compounds*

B - botanicals quality validation

1. *Variability studies*
2. *Stability studies*
3. *Batch plant validation*

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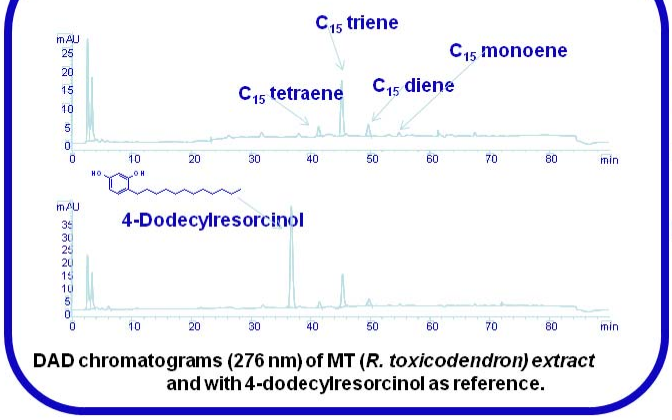
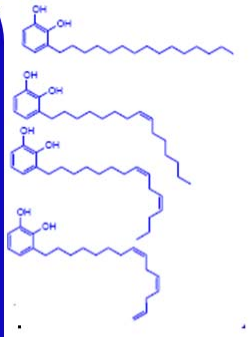
Biomarkers = bioactive compounds
example: Urushiol congeners of *Rhus sp.* extracts





DAD chromatograms (276 nm) of MT (*R. toxicodendron*) extract and with 4-dodecylresorcinol as reference.

11

C. Bertrand, F. Bellvert, A Boisroux, G. Comte, K Taoubi. 2008. Planta Medica, 74, 1095.

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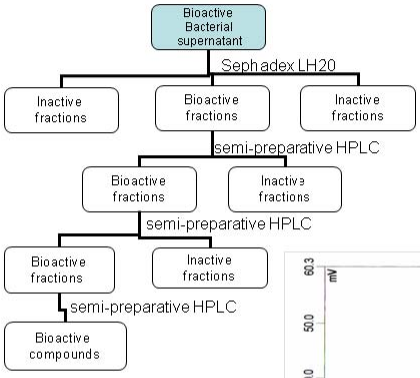
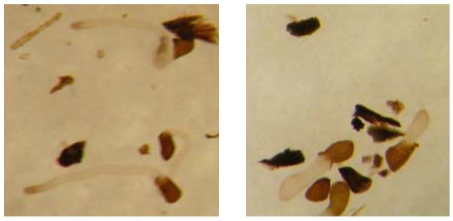
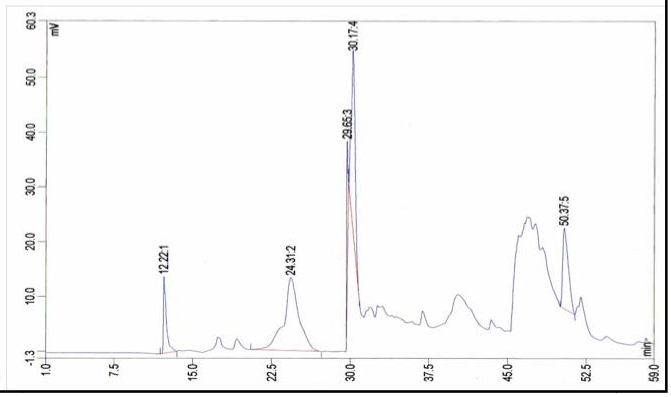
Bio-guided purification of the bioactive compound

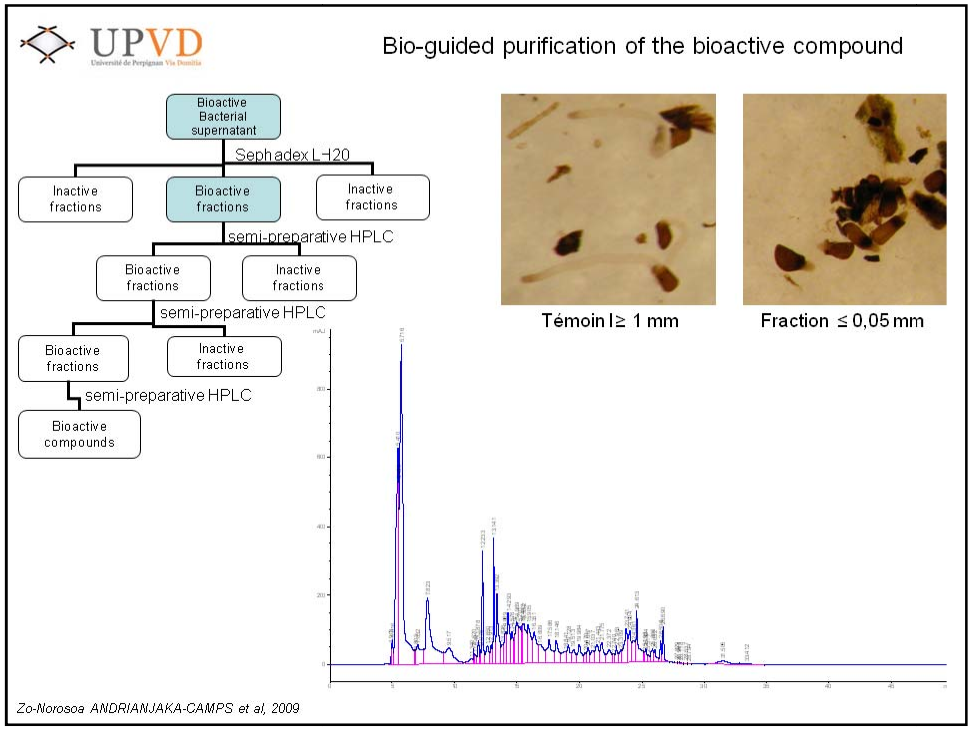




control I ≥ 1 mm



Zo-Norosoa ANDRIANJAKA-CAMPS et al, 2009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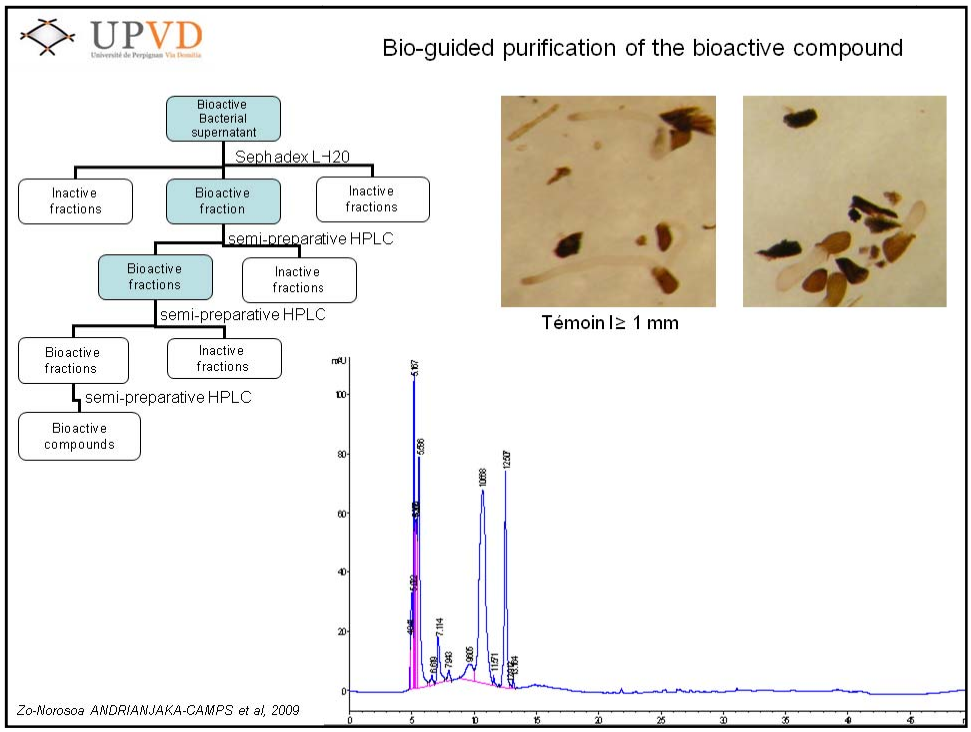
Bio-guided purification of the bioactive compound
Bioactive Bacterial supernatant
Sephadex L-H20
Inactive fractions
Bioactive fractions
Inactive fractions
semi-preparative HPLC
Bioactive fractions
Inactive fractions
semi-preparative HPLC
Bioactive fractions
Inactive fractions
semi-preparative HPLC
Bioactive compounds
Témoin I ≥ 1 mm
Fraction ≤ 0,05 mm
Zo-Norosoa ANDRIANJAKA-CAMPS et al, 2009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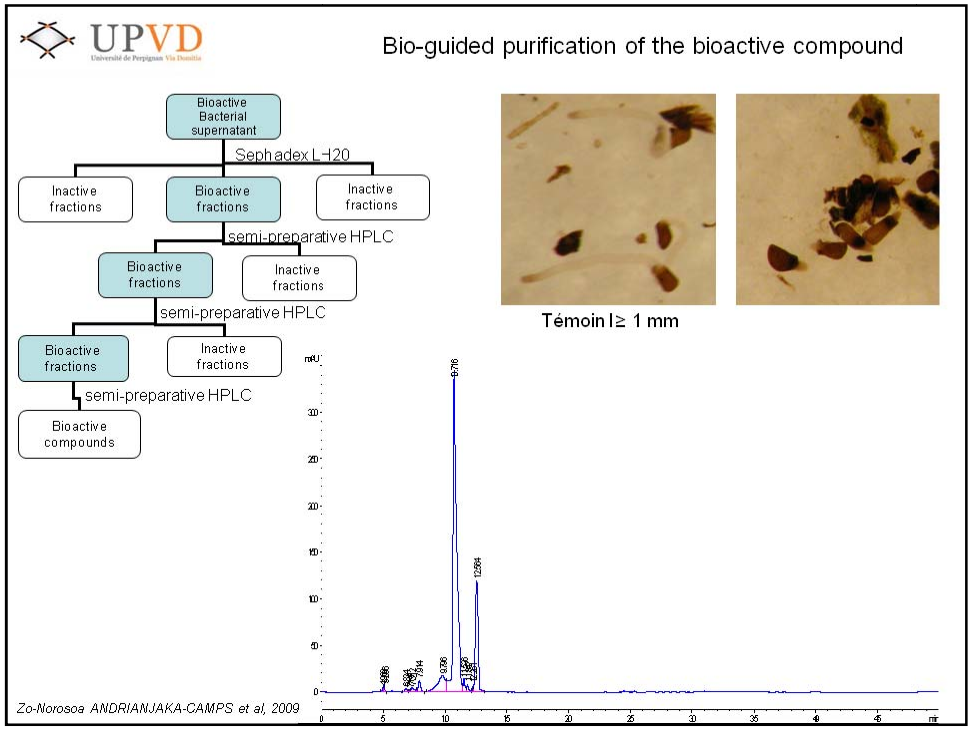
Bioactive fractions
Inactive fractions
semi-preparative HPLC
Bioactive compounds
Témoin I ≥ 1 mm
mAU
Zo-Norosoa ANDRIANJAKA-CAMPS et al, 2009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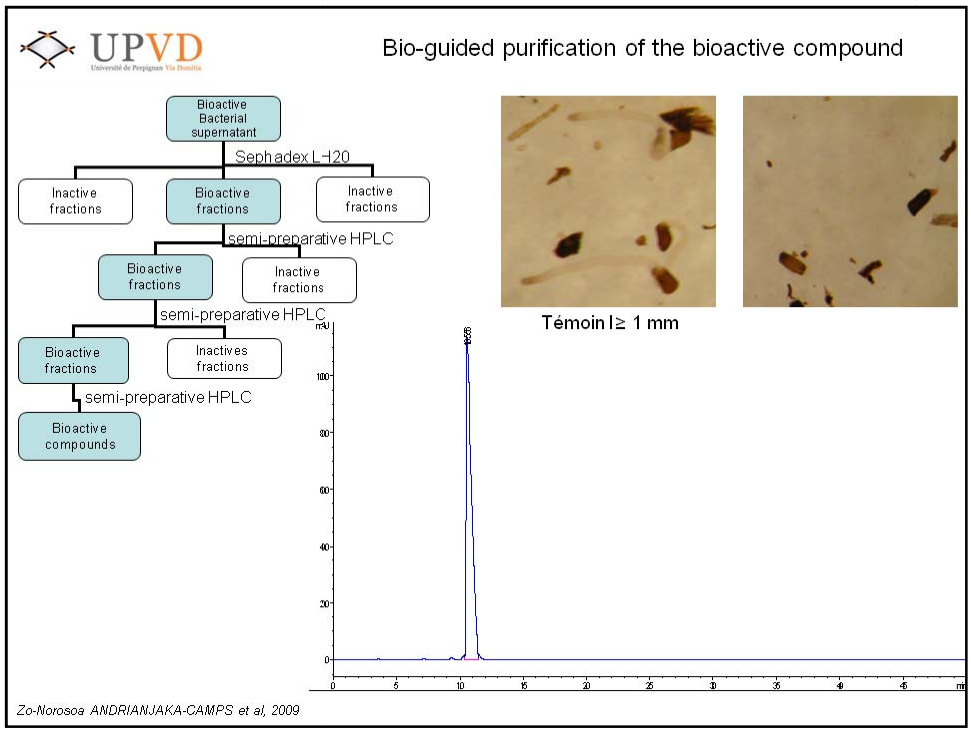
UPVD
Université de Perpignan Via Domitia
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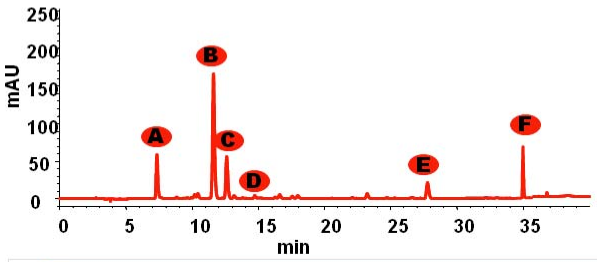
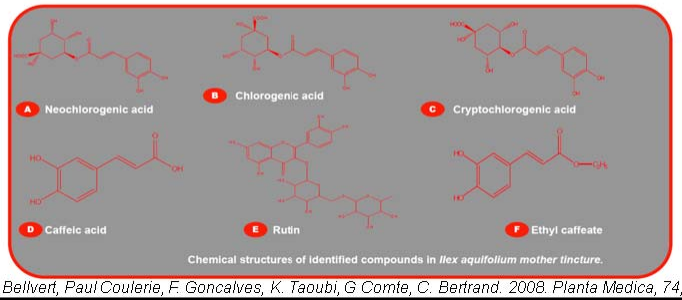
semi-preparative HPLC
Bioactive fractions
Inactives fractions
semi-preparative HPLC
Bioactive compounds
Témoin I≥ 1 mm
Zo-Norosoa ANDRIANJAKA-CAMPS et al, 2009

Major compounds of *Ilex aquifolium* hydroalcoolic extract



HPLC/DAD/MS analysis *Ilex aquifolium* mother tincture



Chemical structures of identified compounds in *Ilex aquifolium mother tincture.*

F. Bellvert, Paul Coulerie, F. Goncalves, K. Taoubi, G Comte, C. Bertrand. 2008. Planta Medica, 74, 1103.

Highlighting variation of composition

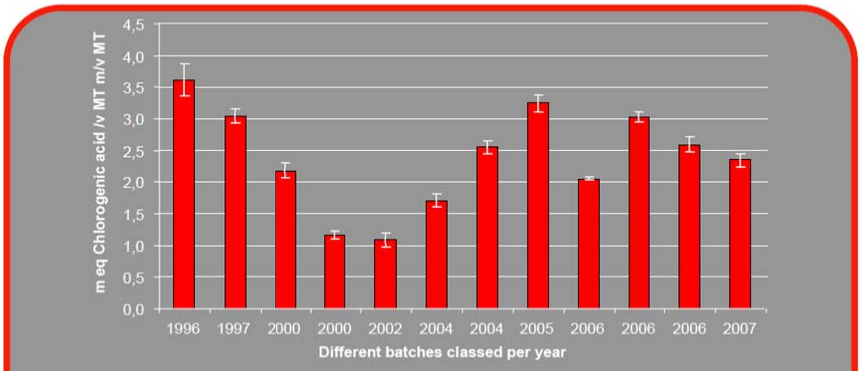
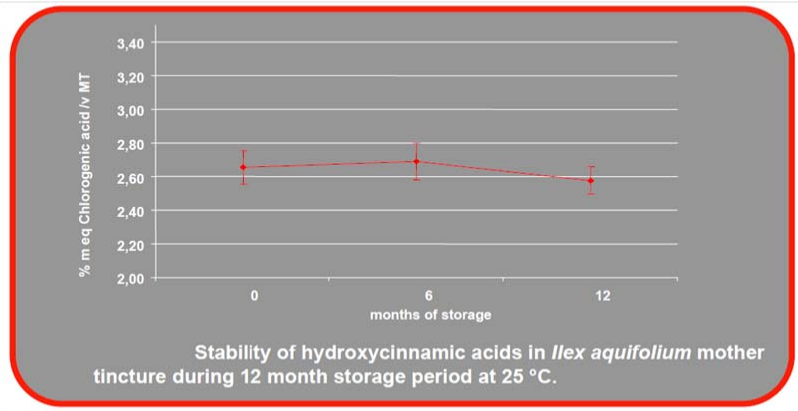


Figure : Variability of total hydroxycinnamic acids in *Ilex aquifolium* mother tincture produced from different batches and year of production.

18

F. Bellvert, P. Coulerie, F. Goncalves, K. Taoubi, G Comte, C. Bertrand. 2008. Planta Medica, 74, 1103.

Stability of *Ilex aquifolium* extract



Stability of hydroxycinnamic acids in *Ilex aquifolium* mother tincture during 12 month storage period at 25 °C.

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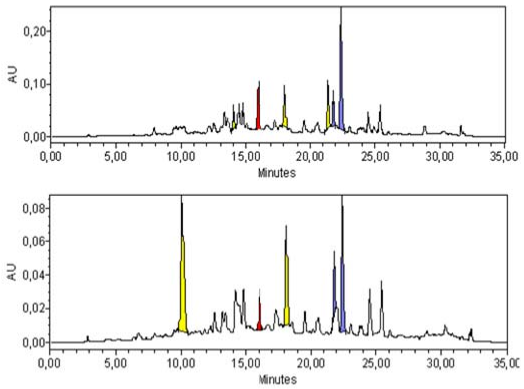
F. Bellvert, P. Coulerie, F. Goncalves, K. Taoubi, G Comte, C. Bertrand. 2008. Planta Medica, 74, 1103.

Highlighting plant extract stability

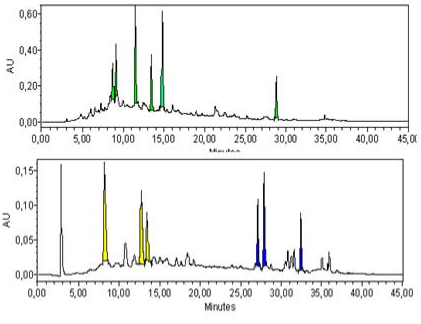


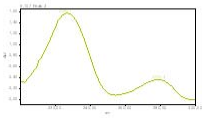
Equisetum arvense (horsetail) aqueous extract

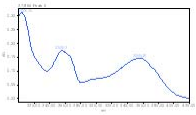
20

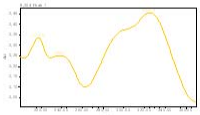
Bertrand and al, 2010. unpublished work

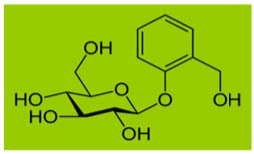
Quality of plant material

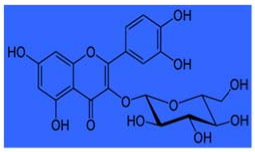


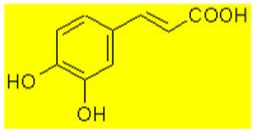












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Bertrand and al, 2010. unpublished work

Environmental fate

Same systems could be used for environmental :-

1. *Soil degradation*
2. *Biodegradation*
3. *Phtotodegradation*

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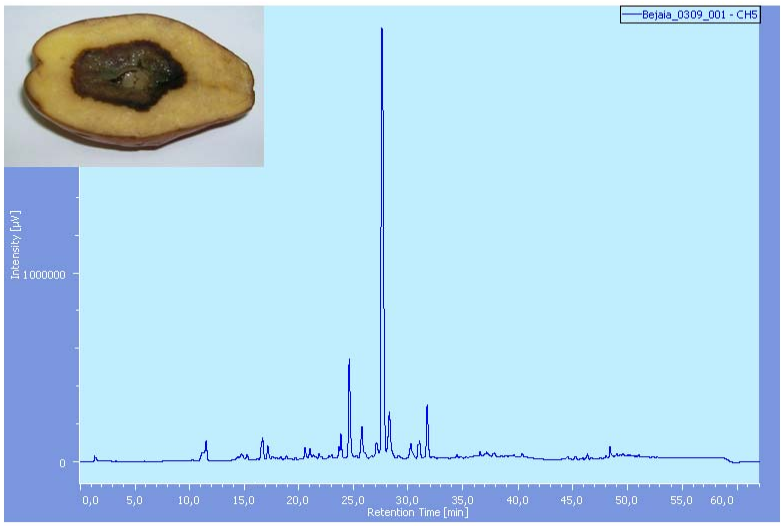
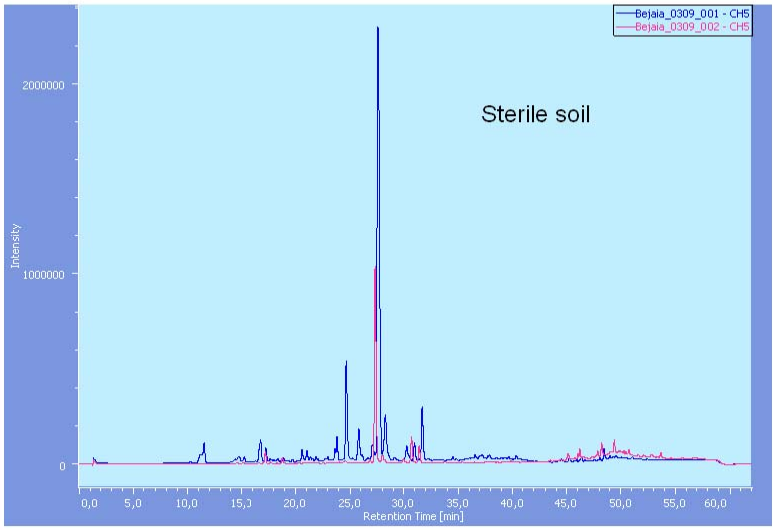
Environnemental fate : *Olea sp* leaves extract



23

Meziani, Bertrand and al, 2011. unpublished work

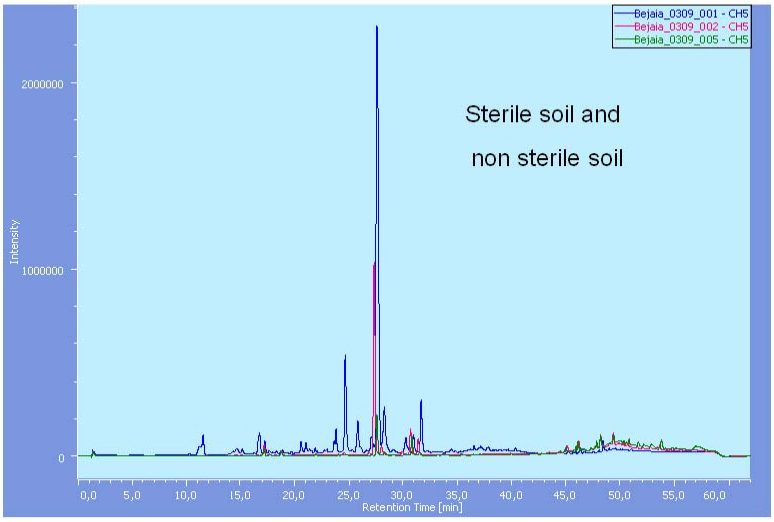
Abiotic degradation of *Olea sp* leaves extract



24

Meziani, Bertrand and al, 2011. unpublished work

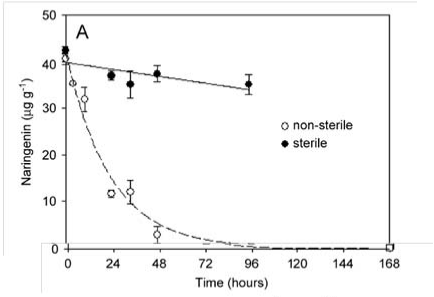
Biotic degradation of *Olea sp* leaves extract

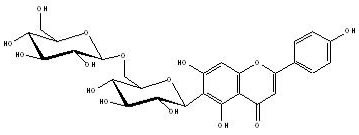


25

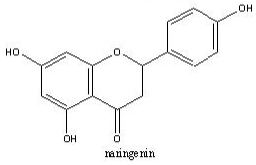
Meziani, Bertrand and al, 2011. unpublished work

Biodegradation on soil of flavonoïds





Hal-live isovitexin-6''-O-glucoside :
Sterile soil : 33,9h
Non-sterile soil : 8,9h



Shaw and Hooker. 2008. The fate and toxicity of the flavnoids naringenin and formononetin in soil. 40, 528-536.
Barto EK, Cipollini. 2009. Half-live and field soil conen trattions of Alliria peytiola secondary metabolites. Chemosphere, 76: 71-75.

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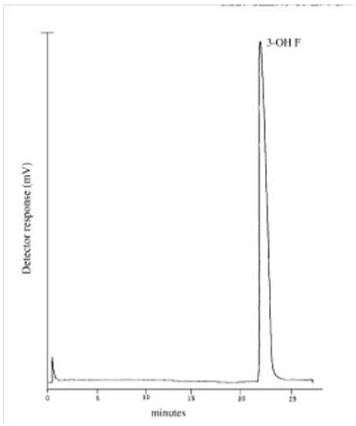
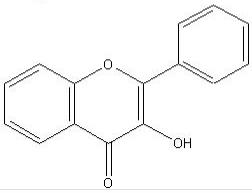
3-hydroxyflavone photodegradation





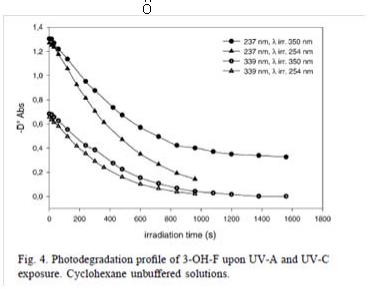
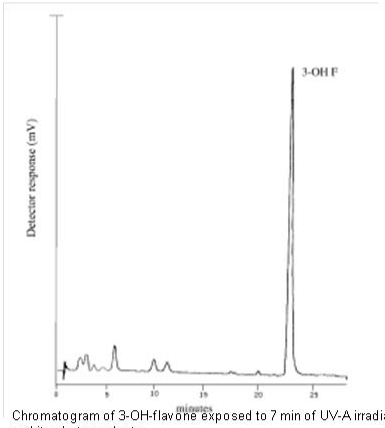


Fig. 4. Photodegradation profile of 3-OH-F upon UV-A and UV-C exposure. Cyclohexane unbuffered solutions.

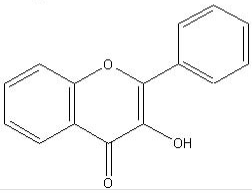
27

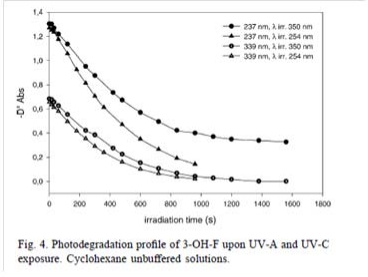
Tommasini et al. 2004. Comparative photodegradation studies on 3-hydroxyflavone. Journal of pharmaceutical and Biochemical Analysis. 35, 389-397.

3-hydroxyflavone photodegradation



Chromatogram of 3-OH-flavone exposed to 7 min of UV-A irradiation and its photoproducts.





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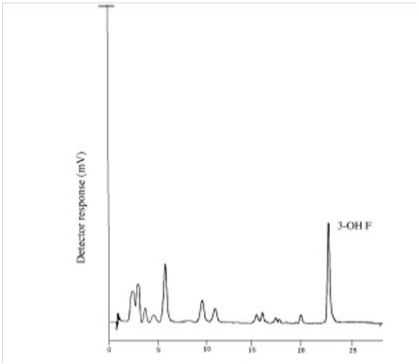
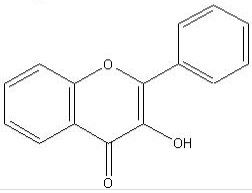
3-hydroxyflavone photodegradation



Chromatogram of 3-OH-flavone exposed to 11 min of UV-A irradiation and its photoproducts



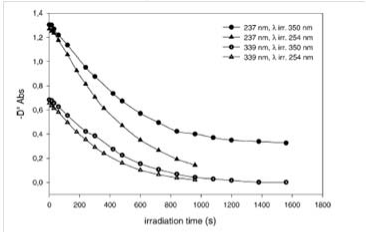


Fig. 4. Photodegradation profile of 3-OH-F upon UV-A and UV-C exposure. Cyclohexane unbuffered solutions.

29

Tommasini et al. 2004. Comparative photodegradation studies on 3-hydroxyflavone. Journal of pharmaceutical and Biochemical Analysis. 35, 389-397.

Natural extracts characterization and application of analytical methods for botanical quality determination

→ Natural extract are very complex mixture of compounds

→ High perpformant analytical systems are available for botanicals characterization

→ This analytic systems allow the characterization of botanicals, quality validation and environmental fate studies

→ And the critical point is the definition of the biomarkers

30

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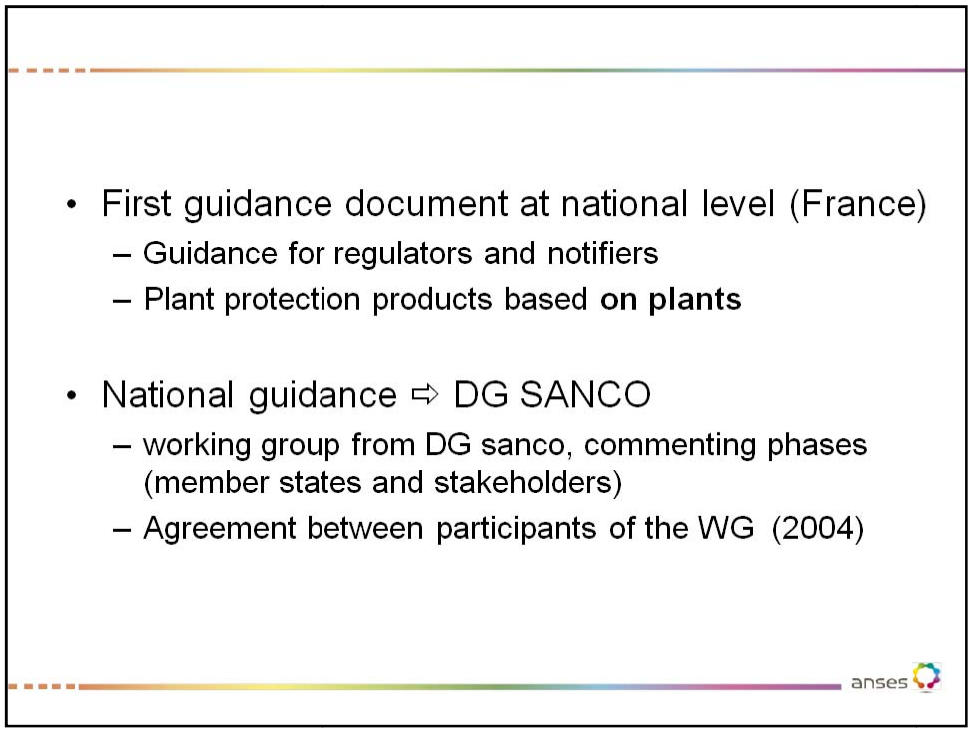
lcbe
Laboratoire de Chimie des Biomolécules et de l'Environnement

Thank you for your attention.

Contact : cedric.bertrand@univ-perp.fr

31

Presentation 4
EU Draft Working Document on Plant Extracts SANCO/10472/2003 - key points and practical experiences.
ByThierry Mercier (French Agency for Food, Environmental and Occupational Health & Safety [ANSES], Paris; France)



– Goals of the Sanco document

- to propose on a weight of evidence basis a tiered approach to the data requirements for plant protection products made from plants or plant extracts
- to maintain harmonised assessment schemes and decision making in Member States
- Submission : Annexes II and III Directive 91/414/EEC
  ↳studies
  or
  ↳ scientific justification

anses

Definitions in the framework of this document

- in the framework of this document
  - live or dried plants and live or dried parts of plants, including fruits and seeds excluding GMO
- ***Plant protection products made from plants or plants extracts***
  - Product intended for use in plant protection which contains plants, plant extracts and possibly formulants
- ***Plant extracts***
  - An extract is obtained
  - from a solution achieved by treating plants or parts of them, with a solvent, which is further concentrated through evaporation, distillation or some other process
  - Only soft extraction with water and/or ethanol (excluding other solvents) are covered in the framework of this document

Basic principle : ethanol/water extraction does not increase significantly the risk

anses

DATA SET REQUIREMENTS

- Category 1 :
  - Plant protection products made from one or several plants included in the reference list and mixed with water and possibly with formulants added.
- Category 2 :
  - Plant protection products prepared with one or several ethanol/water based extracts made of plants included in the reference list and possibly with formulants added
    - -category 2.1 : water/ethanol plant extracts made of plants included in the reference list
    - -category 2.2: plant protection products prepared with one or several ethanol/water extracts made of plants included in the reference list and possibly with formulants added.

anses

Reference list contains :

- all edible parts of plants used for animal or human feed
- parts of plant currently authorised as herbal drugs in EU phamacopoeia
- List established on the basis of available information :
  - including literature
  - evaluation done in OECD countries
  - European pharmacopoeia
  - weight of evidence which indicates that the plant is not harmful to human, animal and environment
- Advisory list

anses

- **Key points of the document**

- **Data requirements for Category 1**
  - Plant protection products made from one or several plants included in the reference list and mixed with water and possibly with formulants added.

anses

Plant protection product : identity-specification

- Description of the known active plant protection substances
  - Provide the active substances' concentration range
- For the other substances, provide a percentage of the total weight (or a percentage range)
- For any toxic substances that are relevant for human, animal health and environment provide a maximum content limit.

anses

Plant protection product : identity-specification

- If the active substance(s) is (are) not identified, define a representative marker *.
- Representative marker : *a chemical naturally present in a known proportion in the plant in order to identify the plant protection product*
- Analysis report of 5 batches of different manufacture, collected over several periods

anses

- <u>Full list of ingredients :</u>
- The plant protection product's trade name, physical state and function must be specified.
- A precise quantity of the plant, or an upper and lower limit must be submitted.

	Quantity
• Plant (whole or part)	[ ] g/kg or g/l (expressed as fresh weight and dry weight or as a weight interval)
Other ingredients	[ ] g/kg or [ ] g/l
Water	[ ] g/kg or [ ] g/l

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PHYSICAL AND CHEMICAL PROPERTIES OF THE PLANT PROTECTION PRODUCT

- Type, appearance, pH, oxidising properties, particle sizes distribution (powders) etc.

DATA ON APPLICATION :

- *GAP*

FURTHER INFORMATION ON THE PLANT PROTECTION PRODUCT

- Packaging, cleaning of the spraying equipment, etc.

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ANALYTICAL METHODS

- If the substances are identified :
  - Validated method for analysing the identified active substance in the plant protection product,
- If the active substances are not identified,
  - a validated method of analysis of the marker in the plant protection product should be available.
- A validated method for analysing the active substance in water, soil and air can be judged necessary if exposure of the concerning compartment is likely and the contribution compared to natural background levels is substantial.
- If any toxic substances that are relevant for human or animal health and the environment are detected in the plant protection product, validated methods of analysis must be provided.

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EFFICACY DATA : information has to be provided

- *e.g. Since 2010 testing protocols available in France*
- *AFPP–CEB : document technique N° 18*

TOXICOLOGICAL STUDIES

- The information provided must be of sufficient quality to enable an evaluation of the plant protection product,
- Risk assessment for the operator and worker must be addressed and personal protective equipment where relevant indicated.

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RESIDUES IN OR ON TREATED PRODUCTS FOOD AND FEED

- exposure due to the use as plant protection product, compared to the exposure due to consumption of the plant itself.
- where relevant residues supervised field trials must be carried out

FATE AND BEHAVIOUR IN THE ENVIRONMENT

- If exposure of water, soil or air is likely to occur available information from literature on natural background levels should be provided. If there is a substantial increase more information may be required based on expert judgement.

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ECOTOXICOLOGICAL STUDIES

- Provide all ecotoxicological information available,

- If classification according to
  - Directive 67/548/EEC or
  - 1999/45/EC is applicable,
  - the following studies must be provided: acute effects on fish, daphnia and algae

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Exemple : Fenugreek seedpowder

- Draft assessement report in December 2004
- Currently : annex I directive 91/414/CEE
- Target : powdery mildew (vine)

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Acute toxicity (Annex IIA, point 5.2)

- Rat LD50 oral> 5000 mg/kg (published data) (Defatted fenugreek seed powder)
- Rat LD50 dermal : not required
- Rat LC50 inhalation : not required

Genotoxicity

- Ames test
- Literature data on components

anses

Long term toxicity and carcinogenicity

- The long term exposure of human to the compound is unlikely. No residues were expected after treatment of vineyards
- Carcinogenicity : not required

- Consumer
  - No residue expected at levels higher than exposure due to the consumption of the plant as medical product or dietary supplements

anses

Fate and behaviour

– No experimental data were submitted on fate and behaviour : justification given

Ecotoxicological studies

– **Acute toxicity to birds**	LD50 > 2 000 mg a.s./kg bw
– **Dietary toxicity to birds**	not required
– ***Oncorhynchusmykiss***	96 h LC50 283 mg/L
– ***Daphnia magna***	48 h EC50 > 100 mg/L
– ***Scenedesmus subspicatus***	96 h EbC50 >160 mg/L
– ***Bees contact and oral***	> 50 & > 200 microg/bee
– ***Earthworms LC50 (14d)***	5 000 mg a.s./kg soil

anses

Conclusion

- Useful document : data provided proportional to the risk
- Not applicable to all plant extracts
- Fully applicable : EU fourth list of existing substances and new active substance
- Update would be necessary based on current experience

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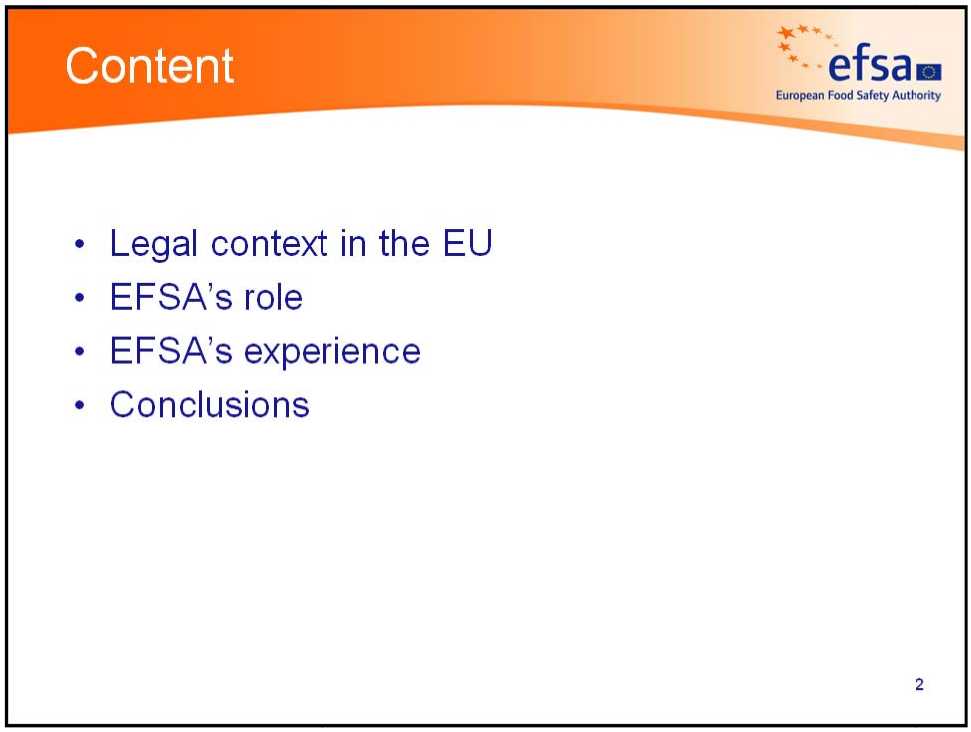
Presentation 5
EFSA's experiences in evaluating 'botanicals'
By Herman Fontier (European Food Safety Authority [EFSA], Parma; Italy)



Legal context in the EU

- Directive 91/414/EEC (as of 14/06/11 Regulation (EC) No 1107/2009 will apply)
- EU evaluation and approval of active substances (a.s.)
- National evaluation and authorisation of plant protection products (PPP)
- Harmonised data requirements for the a.s. and the PPP

3

Legal context in the EU

- Harmonised assessment methodology and authorisation criteria (Uniform Principles)
- Data requirements:
  - Part A: chemicals
  - Part B: micro-organisms
- No specific data requirements for botanicals; part A is applicable to botanicals
- It is always possible to waive data for technical or scientific reasons

4

Legal context in the EU

- EC has developed a draft working document on data requirements for botanicals
- This draft has never been finalised; however, it is available on EC DG SANCO's website
- This document does not supersede the legal data requirements

5

EFSA's role

- EFSA is only involved in the a.s. evaluation procedure
- A rapporteur Member State (RMS) receives and evaluates the application dossier
- The results of the evaluation are reported in the draft assessment report (DAR)
- EFSA circulates the DAR for comments by the applicant, the MSs, and makes it publicly available; EFSA also comments on the DAR

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EFSA's role

- Where necessary EFSA organises expert meetings in order to further discuss issues identified during the commenting
- EFSA, as an independent scientific organisation, drafts its conclusions and submits them to the EC for decision making
- EFSA's role is that of a risk assessor; in the EU, the risk assessment is clearly separated from the risk management

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EFSA's role

- EFSA cannot integrate in its conclusions risk management aspects, such as socio-economic importance
- Although EFSA is independent as a risk assessor, it is bound by the EU legislation
- EFSA has to take into account the data requirements and the risk assessment methodology as defined by the legislator

8

EFSA's experience

- EFSA has drafted two conclusions on botanicals:
  - Fenugreek seed powder (new a.s.)
  - Azadirachtin (existing a.s.)
- The commenting on botanicals listed in Annex I ("green track" a.s.) is ongoing or finalised; conclusions to be delivered by end 2012
- EFSA drafted several conclusions on synthesised chemicals that also occur naturally

9

Fenugreek seed powder

- 100% seed powder
- 3 marker compounds identified, but concentration ranges are missing
- Analytical methods available
- Toxicology: mostly published data
- From literature it appears that hormonal effects are possible (effects on milk production, uterine contractions)

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Fenugreek seed powder

- No AOEL can be set; operator, worker and bystander exposure are inconclusive
- Residues: no consensus between experts; inconclusive
- Environmental fate and behaviour: no studies, just waivers, which have been accepted
- Ecotoxicology: some acute studies available; accepted as low risk substance
- Risk managers decided to approve the a.s.

11

Azadirachtin

- Extract of neem tree seeds
- 1 lead molecule proposed (azadirachtin A); however, the mixture contains other biologically active substances
- Azadirachtin A concentration varies with origin
- Analytical methods available for azadirachtin A
- Toxicology: sufficient information to set ADI, AOEL and ARfD for azadirachtin extracts

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Azadirachtin

- Residues: nothing in the dossier, nature of residues not elucidated; therefore no conclusion possible (but impossible to radio-label the molecules)
- Environmental fate and behaviour: some studies available; data gap about potential groundwater contamination by some compounds (not possible to radio-label them)

13

Azadirachtin

- Ecotoxicology: very toxic for aquatic organisms
- Risk managers decided to approve the a.s.

14

Synthesised "botanicals"

- No reason to treat them differently from other synthesised chemicals (no problem of complex mixtures, molecule can be radio-labelled)
- Natural occurrence can potentially be used for data waiving (considering natural background levels)

15

Conclusions

- The word "botanicals" can cover very different types of substances, with very different properties, going from commonly consumed to very toxic
- Some data requirements do not seem very adequate
  - Specific set(s?) of data requirements (but substances very different)?
  - Guidance on waivers (to be built up with growing experience)?

16

Conclusions

- The non-availability of radio-labelled material is an obstacle for the risk assessment (relevant to toxicology, residues, fate and behaviour)
- Normal risk assessment methodology is not always adequate (for instance a rough estimation to make a comparison with the natural background level can be sufficient) => guidance needed

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Conclusions

- Guidance on lead compound concept needed (1 lead compound is not always enough; lead compound(s) for risk assessment may be different from lead compound(s) for monitoring)
- Level of uncertainty is very often higher
- Is this acceptable, or should the uncertainty be addressed with higher safety factors, risk management measures, bioassays,… ?

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Presentation 6
Neem/*Margosa* extract¨-and its constituents – experience in EU evaluation and registration
By Hubertus Kleeberg (Trifolio-M; Germany)

Trifolio-M GmbH

IBMA International Biocontrol agent Manufacturers' Association

IBMA D/A

Neem/Margosa Extract - and its Constituents - Experience in EU-Evaluation and Registration

Hubertus Kleeberg

Trifolio-M - IBMA D/A

OECD-Seminar 30th March 2011, Paris

1

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/A

Introductory Statement

It is self understood that the same high quality and safety/risk criteria have to be guaranteed for Synthetics and Natural Products (Plant Extracts or Microbial Substances) - and of course other means of Biol. Plant Protection as well (like Micro-organisms, Pheromones, Macrobials etc.).

However, these aims usually can't be fulfilled by the same registration procedure.

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Developmental cost of Biological Plant Protection Products (estimated) in Comparison to Synthetics

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(IVA Data 2006)

Years	1 2 3	4 5 6 7 8	9 10	million Euro
Active ingredient **Chemistry** Formulation	Extraction/Fermentation - Synthesis Lab-scale Development	Process development Development of Packing	Production* Production*	2
Research **Biology** Development	Screening Lab/Greenhouse Small plot trials Field trials		Registration	2
Degradation and residues	plant, animal, soil, water, air		Registration	8
Toxicology	acute u. chronic toxicity, cancerogenicity, mutagenicity, teratogenicity, reproduction			
Eco-Toxicology	algae, daphnia, fish, birds, micro-organisms, bees, beneficials		Registration	
Million Euro	2 (102)	10 (98)		12 (200)
Number of substances	5 – 10 (140000)	1 (1)		

* without cost for production plants

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Two examples for plant extracts may be:

Azadirachtin according to 91/414 EEC (or Margosa - according to Biocide regulations)

and

Quassia extract according to SANCO 10472

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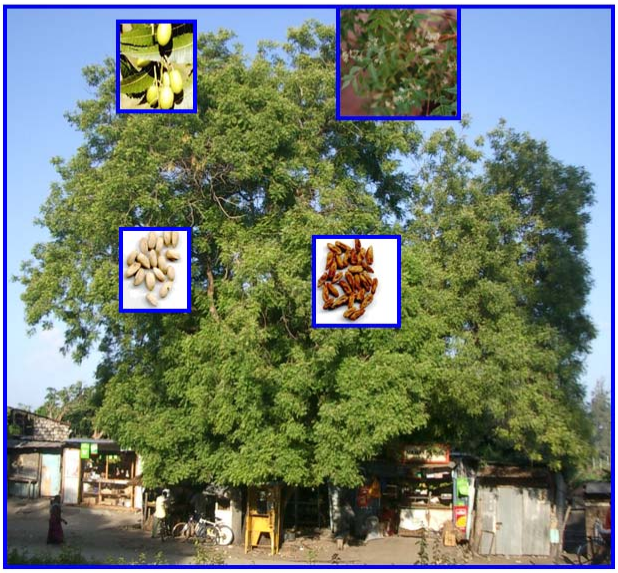
***Azadirachta indica* – Indian Tree**



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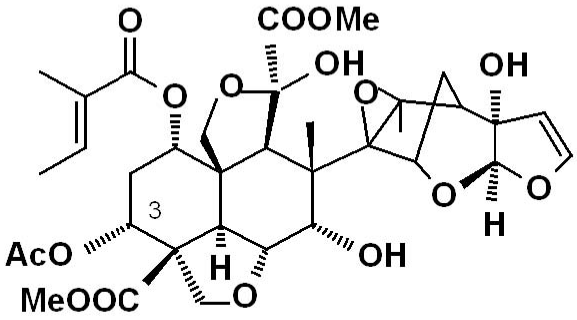

Azadirachtin A

Main active substance and analytical lead compound

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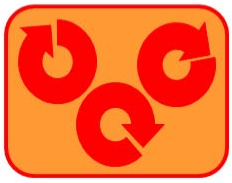
Azadirachta indica - Indian neem tree

Extraction of Azadirachtin A

Neemfruits (100 kg)

Neemseeds (20 kg)

Seedkernels (10 kg)



Extraction

NeemAzal® (0,1 kg)

34% Azadirachtin A

Neemoil (4 kg)

Neemcake (5 kg)

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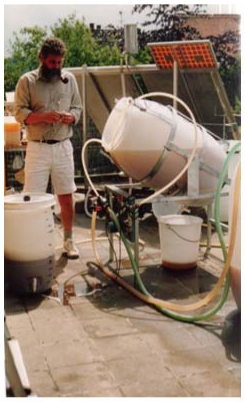
NeemAzal Extraction Process



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Active Ingredient: Azadirachtin (NeemAzal technical) or Margosa extract

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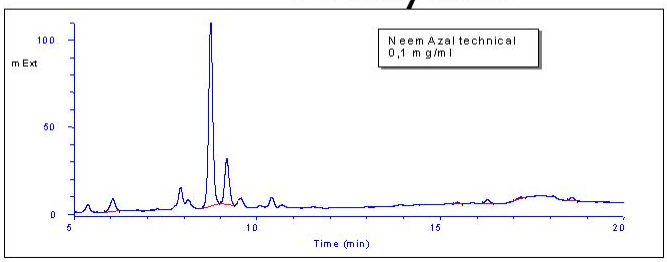
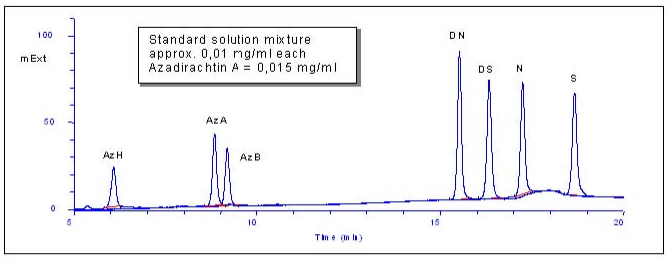
Analytics





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Composition NeemAzal

Substance	av. content in NeemAzal By weight (%)
Azadirachtins:	
Azadirachtin A	34
Azadirachtin B	approx. 5.5
Azadirachtin D	aprrox. 2.1
Azadirachtin E	≤ 1
Azadirachtin F	≤ 1
Azadirachtin G	≤ 1
Azadirachtin H	approx. 2.3
Azadirachtin I	approx. 0.8
Azadirachtin K and other Azadirachtins	≤ 2
Azadirachtinin	approx. 2
Sum of Azadirachtins:	**51,7**

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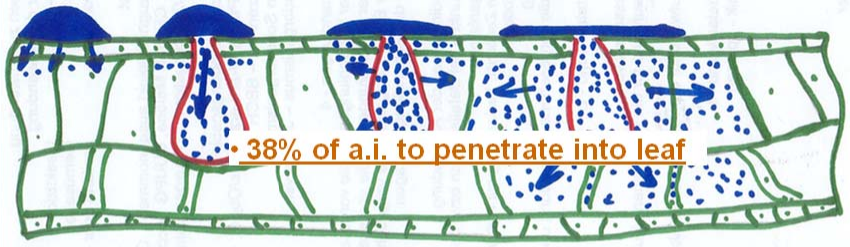
Formulation: "NeemAzal-T/S"

2 hours after application:

- 62% of a.l. spread on the leaf surface



- 38% of a.i. to penetrate into leaf

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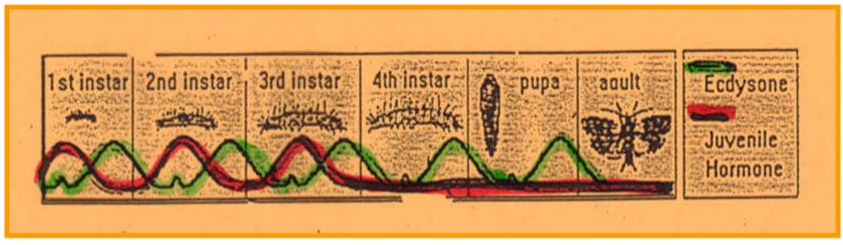
Slow mechanism of action:

Production of hormon Ecdyson - (Rembold, 1995)



Two hormons are very important during the develop of insect:

- Juvenile – development hormon
- Ecdyson – moulting hormon

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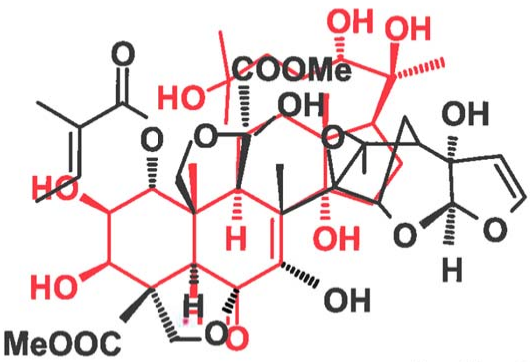
Mode of Action



Azadirachtin A

20-Hydroxyecdysone

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Slow mode of action

⇒ **Feeding inhibition**

⇒ **Moulting inhibition**

⇒ **Fertility reduction**
(contraceptive activity)

⇒ **Mortality**

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Feeding inhibition

Without NeemAzal-T/S With NeemAzal-T/S

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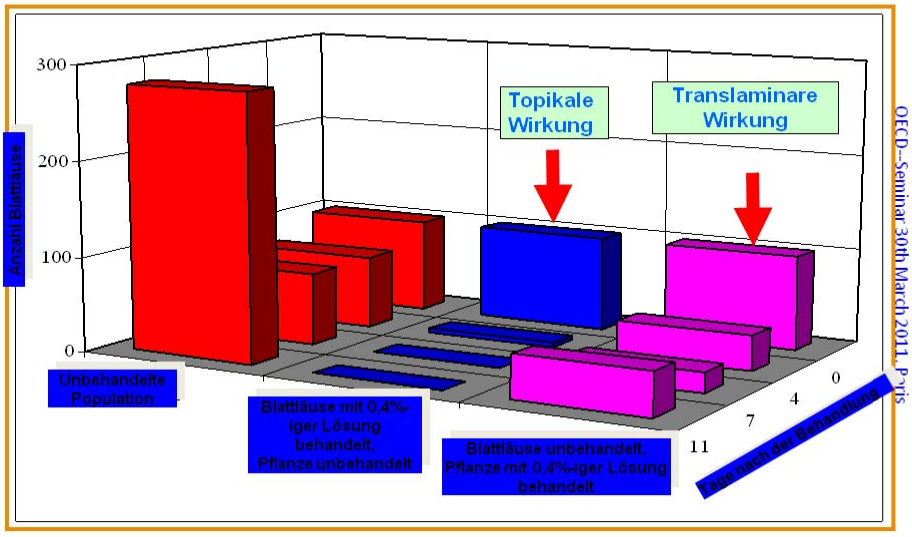
Translaminare und topikale Wirkung auf saugende Schädlinge

(E.Hummel, 1994)

Wickenblattlaus *Megoura viciae* (Hom., Aphididae)...



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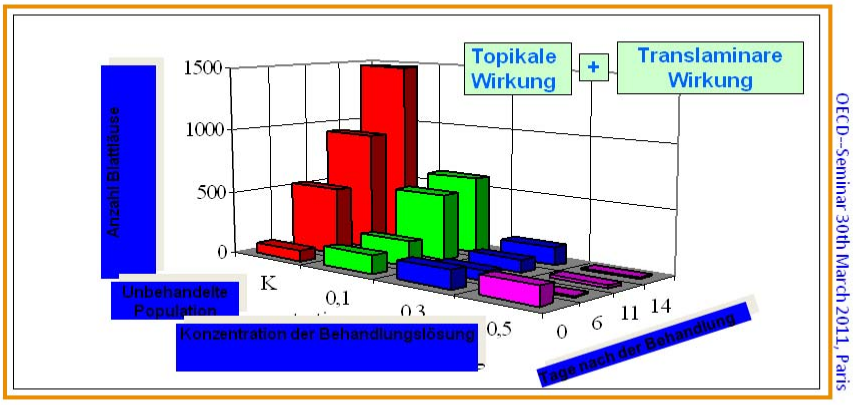
Translaminare und topikale Wirkung auf saugende Schädlinge

(E.Hummel, 1994)

Wickenblattlaus *Megoura viciae* (Hom., Aphididae)...



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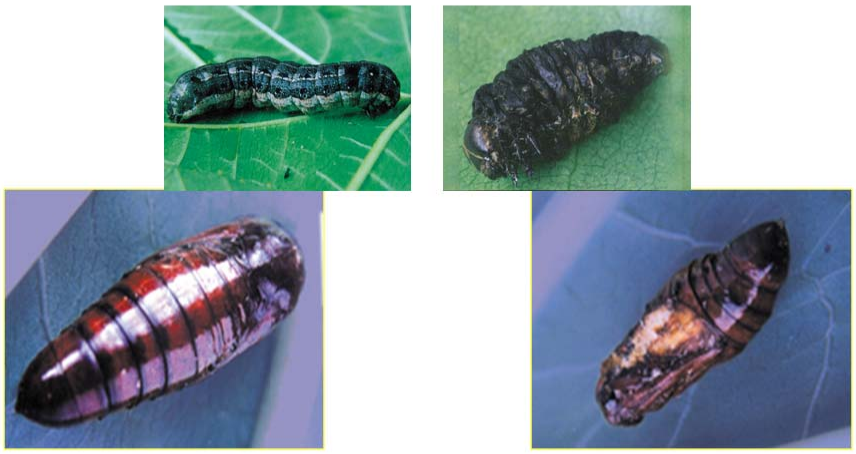
Effects on the development of Pest Insects



Without NeemAzal-T/S With NeemAzal-T/S

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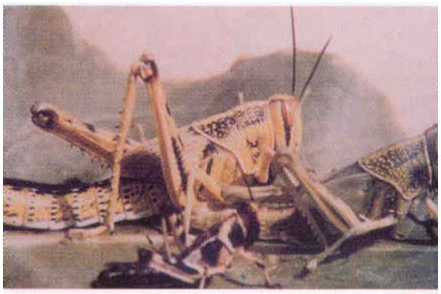

A Normal Locust Nymphs

Malformed Adult Locust

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Without NeemAzal-T/S With NeemAzal-T/S

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Mode of action:
3. Fertility reduction

Fertility of potato beetles after 24 h uptake of potato leaves from cut stems, synergistic infiltrated with "NeemAzal W" (100 ppm)

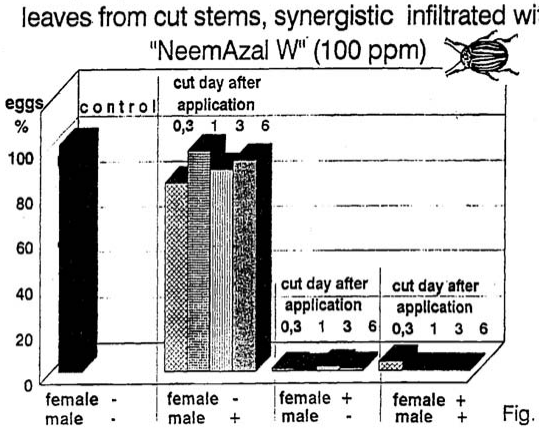

Fig. 5

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Fertilityreduction (Otto, 1994)

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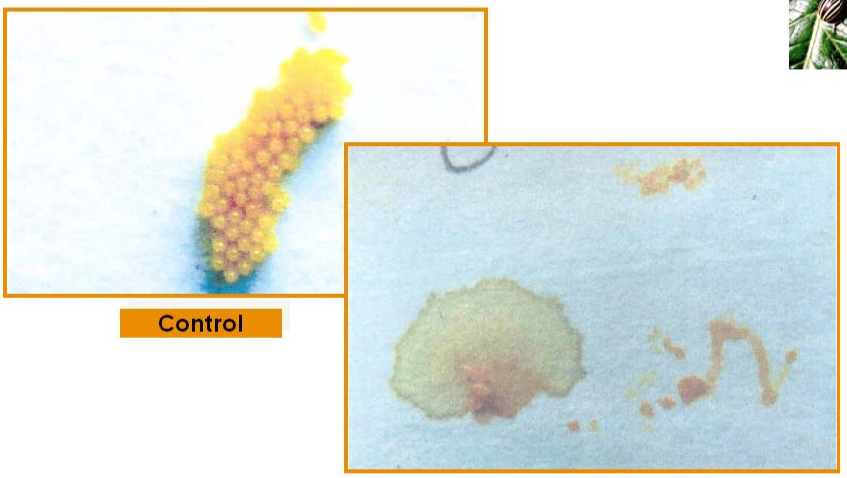
Kartoffelkäfer *Leptinotarsa deccemlineata.*



Control

NeemAzal-T/S

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Target pests (Especially sucking and biting insects) :

- aphids
- caterpillars
- white flies
- thrips
- miners
- beetles, and other insects and
- mites

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Toxicological properties

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Table A. Toxicology profile of azadirachtin TK (NeemAzal®), based on acute toxicity, irritation and sensitization

Species	Test	Duration and conditions	Result	References
Rat (m,f)	oral	15 d (single dose), EPA guideline 152-10	LD_{50} >5000 mg/kg bw	EIP 6/950799/AC
Mouse (m,f)	oral	14 d, FIPPAT, India, standard protocol	LD_{50} >3365 mg/kg bw	Unnumbered A; 1749
Rat (m,f)	dermal	15 d, EPA guideline 152-11	LD_{50} >2000 mg/kg bw	EIP 7/950800/AC
Rat (m,f)	inhalation	4 hr inhalation (observation 14 d), EPA guideline 152-12	LC_{50} >720 mg/m^3	EIP 5/951566
Rabbit, white (m,f)	skin irritation	4 d, EPA guideline 152-14	Non-irritant (0.5 g/animal)	EIP 8/950822/SE
Rabbit, white (m,f)	eye irritation	7 d, EPA guideline 152-13	Slight (70 mg/eye)	EIP 9/950823/SE
Guinea pig (f)	skin sensitization	3 d, EPA guideline 152-15	Slight sensitization	EIP 10/950818/SS

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Table B. Toxicology profile of azadirachtin TK (NeemAzal®), based on repeated administration (sub-acute to chronic)

Species	Test	Duration and conditions	Result	References
Rat (m,f)	Oral	90 d, EPA guideline 152-153	NOEL: 100 ppm in feed NOAEL = 10 mg/kg bw/d LOEL = 400 ppm	EIP 4/963100
Rat (m,f)	Feeding, carcinogenicity	105 wk, Gaitonde committee (India) guidelines No. 6.3.0.C.4	Non-carcinogenic	7291
Rat (m,f)	Feeding, 2-generation reproduction	105 wk, Gaitonde committee (India) guidelines No. 6.3.0.C.4	NOEL: 750 mg/kg bw (highest dose)	4826
Rat (f)	Teratogenicity	105 wk, Gaitonde committee (India) guidelines No. 6.3.0.C.4	Not teratogenic NOEL: 500 mg/kg bw	4824
Rat (f)	Developmental toxicity	20 d, EPA guideline 152-23	Not teratogenic NOAEL: 50 mg/kg bw	EIP 2/952493

The sub-acute to chronic oral toxicity of azadirachtin/NeemAzal® TK is relatively low. Azadirachtin was not carcinogenic in rats after administration via the diet, and did not lead to any malformations in rats and their offspring.

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Table C. Mutagenicity profile of azadirachtin TK (NeemAzal®) based on *in vitro* and *in vivo* tests.

Species	Test	Conditions	Result	References
Salmonella typhimurium	Point mutation, Ames test	Dose range: 5, 1.5, 0.5, 0.15 or 0.05 mg/plate, EPA FIFRA Guideline 152-17	Not mutagenic	EIP 11/950642
Chinese hamster ovary (CHO) cell line (HGPRT locus)	Point mutation, HO/HGPRT test	Dose range (µg/ml ethanol: 25, 50, 100, 200, 400, 800, 1000, 1250), EPA FIFRA Guideline 152-17	Not mutagenic	7291
CD-1 Mouse bone marrow cells	Chromosome aberration, micronucleus test, *in vivo*	Oral administration, dose range: 1250, 2500, 5000 mg/kg bw, EPA Subdiv. M Guideline 152-17	Not mutagenic	EIP 13/952782

The genotoxic potential of azadirachtin/ NeemAzal® TK was tested covering the endpoints gene mutation and chromosome damage. *In vivo* studies performed with CD-1 mice gave no indication of chromosome aberration. Azadirachtin/NeemAzal technical was thus found to be devoid of mutagenic activity on the basis of the studies performed.

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Toxicological properties of NeemAzal-T/S – acute toxicity

Rat LD50 oral	> 5000 mg/ kg bodyweight
Rat LD50 dermal	> 2000 mg/ kg bodyweight
Rat LC50 inhalation	> 5,4 mg/L
Skin irritation	Not irritating
Eye irritation	Not irritating

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Toxicological Values

ADI	0,1 mg/kg/d
AOEL	0,1 mg NA/kg/d
NOAEL	3,7 mg/kg/d
ARfD	0,75 mg/kg/d
MRLs	0,01 - 1 mg AzA/kg

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Risk Estimation Conclusion!!

„For NeemAzal technical or its commercial formulation NeemAzal-T/S, no evidence of neither acute toxicity nor reproductive effects was obtained in valid studies.

However, in no way can it be concluded that other neem products are generally devoid of a significant risk since NeemAzal is considered as safe."

Niemann,L. (2001): Regulatory Data Requirements for Health Evaluation of Biological Plant Protection Products in: ‚Practice Oriented Results on Use of Plant Extracts and Pheromones in Integrated and Biological Pest Control', Proceedings of the 10th Workshop, p 95

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Ecotoxicological Properties:

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Table D. Ecotoxicology profile of azadirachtin TK (NeemAzal®)

Species	Test	Duration and conditions	Result	References
Scenedesmus subspicatus (green alga)	Effect on growth, static water	72 h, static water, 22°C; pH 7.6–8.5; OECD (201)	EC_{50} (biomass) = 530 mg/l EC_{50} (growth rate) = 996 mg/l NOEC (biomass) <13.5 mg/l NOEC (growth rate) <13.5 mg/l	TRF-001/4-30
Daphnia magna (water flea)	Acute toxicity	24/48 h, static water; OECD (202) Part I	EC_{50} (24 h) = 107.55 mg/l EC_{50} (48 h) = 23.63 mg/l	TRF-001/4-21
Daphnia magna (water flea)	Chronic toxicity	21 d, semi-static; OECD guideline 202 Part II	NOEC = 2.5 mg/l (highest test concentration)	TRF-001/4-21
Danio rerio (zebra fish)	Acute toxicity (early life stage test)	37 d; OECD (210)	NOEC = 6.4 mg/l	TRF-001/4-60
Danio rerio (zebra fish)	Reproductive toxicity (full life cycle)	134 d; OECD (210)	NOEC (lethal) = 6.4 mg/l NOEC (sub-lethal) = 6.4 mg/l	TRF-001/4-60
Colinus virginianus (bobwhite quail)	Acute oral toxicity	14 d; EPA Subdiv. E 71-1	NOEL = 4000 mg/kg bw (highest dose)	EIP 21/960383
Colinus virginianus (bobwhite quail)	Dietary toxicity	8 d; EPA Subdiv. E 71-2	NOEL = 5200 mg/kg diet (highest dose)	EIP 22/960382

Azadirachtin/ NeemAzal® TK is of low toxicity to aquatic and terrestrial organisms including fish, aquatic invertebrates and birds.

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Ecotoxicological properties

NeemAzal:

Fish - acute toxicity
NOEL: 2.29 mg/L
LC_{50} > 6.18 mg/L

Daphnia - acute toxicity
NOEL: ≥2.5 mg/L
LOEC: >2.5 mg/L

NeemAzal-T/S:

Fish - acute toxicity
NOEL: 100 mg/L
LC_{50}: 160 mg/L

Daphnia - acute toxicity
NOEL: 197.5 mg/L
LC_{50} 1000 mg/L

No labelling of the product with hazard symbols required!

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Ectoxicological behaviour – Acute toxicity to the honeybee

Acute oral toxicity (NeemAzal-T/S)	48-Hour Oral LD50 > 5.89 µg a.i. * /bee
Acute contact toxicity (NeemAzal-T/S)	48-Hour Dermal LD50 > 21.00 µg a.i. * /bee
Conclusion: not toxic to bees (B4)	* a.i. refers to analytical leading compound Azadirachtin A

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Ecotoxicological behaviour – degradation of Azadirachtin

Degradation in water - DT50	Natural river water DT50: 8.8 days (25 °C)
photochemical oxidative degradation	halflife time: 1.69 hrs

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Resistancies?????????

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Resistance – Feng, Isman, 1995

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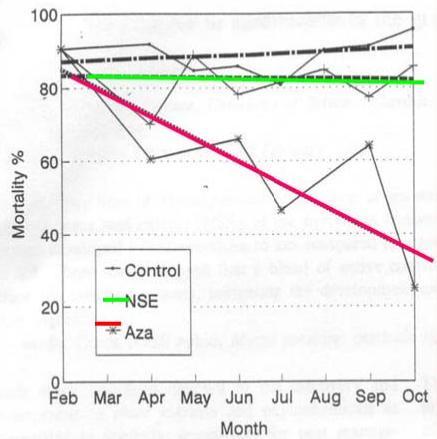

Figure. Susceptibility to a discriminating concentration of azadirachtin (8 ppm) in control, NSE-selected and aza-selected *M. persicae* lines in leaf disc assays. Dashed lines represent regression lines for mortality data versus date of bioassay.

Results:

- 40 generations *Myzus persicae (Hom., Aphididae)*
- 9x resistence of AzA pur
- NO after using of NSE

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Selection for resistance to azadirachtin in the green peach aphid, *Myzus persicae*

R. Feng and M. B. Isman*

Department of Plant Science, University of British Columbia, Vancouver, B.C. (Canada, V6T 1Z4), Fax +1 604 822 8640

Received 2 January 1995; accepted 2 February 1995

Abstract. Two lines of *Myzus persicae* of the same origin were treated repeatedly with pure azadirachtin (aza), or a refined neem seed extract (NSE), at the equivalent concentration of aza. After 40 generations, the aza-selected line had developed 9-fold resistance to aza compared to a non-selected control line, whereas the NSE-selected line did not. These results suggest that a blend of active constituents in a botanical insecticide such as neem might diffuse the selection process, mitigating the development of resistance compared to that expected with a single active ingredient.

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Standardisation of BCA's

Example: Azadirachtin

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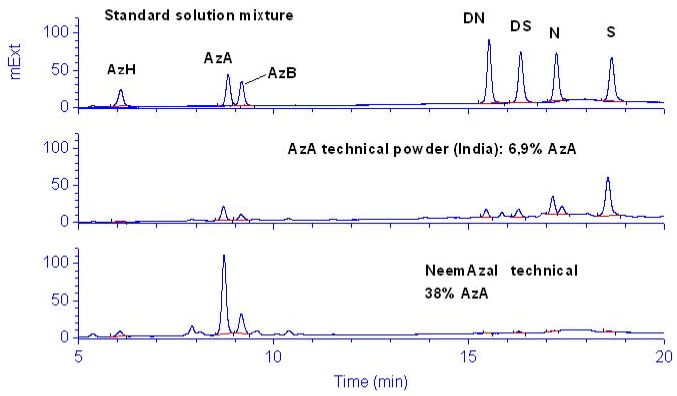
Identification of the active ingredient, Analytics, Standardisation



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Analysis of 10 batches of NeemAzal

Code or name	270295/II	CC223	CC224	CC225	CC226	CC227	IA	IIA	IB	IIB	Mean content % [w/w]
AzA	34.66	39.1	42.1	39.1	43.3	42.0	33.7	26.4	29.0	27.5	35.7
AzB	5.4	6.13	5.7	5.51	6.22	6.09	5.9	4.6	5.2	4.8	5.6
AzF(H)	2.12	2.51	2.8	2.67	2.86	2.69	2.42	1.92	2.13	1.91	2.4
DNim	0.19	0.75	0.67	0.55	0.6	0.59	0.35	0.35	0.33	0.3	0.5
DSal	0.51	1.03	0.99	0.93	0.89	0.84	0.43	0.42	0.36	0.33	0.7
Nim	0.22	1.1	1.09	0.92	1.02	1.01	0.45	0.56	0.47	0.44	0.7
Sal	1.09	5.08	5.04	4.52	4.6	4.39	1.93	2.32	1.87	2.02	3.3

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5 batch analysis

5 batch analysis data

Code or name	Batch 1 (IV 97.1a), g/kg	Batch 2 (VII 97.1b), g/kg	Batch 3 (XXVIII 96.1a), g/kg	Batch 4 (XXIX 96.1b), g/kg	Batch 5 (XXX 96.1c), g/kg	Manufacturing QC limit (technical specification), g/kg
Azadirachtin A	321.3	251.5	322.6	314.9	334.7	340 ± 90
Azadirachtin B	63.7	45.4	59.7	61.3	57.6	55 ± 10
Azadirachtin H	26.1	19.6	23.1	22.9	25.2	23 ± 10
Desacetyl-Nimbin	3.7	2.9	5.3	3.9	4.2	5 ± 4
Desacetyl-Salannin	8.7	4.0	11.1	8.2	8.5	7 ± 4
Nimbin	3.6	4.1	9.8	8.1	8.0	7 ± 6
Salannin	16.1	18.1	41.5	28.6	35.3	30 ± 20
Aflatoxins	Batch 1 (IV/97-98),	Batch 1 (CC007/99),	Batch 1 (9912-024)	Batch 1 (017/1999-2000RP)	Batch 1 (0008-06/2001)	
Aflatoxin B1	0.000051	0.000048	0.000054	0.0000038	0.0000019	<0.000025
Aflatoxin B2	0.0000083	0.0000077	0.0000091	0.0000006	0.0000022	<0.000025
Aflatoxin G1	0.0000022	0.0000030	0.0000035	0.0000008	0.0000066	<0.000025
Aflatoxin G2	<0.0000005	<0.0000005	<0.0000005	<0.0000005	<0.0000005	<0.000025
Total aflatoxins (sum)	<0.000062	<0.000592	<0.0000671	<0.0000057	<0.0000283	<0.0001

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Residue situation

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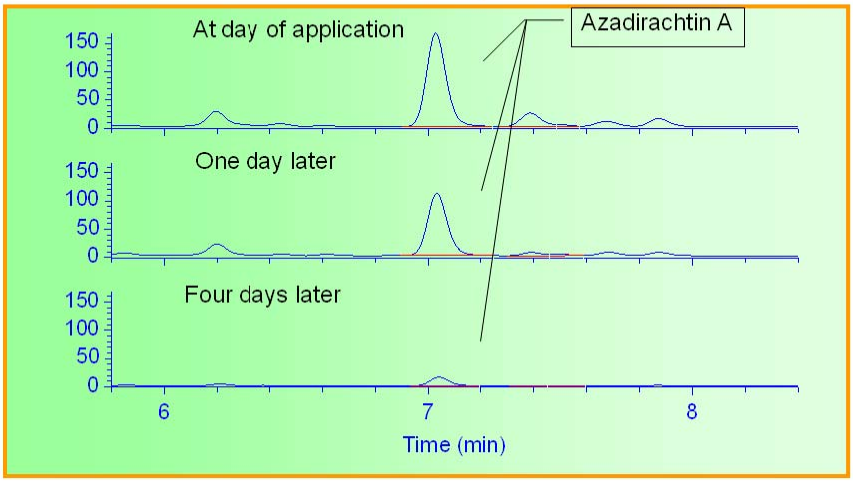
Residue situation - spinach

Degradation of Azadirachtin A/NeemAzal-T/S (0,5%)



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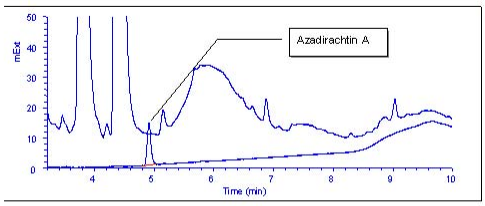
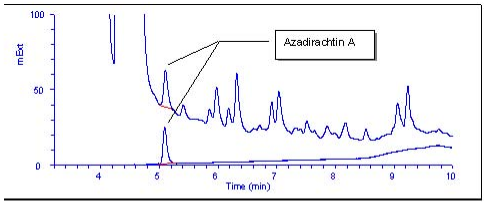


Residue situation Tomato

HPLC-residue Analytics of Tomato:
top: control matrix; bottom: AzA-standard



Verification of the determination limit of 0,1 ppm:
Top AzA in tomato-matrix on day zero;
Bottom: AzA-standard of 0,0073 mg/ml

Quelle: Bericht 7M 0500.01

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Residue situation

Residue Studies (confidential information) BR / 04.06.2009

Crop	Crop growth stage at application	LOQ [mg AzA / kg]	AzA content[1] [mg AzA / kg]	MRL[2] [mg AzA / kg]	waiting period[3] [d]
Fruits:					
Apple	BBCH 87-89	0.1	< LOQ	1	0
Apple	BBCH 56-69	0.02	< LOQ		
Cherry	BBCH 81-87	0.02	0.26	1	0
Orange, peel and pulp	BBCH 87-98	0.02	peel: 0.055	0.5	0
			pulp: < LOQ	0.5	0
Peach	BBCH 83-86	0.02	0.049	1	0
Strawberry	BBCH 84-87	0.02	0.032	1	0

1) mean AzA content
2) MRL according to regulation No 149/2008
3) proposal by Trifolio-M GmbH

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Residue situation

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Residue Studies (confidential information) BR / 04.06.2009

Crop	Crop growth stage at application	LOQ [mg AzA / kg]	AzA content[1] [mg AzA / kg]	MRL[2] [mg AzA / kg]	waiting period[3] [d]
Vegetables:					
Cabbage	BBCH 48-49	0.02	0.020	1	0
Cucumber, field	BBCH 75-76	0.02	< LOQ	1	0
Cucumber, greenhouse	BBCH 69-89	0.02	< 0.020		
Head lettuce	height: 25 cm	0.02	0.13	1	0
Potato	BBCH 40-70	0.01	< LOQ	1	0
Spinach	BBCH 11-49	0.1	1.01	1	1
		0.02	0.86		
Sweet pepper	BBCH 72-76	0.02	0.17	1	0
Tomato, field	ripe fruit; BBCH 82-84	0.1	< 0.043* from study with 10fold application rate	1	0
Tomato, greenhouse	fruit producing		< LOQ		

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Residue situation

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Residue Studies (confidential information) BR / 04.06.2009

Crop	Crop growth stage at application	LOQ [mg AzA / kg]	AzA content[1] [mg AzA / kg]	MRL[2] [mg AzA / kg]	waiting period[3] [d]
Herbs:					
Basil	height: 40-45 cm	0.02	0.43	1	0
Dill, fresh	BBCH 45-49	0.02	0.7	1	1
Dill, dried		0.02	1.38		
Fennel seeds	BBCH 92	0.02	< LOQ	0.01	0
Lemon balm, dried	BBCH 49	0.02	4.60	1	3
Lemon balm, fresh		0.02	0.81		
Parsley, dried, field	height: 25 cm	0.02	6.84	1	3
Parsley, fresh, greenhouse	46-49; height: 15 cm	0.02	3.06	1	?[4]
Parsley, fresh, field	height: 25 cm	0.02	1.39	1	1
Sage	height: 35 cm	0.02	1.04	1	7
Savory, fresh	not stated	0.02	1.43	1	1
Savory, dried		0.02	5.39		

4) results on degradation on/in parsley differ variably, see: overview - fresh parsley

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Anwendungshinweise/Gebrauchsanleitung

NeemAzal®-T/S

1% Azadirachtin A

Pflanzenextrakt aus den Kernen des tropischen Neem-Baumes für

Obst-, Zierpflanzen-, Gemüse-, Wein- und Ackerbau

gegen frei lebende saugende und beißende Schadinsekten und Spinnmilben

- nicht bienengefährlich
- Wartezeit nicht erforderlich
- nicht schädgend für bestimmte Nützlingsarten
- kein Abstand zu Gewässern erforderlich
- für ökologischen Anbau zugelassen

Herstellung und Vertrieb: Trifolio-M GmbH

Vertrieb für die BRD: BIOFA Bio-Farming-Systeme

In the professional area the cooperation with Biofa - and other partners in other countries is very effective

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Bayer Garten

Bio-Schädlingsfrei Neem
- Produkteigenschaften -

- Natürlicher Wirkstoff (Neembaumsamen-Extrakt)
- Zugelassen an Zierpflanzen, Obst Gemüse sowie Kartoffeln
- Erfolgreich verwendet im biologischen Landbau
- Breite und zuverlässige Wirkung gegen Schädlinge
- Lange wirksam, bis zu 12 Tage

Since 2010 Bayer Crops Science is our distributor of Bio-Schädlingsfrei Neem in Home&Garden

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Biocides: to reduce health risks

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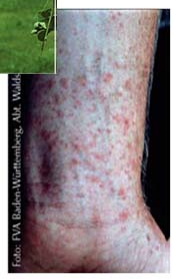
- Itching
- Dermatitis
- Coughing, Asthma
- Eye irritation
- General Symptoms of disiness, feaver, allergies

(Of sensitive persons)



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Registrations of NeemAzal-T/S

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Country	Registration No and date
India (EID)	CIR-22,388/95 dated 22.01.1996
Sri Lanka (EID)	947 dated 01.01.1996
Germany	4436-00 dated 18.10.1998
Austria	2699-01 dated 30.05.2000 (valid until 2008-12-31)
Switzerland	W 5351 dated 06.03.2000
USA (EID)	EPA 71908-1 dated 21.04.2000
New Zealand	5412 cated 21.12.2000
Turkey	3792 cated 20.06.2000 (valid until 2010)
Georgia	459 dated 4.08.2000 (valid 5 years)
Bulgaria	951 dated 03.05.2000
Estonia	0211 cated 12.10.2001
Kingd. of Saudi Arabia	356 159 241 dated 16.07.2003 (valid 5 years)
The Netherlands	12455 N dated 20.06.2003
Italy	11561 dated 20.01.2003
Slovenia	32702293/02 dated 15.04.2003
Lithuania	02401/02 and 07-368 dated 02.05.2003
Greece	119157 of 24.Dec.2003 valid till 24.12.2007
Latvia	0241 cated 14 Oct. 2004 valid till 14.10.2014
Luxemburg	LO1626-103 9 July 2004 valid till 31. Dec. 2008

EU: according to 91/414/EEC, & article 4(2)1869/2000 EC biocide notification No. N611

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On 11th March 2011 the inclusion of Azadirachtin into EU-Annex I was decided!!

But still some questions are open concerning especially:

degradation and metabolites!

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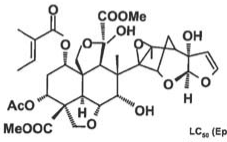
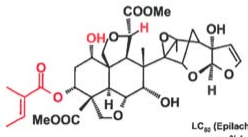
Comparison of some structures of AzadirachtinS



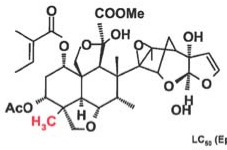
Azadirachtin A
mw: 720

LC_{50} (Epilachna varivestis bio assay): 1.3 ppm
average % in NA: 34



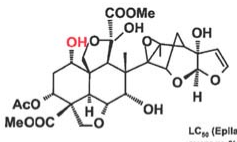
Azadirachtin B
mw: 662

LC_{50} (Epilachna varivestis bio assay): 1.6 ppm
average % in NA: 5.5



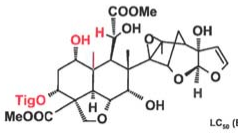
Azadirachtin D
mw: 676

LC_{50} (Epilachna varivestis bio assay): 1.6 ppm
average % in NA: 2.1

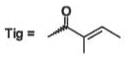


Azadirachtin E
mw: 638

LC_{50} (Epilachna varivestis bio assay): 1.3 ppm
average % in NA: <1

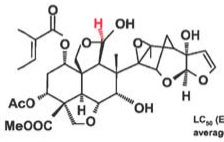


Tig =



Azadirachtin F
mw: 664

LC_{50} (Epilachna varivestis bio assay): 1.1 ppm
average % in NA: <1



Azadirachtin H
mw: 662

LC_{50} (Epilachna varivestis bio assay): 0.5 ppm
average % in NA: 2.3

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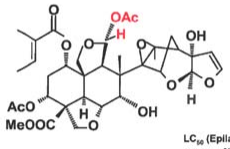
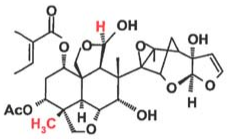
Comparison of some structures of AzadirachtinS



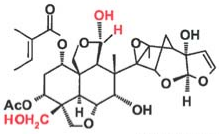
Azadirachtin L
mw: 694

LC_{50} (Epilachna varivestis bio assay): 0.3 ppm
average % in NA: approx. 0



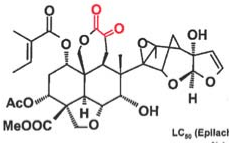
Azadirachtin I
mw: 618

LC_{50} (Epilachna varivestis bio assay): 0.3 ppm
average % in NA: 0.8



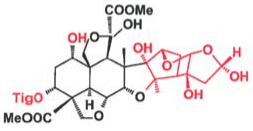
Azadirachtin M
mw: 633

LC_{50} (Epilachna varivestis bio essay): not investigated
average % in NA: approx. 0



Azadirachtin K
mw: 688

LC_{95} (Epilachna varivestis bio assay): 1.1 ppm
average % in NA: <2 (and some other Azas)



Tig =

Azadirachtin N
mw: 680

LC_{50} (Epilachna varivestis bio essay): not investigated

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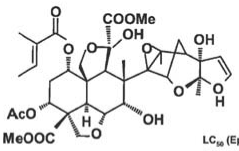
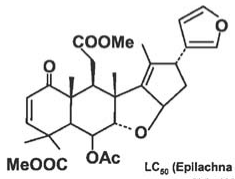
Comparison of the structure of AzA and other limonoids (Salannin & Nimbin)



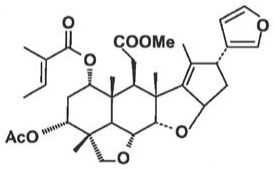
Azadirachtin A
mw: 720

LC_{50} (Epilachna varivestis bio assay): 1.3 ppm
average % in NA: 34



Nimbin
mw: 540

LC_{50} (Epilachna varivestis bio assay): not investigated
average % in NA: 0.7



Salannin
mw: 596

LC_{50} (Epilachna varivestis bio assay): >100 ppm
average % in NA: 3

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Since the polar side chains in which the Azadirachtins differ structurally are cleaved easily, it can quite reasonably be assumed, that the main degradation products and metabolites of all the Azadirachtins present in NeemAzal are very similar or even identical.

This means that it would be of highest interest to look at degradation products and metabolites of AzadirachtinA (the analytical lead substance).

We have the impression that this is not understood by all EU-authorities!

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Since the important Carbon-skeleton of Azadirachtins can't be labled radioactively (Ley...!) it is extremely difficult (practically impossible) to identify metabolites or degradation products under different conditions.

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One realistic possibility in addition to fundamental analytical research in this respect may be trials with aged substance/residues or mesocosme investigations

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IBM A
International Biocontrol agent Manufacturers' Association

MA D/A

Quassia extract according to SANCO 10472

Bitterwood (*Quassia amara*)

Analytical lead substance:
Quassin: 0.01 – 0,2%

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Quassia amara,
Picrasma excelsa

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Quassia-MD

Trifolio-M GmbH

Requested for EU/91/414 Annex I

(RMS Italien; SANCO 10472)

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International Biocontrol agent Manufacturers' Association
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USA: Quassin is included in the GRAS-List (Generally Regarded As Save) (Lebensmittel-Zusatz)

Germany:

Aroma Guidelines (2006):
Quassin ≤ 50 mg/kg is permitted in alcoholic Beverages.

In homöopathic medcins max: D2/C1

Cosmetics: INCI-Liste: Quassia amara Extract: Skin conditioning/Denaturating agent/ body lotion

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IBMA

Quassia Extract MD: Acute Toxicity

Type	Species	Results
Oral route	Rat	LD_{50} > 2000 mg/kg bw
Dermal route	Rat	LD_{50} > 2000 mg/kg bw
Inhalation	Rat	LC_{50} at 4 hours > 13.542 mg/L
Primary skin irritation	Rabbit	Not irritating
Eye irritation	Rabbit	Not irritating. However, slight changes on the conjunctivae: hyperemia (3/3eyes) and edema (1/3 eyes) grade 1, at 1h. All irritation signs had returned to normal by the 24-hr time point following treatment
Skin sensitisation	Guinea pig	not sensitizing

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Quassia Extract MD: Aquatic Toxicity

Quassia – Extract – MD: Acute Toxicity - aquatic organisms

Type	Species	Results
Fish	*Danio rerio*	LC_{50} (96-hours): >100 mg /l
Daphnia	*Daphnia magna*	EC_{50} (48 hours): 213.34 mg/l NOEC (48 hours): 65 mg/l.
Brine Shrimp	*Artemia salina*	EC_{50} (24 hours): 409 mg/l
Algae	*Pseudokirch-neriella subcapitata*	EC_{50} (72-hour): 179.93 mg/L. NOEC: (72-hour): > 100 mg/L

Quassia Extract

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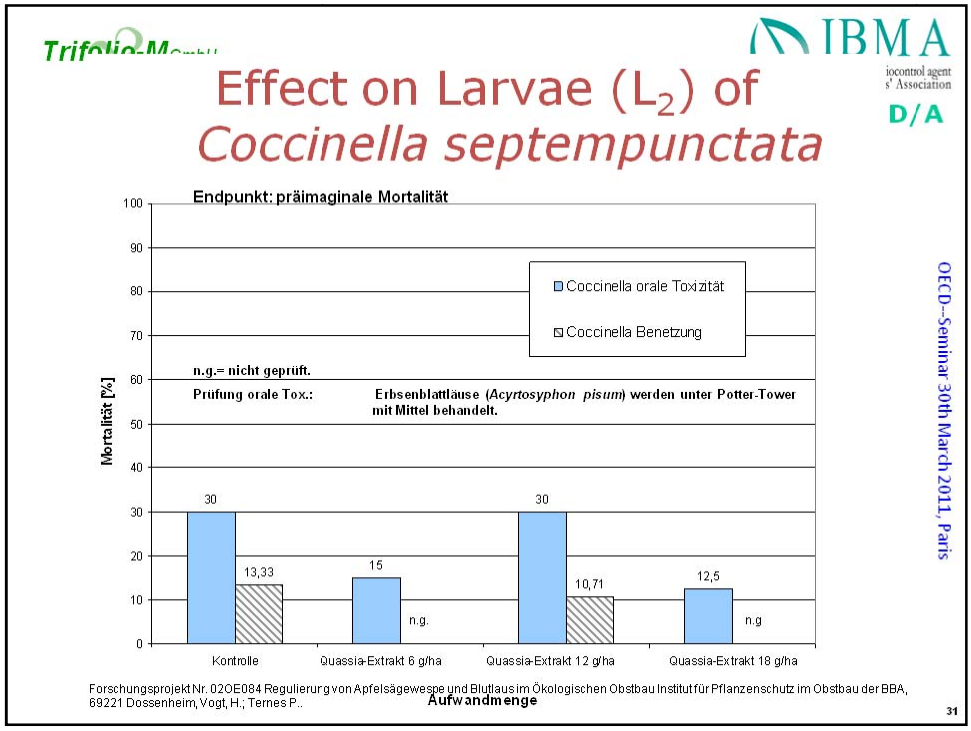
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IBMA
biocontrol agent s' Association
D/A
Effect on Larvae (L_2) of
Coccinella septempunctata
Endpunkt: präimaginale Mortalität
Coccinella orale Toxizität
Coccinella Benetzung
n.g.= nicht geprüft.
Prüfung orale Tox.: Erbsenblattläuse (Acyrtosyphon pisum) werden unter Potter-Tower mit Mittel behandelt.
Mortalität [%]
100
90
80
70
60
50
40
30
20
10
0
30
13,33
15
n.g.
30
10,71
12,5
n.g
Kontrolle
Quassia-Extrakt 6 g/ha
Quassia-Extrakt 12 g/ha
Quassia-Extrakt 18 g/ha
Aufwandmenge
Forschungsprojekt Nr. 02OE084 Regulierung von Apfelsägewespe und Blutlaus im Ökologischen Obstbau Institut für Pflanzenschutz im Obstbau der BBA, 69221 Dossenheim, Vogt, H.; Ternes P..
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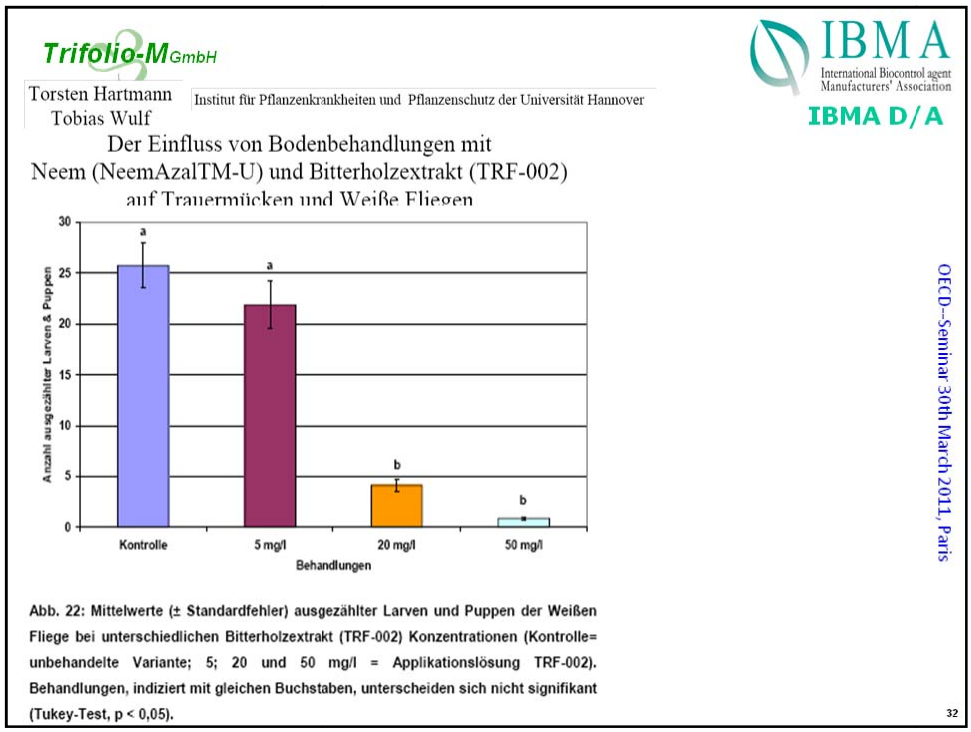
Trifolio-M GmbH
IBMA
International Biocontrol agent Manufacturers' Association
IBMA D/A
Torsten Hartmann
Tobias Wulf
Institut für Pflanzenkrankheiten und Pflanzenschutz der Universität Hannover
Der Einfluss von Bodenbehandlungen mit
Neem (NeemAzalTM-U) und Bitterholzextrakt (TRF-002)
auf Trauermücken und Weiße Fliegen
Anzahl ausgezählter Larven & Puppen
30
25
20
15
10
5
0
a
a
b
b
Kontrolle
5 mg/l
20 mg/l
50 mg/l
Behandlungen
Abb. 22: Mittelwerte (± Standardfehler) ausgezählter Larven und Puppen der Weißen Fliege bei unterschiedlichen Bitterholzextrakt (TRF-002) Konzentrationen (Kontrolle= unbehandelte Variante; 5; 20 und 50 mg/l = Applikationslösung TRF-002). Behandlungen, indiziert mit gleichen Buchstaben, unterscheiden sich nicht signifikant (Tukey-Test, p < 0,05).
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Quassia-MD (0,7% Quassin)

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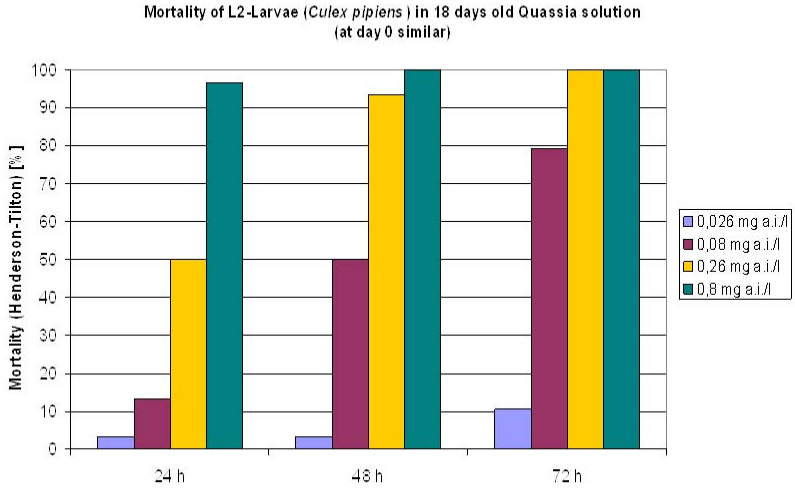

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D/A

Conclusions I

In addition to Azadirachtin/Margosa extract and Quassia we are working on Sweetwood as a copper substitute and on fruits of a Sub-Sahara tree for control of snails and slugs.

In principle these products can only be registered - and made available to the farmers - when reasonable registration requirements are agreed upon. For this SANCO 10472 may serve as a starting point.

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IBMA D/A

Conclusions II

Only a reasonable scientifically based approach in which the special properties of the respective extract are taken into account and waivers for unnecessary requirements are possible.

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IBMA D/A

Thank you for your kind attention!

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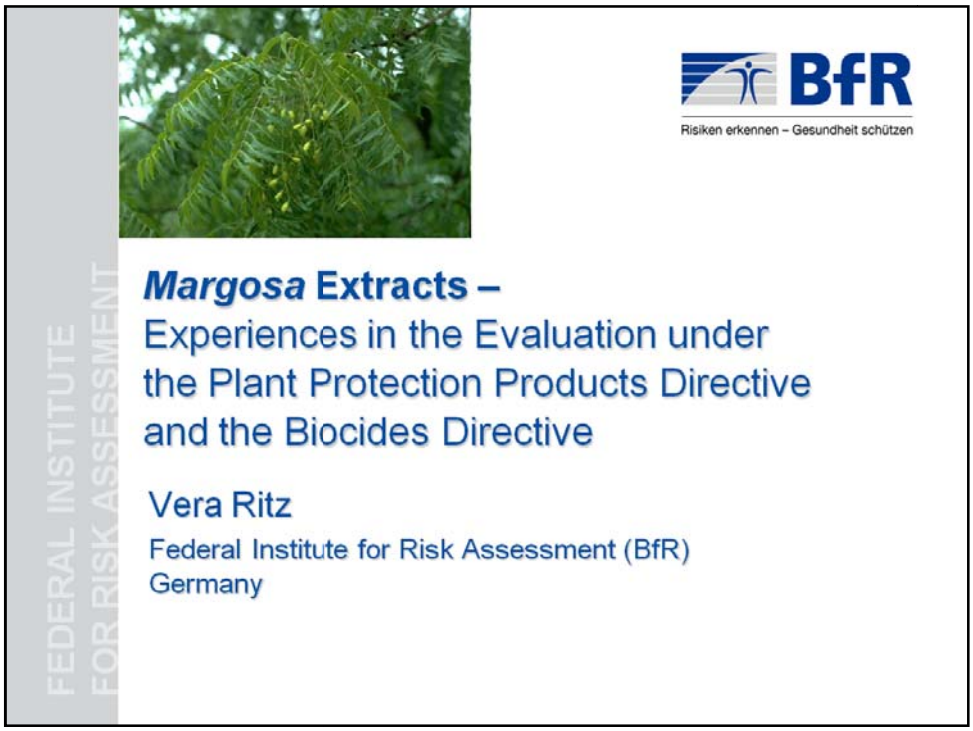
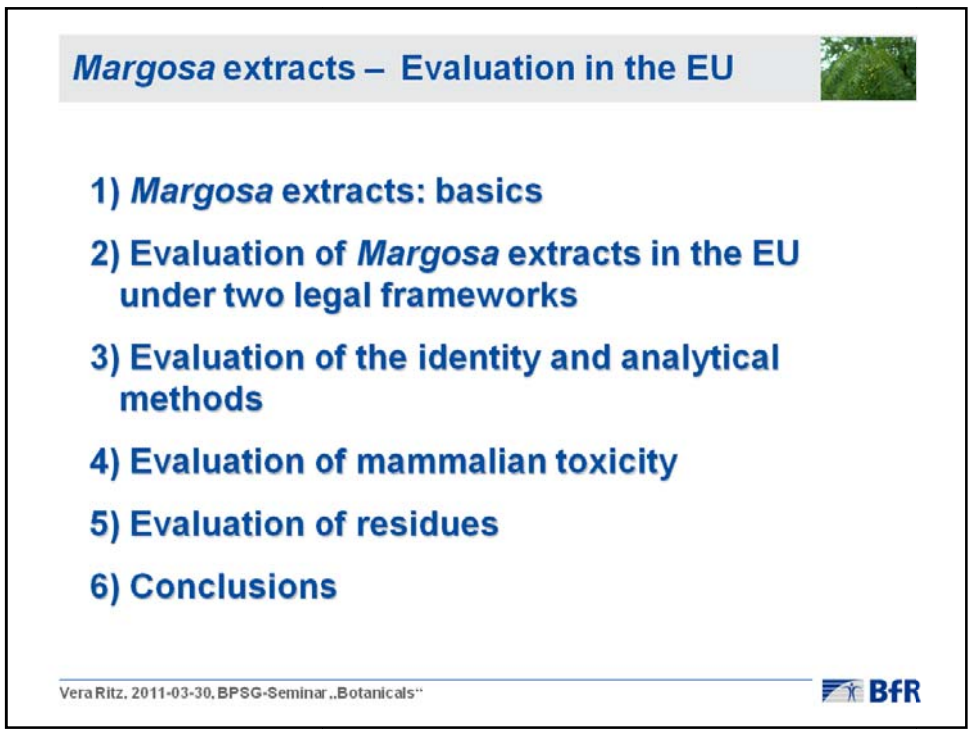
Presentation 7
Neem/*Margosa* extracts – experiences in the evaluation under the Plant Protection Products Directive and the Biocides Directive
By Vera Ritz (Federal Institute for Risk Assessment [BfR], Berlin; Germany)





Margosa extracts – Basics I

Definitions:

Margosa (Neem) extract: product from fruit, seeds, or leaves of *Azadirachta indica;* for biocidal/pesticidal use: seed kernel extract

Azadirachtin: refers to only one compound class in the extract

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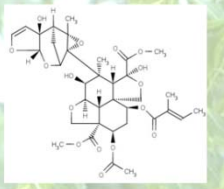
Margosa extracts – Basics II

Biologically active compounds (seed kernels):



Azadirachtin
~2-6 mg/g seed kernel
Azadirachtin A, B, D, E, F, H, I, K, L

further: Azadirachtol, Salannin, Nimbin, Gedunin, Vilasinin, Meliacarpin,…

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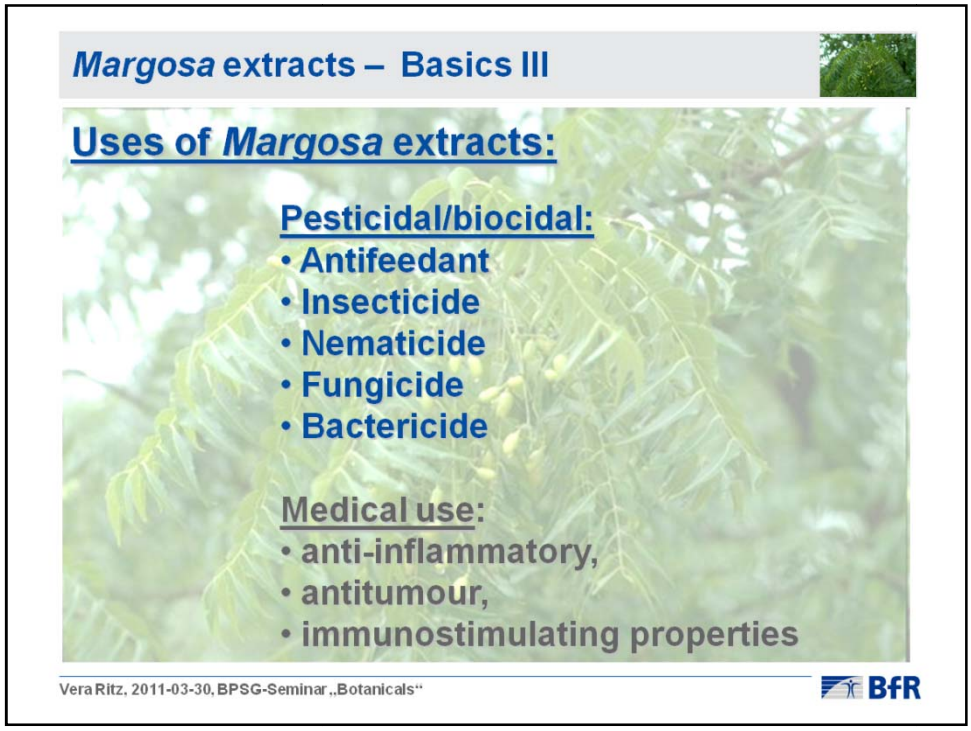
Margosa extracts – Basics III
Uses of *Margosa* extracts:
Pesticidal/biocidal:
• Antifeedant
• Insecticide
• Nematicide
• Fungicide
• Bactericide
Medical use:
• anti-inflammatory,
• antitumour,
• immunostimulating properties
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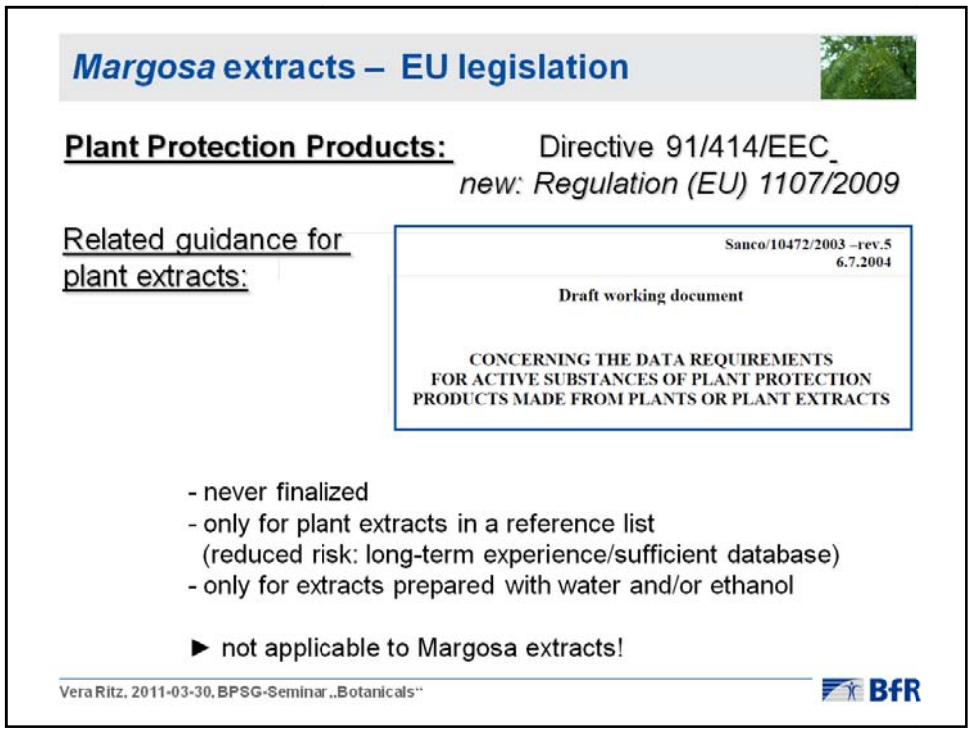
Margosa extracts – EU legislation
Plant Protection Products:
Directive 91/414/EEC
new: Regulation (EU) 1107/2009
Related guidance for plant extracts:
Sanco/10472/2003 –rev.5 6.7.2004
Draft working document
CONCERNING THE DATA REQUIREMENTS FOR ACTIVE SUBSTANCES OF PLANT PROTECTION PRODUCTS MADE FROM PLANTS OR PLANT EXTRACTS
- never finalized
- only for plant extracts in a reference list (reduced risk: long-term experience/sufficient database)
- only for extracts prepared with water and/or ethanol
► not applicable to Margosa extracts!
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Margosa extracts – EU legislation

Biocidal Products: Directive 98/8/EC
new legislation in 2013

Related guidance for plant extracts:

How to deal with extracts and oils of plant or animal origin?

(Addendum to the TNsG on data requirements for active substances endorsed at the 23rd CA meeting, Nov. 2006)

Margosa extracts – Evaluation outcome

Plant Protection Products:
DE RMS for 2 Margosa extracts notified as **Azadirachtin**
under the **Plant Protection Product Directive (91/414/EEC)**:
- **Non-inclusion in Annex I in 2008**
- **Resubmission:**
  Approval; but „**a critical area of concern** is identified“)

Biocides:
DE RMS for 2 Margosa extracts notified as **Margosa extract**
under the **Biocidal Product Directive (98/8/EC)**:
1st extract Foreseeable **Annex I Inclusion** in 2011/12
2nd extract Submission in 2011

What makes the difference?

Margosa extracts – Identity/Analyses

Biocides, Guidance Document

1. **What is regarded as the active substance in an extract/oil?**
   -whole mixture of all constituents
2. **How precisely must an extract/oil be analyzed?**
   - as precisely as possible,
   - constituents ≥ 1.0 % (w/w) to be identified,
   - hazardous constituents down to 0.1 %,
   - default purity of an extract/oil: 100 %

Margosa extracts – Identity/analyses

Biocides, Draft Assessment Report (2010):

The **analytical methods in all relevant matrices** are covered by the **methods of azadirachtin A**. Due to the complex nature of margosa extract, the choice of **azadirachtin A as lead compound** for **residues in soil and water** is accepted.

Margosa extracts – Identity/analyses

Plant Protection Products, Guidance Document

Establish a chemical profile :
- Description of the known active plant protection substances. Provide the active substances' concentration range.
- For the other substances, provide a percentage of the total weight (or a percentage range).

If any active substance has been identified the following information are required :

-Chemical name according to IUPAC, and other information about identity (CAS N°, structural formula, ISO name).
-Physico-chemical properties: vapour pressure, partition coefficient, hydrolysis, photolysis.

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Margosa extracts – Identity/analyses

Plant Protection Products, Guidance Document

- For any toxic substances that are relevant for human, animal health and environment provide a maximum content limit.

If the active substance(s) is (are) not identified, define a representative marker*.
** i.e. a chemical naturally present in a known proportion in the plant in order to identify the plant protection product.*

Analysis report of 5 batches of different manufacture, collected over several periods.

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Margosa extracts – Identity/analyses

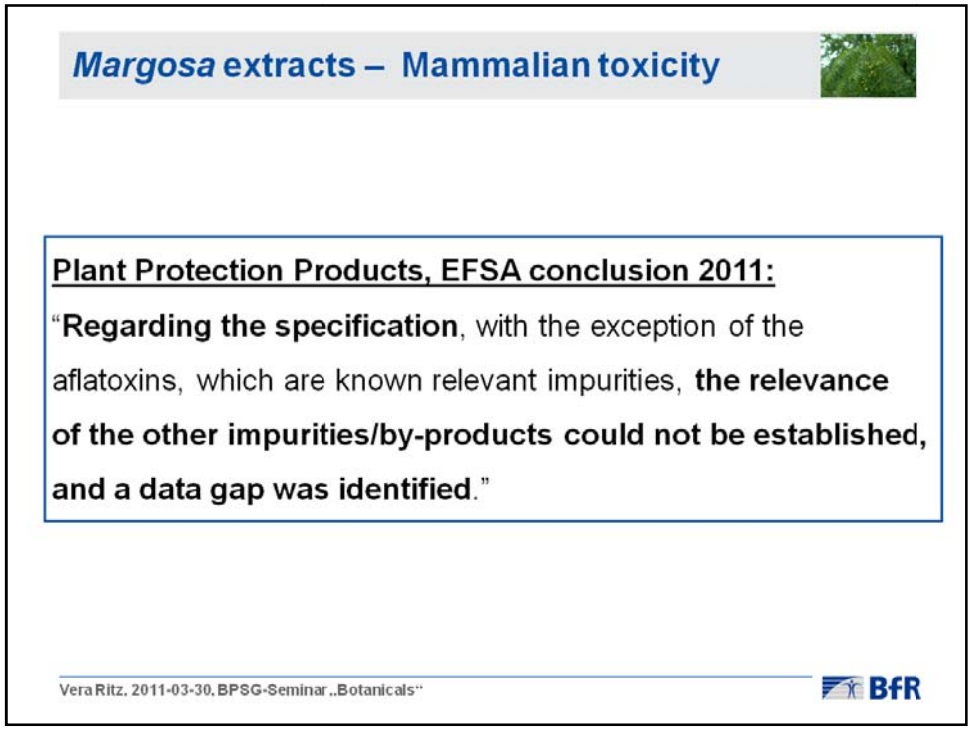
Plant Protection Products, EFSA conclusion (2011):

"**Azadirachtin A** was the proposed **lead substance**, however **this was not accepted** and the content of the total biologically active extract is not yet defined.
A **general data gap is identified for data** in the area of **identity, physical/chemical/technical properties and methods of analysis** for the other biologically active components…"

Margosa extracts – Mammalian toxicity

Biocides, Technical Meeting 2010:

The **technical specification** was **accepted.**
No outstanding issues for the human health part were identified.


Margosa extracts – Mammalian toxicity
Plant Protection Products, EFSA conclusion 2011:
"Regarding the specification, with the exception of the aflatoxins, which are known relevant impurities, the relevance of the other impurities/by-products could not be established, and a data gap was identified."
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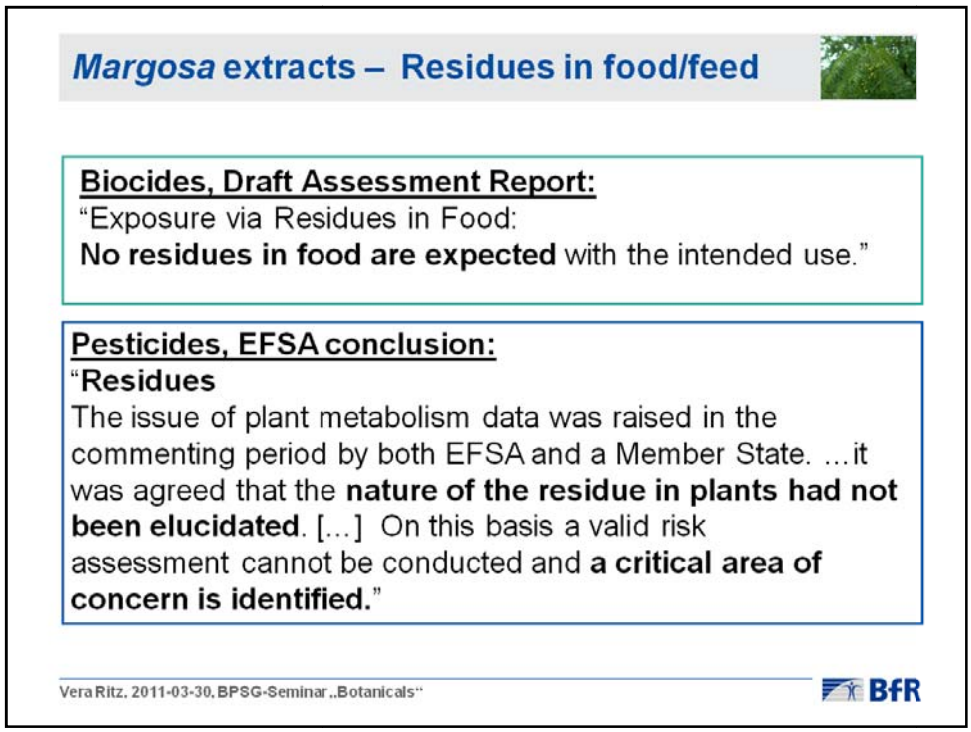
Margosa extracts – Residues in food/feed
Biocides, Draft Assessment Report:
"Exposure via Residues in Food:
No residues in food are expected with the intended use."
Pesticides, EFSA conclusion:
"Residues
The issue of plant metabolism data was raised in the commenting period by both EFSA and a Member State. …it was agreed that the nature of the residue in plants had not been elucidated. […] On this basis a valid risk assessment cannot be conducted and a critical area of concern is identified."
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Margosa extracts – Conclusions

What makes the difference?

-No proper guidance

- Switch from „lead substance“ concept to „whole extract“ concept during the evaluation process

- Residues: How can they be assessed?

Margosa extracts – Conclusions

How much effort and elaboration in analytical methods and identification is appropriate for a plant extract with a relatively low toxicity profile?

FEDERAL INSTITUTE FOR RISK ASSESSMENT

BfR
Risiken erkennen – Gesundheit schützen

Thank you for your attention

Vera Ritz

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Tel. +49 30 - 184 12 - 0 • Fax +49 30 - 184 12 - 47 41
vera.ritz@bfr.bund.de • www.bfr.bund.de

Margosa extracts – References

Conclusion of the peer review of the pesticide risk assessment of the active substance azadirachtin. EFSA Journal 2011; 9(3):1858

Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31991L0414:EN:HTML

Directive 98/8/EC of the European parliament and of the council concerning the placing of biocidal products on the market. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1998:123:0001:0063:EN:PDF

Draft Assessment Report Margosa Extract, http://circa.europa.eu/Public/irc/env/bio_reports/library?l=/review_programme/ca_reports/pt18_insecticides&vm=detailed&sb=Title

How to deal with extracts and oils of plant or animal origin. Addendum to the TNsG on data requirements for active substances. http://ecb.jrc.ec.europa.eu/documents/Biocides/TECHNICAL_NOTES_FOR_GUIDANCE/TNsG_DATA_REQUIREMENTS/Addendum-TNsG-Data_Requirements_PT18_PT19_Oils_and_extracts.pdf

Neem: Today and in the new millenium. Ed. O. Koul and S. Wahab. Kluwer Academic Publishers 2004

Regulation (EC) No 1107/2009 of the European parliament and of the council concerning the placing of plant protection products on the market. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:0001:0050:EN:PDF

The neem tree. Ed. H. Schmutterer. Neem Foundation, Mumbai 2002

Photo: *Azadirachta indica:* FAO forestry photos, R. Faidutti, Ref: 1852 WT2 img0075

Presentation 8
Algae extracts and Laminarine – experience in EU evaluation and registration
Jean-Marie Joubert (Laboratoires Goëmar; France)

GOËMAR

- Brief presentation of Laboratoires Goëmar
- Introduction
- Two experiences :
  - ✓ algae extract : Plant Growth Regulator
  - ✓ laminarin : Natural Defence Stimulator
- Sourcing
- Characterization
- Analysis method
- Evaluation of the toxicity, Ecotoxicity, Behaviour in the Environment
- The Approvals in UE

Goëmar, OECD BPSG; 30 March 2011

La nature qui stimule la nature.

GOËMAR

GOËMAR

1 The sea, the origin of a success story

1971: Establishment of the Goëmar laboratory in Saint-Malo – Brittany
Brittany: 800 varieties of seaweed - the strongest tides in the world.
Goëmar: Development and marketing of seaweed-based products destined to plant health: Trading opportunities for foliar stimulants.

1987: New approach to value natural sea water in human health
Activity diversification due to the launching of a seawater-based product range destined to human health : Marketing of *PHYSIOMER* in 1988.

2002: Approval of the 1st plant vaccine
Invention of the plant vaccination, with a 1st vaccine registered for cereals the same year, containing a natural compound resulting from Laminar seaweed.

2008: Reinforcement of the expertise in Plant Health
Sale of the Human Health department (Laboratoire de la Mer).
Goëmar is now concentrating on its agricultural activity

2010: Change the shareholders
Reinforce the company around Sustainable Agriculture

www.goemar.com

Goëmar, OECD BPSG; 30 March 2011

La nature qui stimule la nature.

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2 Our expertise: know how to anticipate needs

To meet the challenges of a responsible and sustainable agriculture

PHYSIO ACTIVATOR™ TECHNOLOGY

Precursor of the development of new pathways of agronomic progress

- Opening on the foliar stimulants' market over 30 years ago.
- Advanced knowledge of plant physiology.
- Identification of the modes of action of our exclusive filtrates

Physio Activator range

Ascophyllum seaweed-based

Cereals, corn, oleoprotagineous, sugar beat, potatoes, vine, fruits, olive, vegetables

NATURAL PROTECT™ TECHNOLOGY

- Need of harmless and reliable solutions to maintain productiveness and profitability of a more respectful agriculture, for Mankind and the Environment.
- Partnership with the CNRS: Discovery of a natural component of *Laminaria digitata* seaweed, Laminarine, containing plant defense stimulants.

Natural defense stimulator range

Laminarine seaweed-based

Authorized against fungus diseases:

- Cereals (septoria, powdery mildew, helminthosporium,
- Strawberry (powdery mildew),
- Apple/pear (fire blight, scab)

we participate to the ongoing process towards better and safer food etc

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Introduction

Why registering the substance?

- to give confidence to the users
- for data protection

How to register a natural substance?

-no "elicitor" procedure

- Directive 91/414/EEC

How to prove its harmlessness?

- identification and quantification of impurities
- toxicological studies

Goëmar, OECD BPSG; 30 March 2011

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	Laminarin	Algae extract
Date of submission	2001	2007
Date of inclusion in Annex I	2005	2009
Member state	Rapporteur: Belgium Co-rapporteur France	Rapporteur : Italy
Applicant	Goëmar	TaskForce of 5 companies
Condition of reviewing	New active substance	Revision of the Annex IV

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	Laminarin	Algae extract
Sourcing = brown algae	*Laminaria digitata*	*Ascophyllum nodosum*
Registration category	91/414/EEC Directive (PPP)	91/414/EEC Directive (PGR)
Category	Plant Protection Product Natural Defense Stimulator	Biostimulant
Active substance	ß 1-3-D- glucan: $(C_6H_{12}O_5)_n$, n=20-30	Not determined
Purity	Nominal purity: 930 g /kg Minimal purity: 860 g/kg	Extract not purified
Impurities	Impurity details: mineral salts, glucans	No impurity detail
Characterization	Phys-chem	Markers : mannitol, alginic acids, fucose
Preparation	Laminarin + co-formulants	Algae extract + only preservatives

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Topics	Laminarin	Algae extract
Analysis method	HPLC/HPIC (see curve)	Several methods for markers
Impurities	Detailed description	Not described
Residues	None (waived)	None
Physico-chemical features	Totally described	Not described
Degradation	Waiver : degradation in glucose	Waiver : edible seaweed
Toxicity	Described + waivers	Waivers
Eco-toxicity	Described + waivers	Waivers
Environment	Described + waivers	Waivers

Goëmar, OECD BPSG; 30 March 2011

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Analytics

- High Performance Ionic Chromatography (HPIC)
- Amperometric detection
- Direct injection of solution
- Validation according to Sanco requirements
- Impurities also validated and quantified ⇨ specifications
- Total quantified > 96% over 5 batches

Goëmar, OECD BPSG; 30 March 2011

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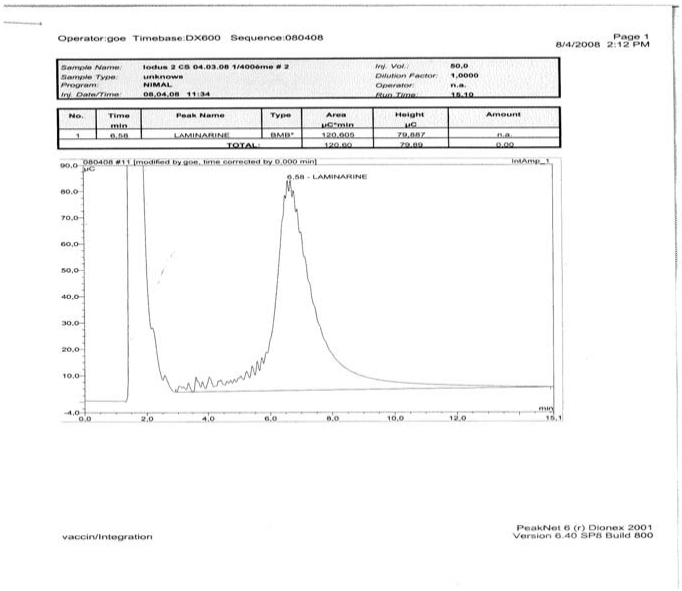
HPLC : laminarin analysis method



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Registration

Authorizations obtained

Country	Commercial name	Approval n°	Disease	Crop	Authorization Date	Expiry date
France	Iodus 2 Céréales	AMM 2020021	Septoria, P. mildew, Helmintho, P. mildew	Wheat Barley	2002	October 2012
UK	Vacciplant	MAP 131 49	Septoria, P. mildew	Wheat	2005	31 March 2015
Switzerland	Iodus 40	W 6436	P. mildew P. mildew, Rhyncosporia,	Wheat Barley	2007	31 May 2017
	Vacciplant	W 6724	Fire blight	Apple, pear trees	2010	2020
France	Iodus 2 Cultures Spécialisées	AMM 2080019	P. mildew Fire blight, Scab	Strawberry Apple, pear trees Apple trees	2008 2008 2011	2018 2018 2021
Belgium	Vacciplant	N° 9661/B	P. mildew P. mildew Fire blight	Strawberry Courgettes Apple, pear trees	2008	17 June 2018
Slovakia	Vacciplant	914/2009-940*	Fire blight	Apple, pear trees	2009	To renew
The Netherlands	Vacciplant	13383 N	P. mildew Fire blight	Strawberry Apple, pear trees	2010	17 June 2018
Greece	Vacciplant SL	11105	P. mildew Fire blight Bacteria *(Pseudo, Xantho, Coryne)*	Strawberry Apple, pear trees Tomato*	2009 2009 26-7-2010	31 March 2015 31 March 2015 26 nov 2010
Germany	Vacciplant	6904-00	All diseases	All crops	2010	March 2020
USA	Vacciplant	EPA 83941-2	All diseases	All crops	2010	March 2020
Morocco	Iodus 2 Cultures Spécialisées	E 03-9-001	Fire blight	Apple, pear trees	2009	24 March 2019

*temporary approval

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Registration

Files already submitted

Country	Commercial name	Disease	Crop	Submitting date	Authorization expected
Europe	Iodus / Vacciplant	Organic		2008	2011
Denmark	Vacciplant	P. Mildew Botrytis	Strawberry	2010	2012
France	Iodus 2 Cultures Spécialisées	Gloeosporium, P. mildew	Apple trees	Dec 2008	2011
Greece	Vacciplant SL	P. mildew	Grapes	2009	2011
Italy	Vacciplant	P. mildew	Strawberry	Dec 2010	2011
	Vacciplant	Fire Blight	Apple, pear trees	Dec 2010	2011
Poland	Vaxiplant SL	Botrytis, P. mildew Bacteria spot	Strawberry Tomato	2009	2013
Portugal	Vacciplant	P. mildew Fire blight	Strawberry Apple, pear trees	2010	2011
Spain	Vacciplant	P. mildew P. mildew Bremia	Table grapes Strawberry lettuce	2009	2013
Switzerland	Vacciplant	P. mildew	Strawberry	2010	2011

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Registration

Files to be prepared in 2011

Country	Zone	Commercial name	Disease	Crop	Submitting expected	Authorization expected
Finland	North	Vacciplant	P. mildew, Botrytis	Strawberry	2011	2012
Sweden						
Norway						
Lithuania						
Belgium	Centrum	Vacciplant	Scab, Gloeosporium	Apple trees	Feb 2011	2011
Germany		Vacciplant	P. mildew, Botrytis Scab, Gloeosporium	Strawberry Apple trees	?	?
France, Spain	South	Iodus 2 cultures spécialisées	Monilia	Peaches	End 2011	2012
Italy		Vacciplant	P. mildew	Table grapes	End 2011	2013
		Vacciplant	Grey mold, Bacteriosos Botrytis	Tomatoe Strawberry	End 2011	2013
Spain		Vacciplant	Grey mold P. mildew Botrytis	Tomato Table grapes Strawberry	End 2011	2013
Greece		Vacciplant	Grey mold	Tomato		
Morocco		Iodus 2 cultures spécialisées	P. mildew	Strawberry	2011	2012
Turkey		Vacciplant	Fire blight	Apples, pear trees	2012	2014

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Presentation 9
Updates to PMRA's regulatory proposal on non-conventional pesticide registration
By Brian Belliveau (Health Canada Pest Management Regulatory Agency, Ottawa, Canada)

Health Canada Santé Canada
Your health and safety… our priority. *Votre santé et votre sécurité… notre priorité.*

UPDATES TO PMRA'S GUIDELINES FOR THE REGISTRATION OF NON-CONVENTIONAL PEST CONTROL PRODUCTS

OECD BPSG Seminar Day
30 March 2011

Brian Belliveau, PhD
Head, Microbial and Biochemical Evaluation Section
Health Evaluation Directorate
Pest Management Regulatory Agency

Canada

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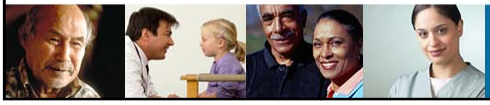
NEW Regulatory Proposal

- Regulatory Proposal (PRO2010-06): *Guidelines for the Registration of Non-Conventional Pest Control Products*
  - Replaces Regulatory Proposal PRO2007-02, *Guidelines for the Registration of Low Risk Biochemicals and Other Non-Conventional Pesticides*
  - Approaches were refined to reflect stakeholder comments on PRO2007-02 and insights gained during the pilot period



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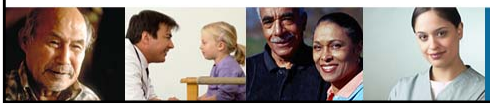
NEW Regulatory Proposal

- PRO2010-06, *Guidelines for the Registration of Non-Conventional Pest Control Products*
  - Published for public comment on 28 October 2010
  - PMRA now considering comments received (mostly from established biopesticide and conventional pesticide industry associations and registrants)
- Criteria for eligibility under this proposal remain unchanged from PRO2007-02



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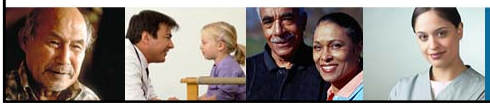
NEW Regulatory Proposal

- Characteristics of products eligible for review under Regulatory Proposal PRO2010-06 are flexible
- Active ingredients must have some (not all) of the following characteristics:
  - Low inherent toxicity to humans and other non-target organisms; metabolites must also be of low toxicity
  - Low potential for use to result in significant human or environmental exposure
  - Not persistent in the environment
  - Already widely available to the public for other uses with a long history of safe use at equivalent exposure levels
  - Pesticidal action is not the result of toxicity to the target organism
  - Unlikely to select for pest resistance



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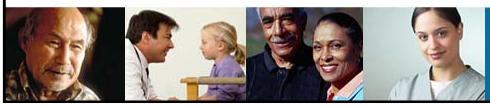
NEW Regulatory Proposal

- Substances eligible for review include:
  - Food items, extracts, preservatives or additives
  - Plant extracts (botanicals) and oils
  - Commodity chemicals that have a range of non-pesticidal uses
  - Fertilizer or other plant growth supplements, commonly used in the agricultural sector
  - Inert materials



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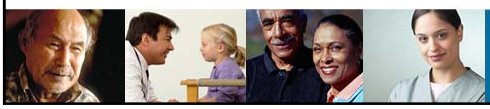
NEW Regulatory Proposal

- Strong focus on product registration
  - Regulatory Proposal PRO2010-06 is a registration guideline and does not address scheduling or exemption as regulatory options
    - No PMRA equivalent to U.S. EPA's list of active ingredients exempted from registration under Section 25(b) of *Federal Insecticide, Fungicide and Rodenticide Act*



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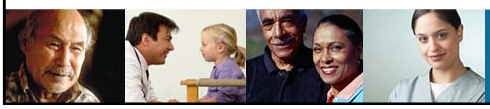
NEW Regulatory Proposal

- Data/information requirements
  - Tables of data (DACO) requirements have been removed from Regulatory Proposal PRO2010-06
    - Allows for greater flexibility in setting registration requirements
    - Eliminates the need for applicants to submit waiver requests for "core" requirements if the Agency does not ID them as required ("R") during the presubmission consultation



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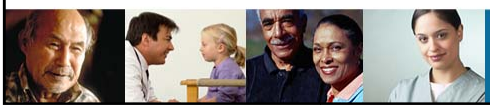
NEW Regulatory Proposal

- Provide more guidance on the submission process, particularly for first-time / inexperienced applicants
  - Pre-submission consultation and preparation of the information package
  - Compile list of frequently asked questions
- Data requirements for Domestic (home and garden) and Commercial/Restricted (agriculture, forestry) class products may differ; the process for domestic uses will usually be simpler



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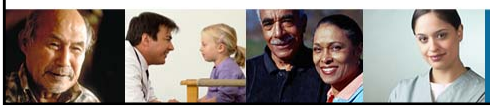
Non-Conventionals: How is the Process Working?

- We are learning through experience
  - "Case-by-case" approach has been adopted and is working well
  - Totality of evidence is considered
    - Flexible, weight-of-evidence approaches to health, environment and value assessments
    - Data requirements and assessment efforts are commensurate with expected level of risk



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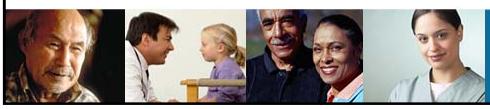
Product Chemistry Data Requirements Overview

- General requirements listed under:
  - Regulatory Directive DIR98-03 (manufacturing concentrates and end-use products)
  - Regulatory Directive DIR98-04 (technical grade active ingredients and integrated system products)
- Presubmission consultation
  - Products evaluated on case-by-case basis, when necessary
- Flexibility in data requirements
  - Provision of valid scientific rationales



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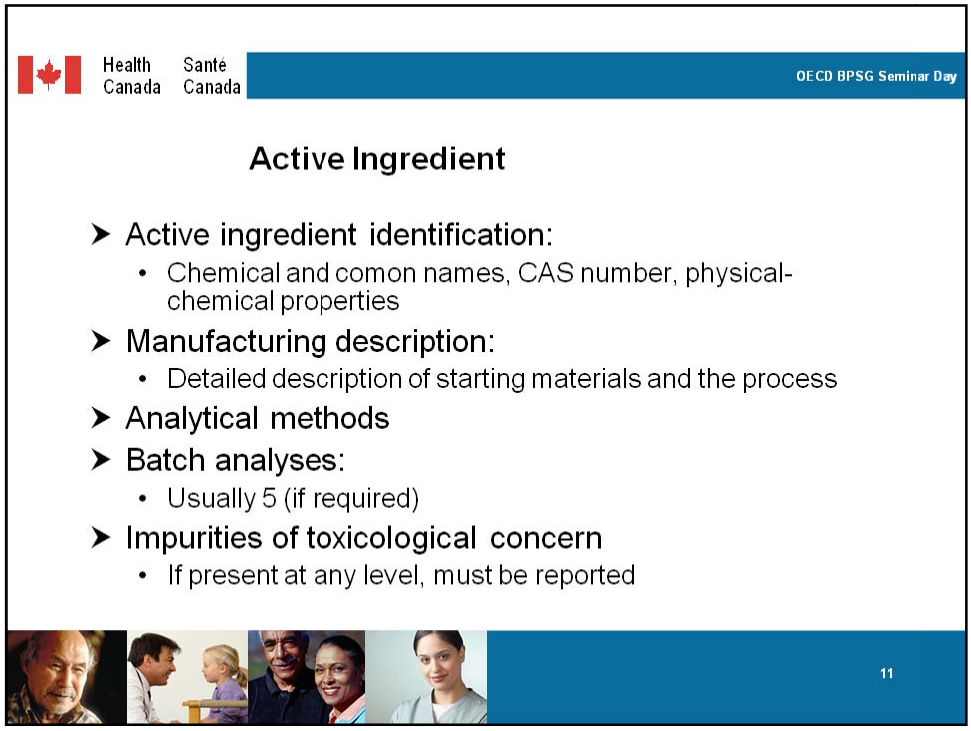
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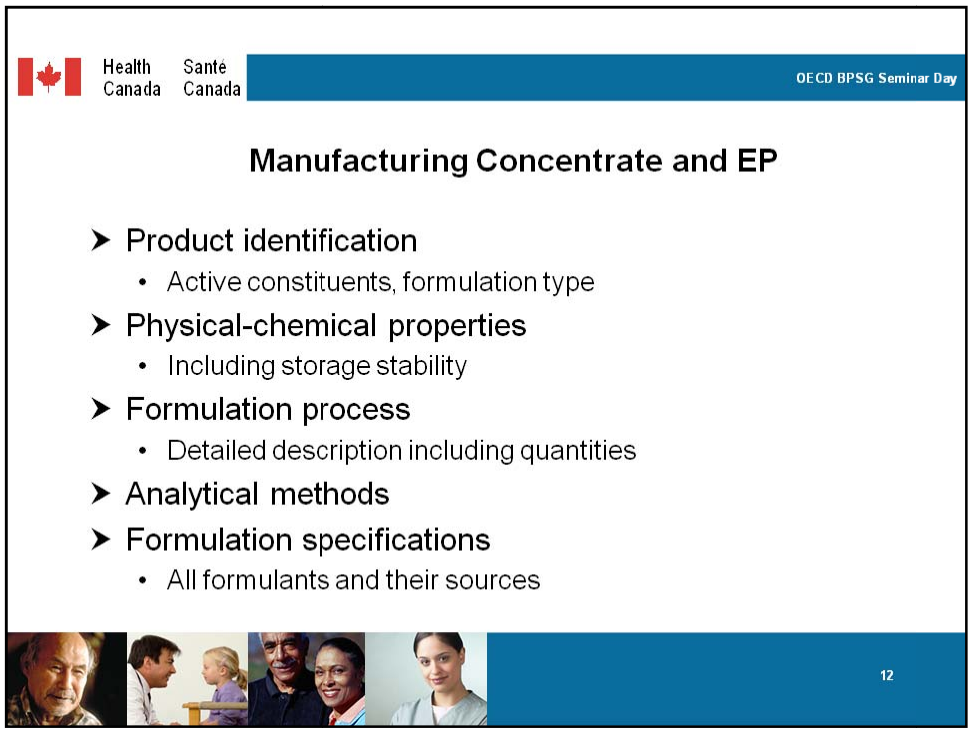
Active Ingredient
Active ingredient identification:
Chemical and comon names, CAS number, physical-chemical properties
Manufacturing description:
Detailed description of starting materials and the process
Analytical methods
Batch analyses:
Usually 5 (if required)
Impurities of toxicological concern
If present at any level, must be reported
11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Manufacturing Concentrate and EP
Product identification
Active constituents, formulation type
Physical-chemical properties
Including storage stability
Formulation process
Detailed description including quantities
Analytical methods
Formulation specifications
All formulants and their sources
12

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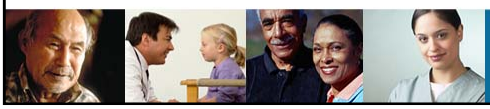
Plant Extracts/Oils

- For plant extracts and essential oils, a method(s) is required to determine the **composition** of the product
- Major and representative components in each extract/oil must be determined and **quantified**
- Standard and literature methods are acceptable



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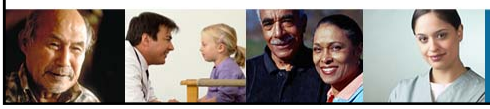
Food Chemicals and Food Grade Edibles

- Food grade products include food grade edibles as well as the chemicals listed in the Food Chemicals Codex (FCC)
- For food grade edibles:
  - A certificate from the supplier/manufacturer that the product is fit for human consumption is required
- For food grade chemicals listed in FCC:
  - FCC requirements and specifications must be met



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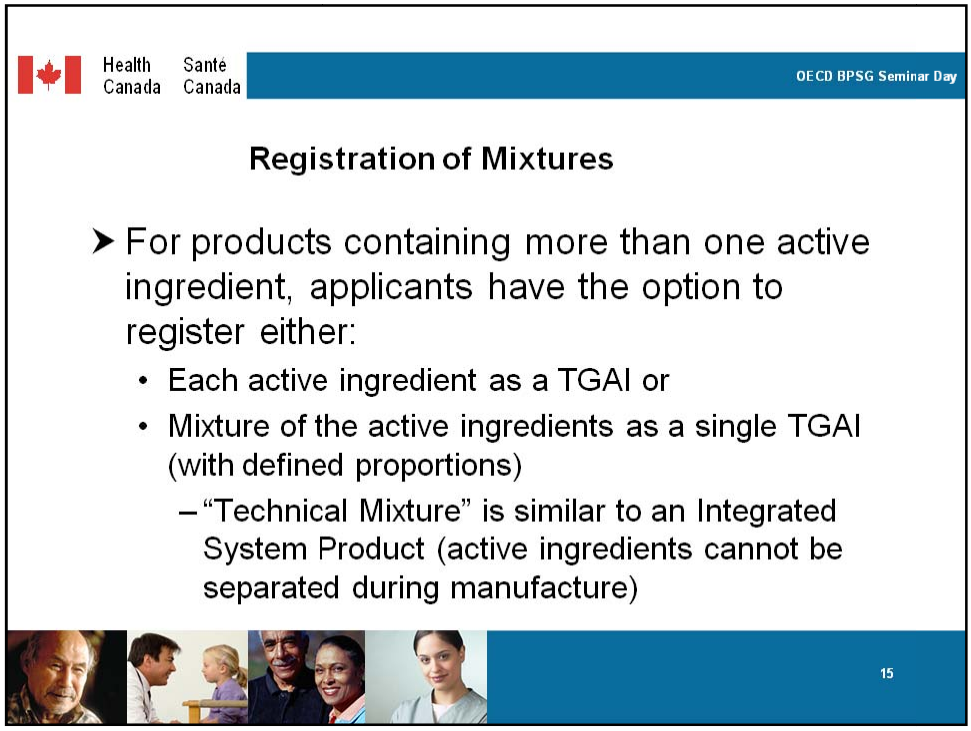
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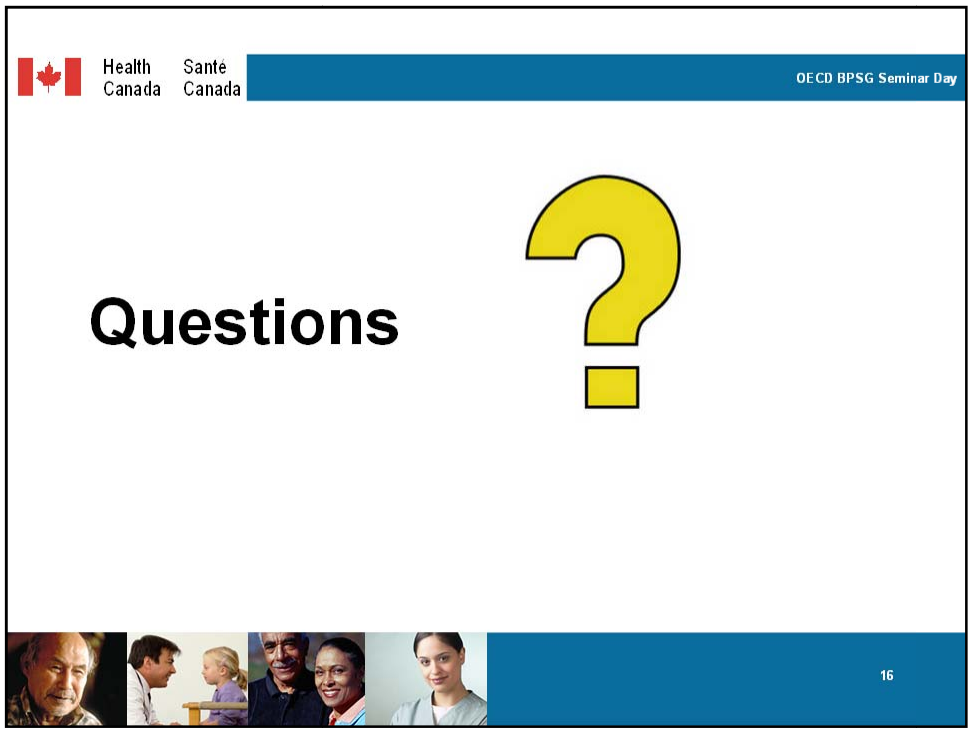
Registration of Mixtures
For products containing more than one active ingredient, applicants have the option to register either:
Each active ingredient as a TGAI or
Mixture of the active ingredients as a single TGAI (with defined proportions)
"Technical Mixture" is similar to an Integrated System Product (active ingredients cannot be separated during manufacture)
15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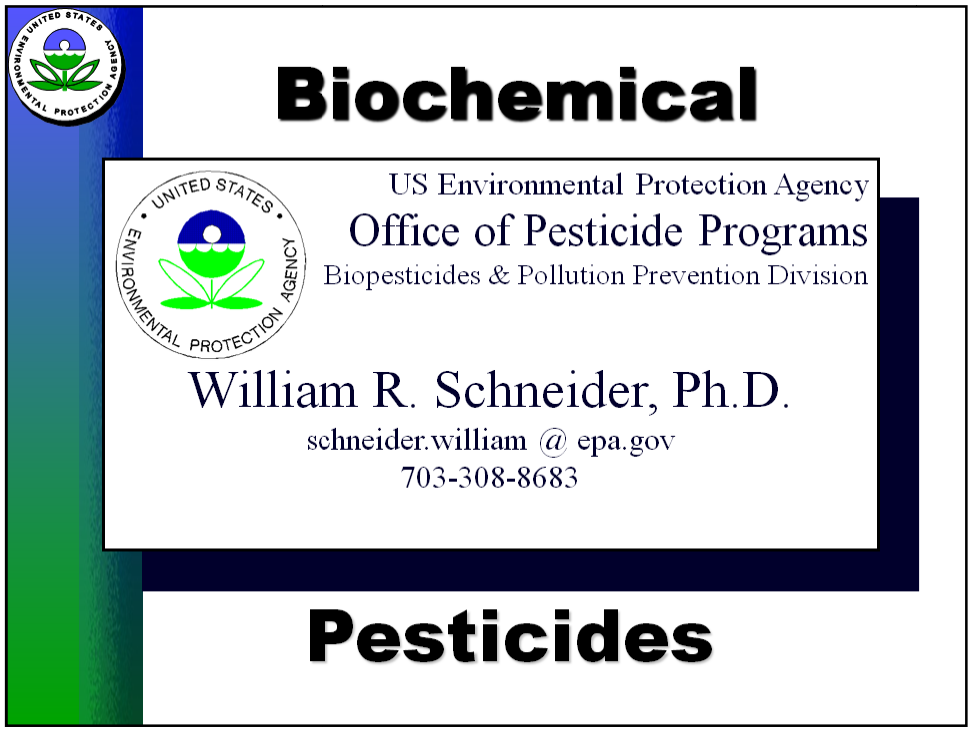
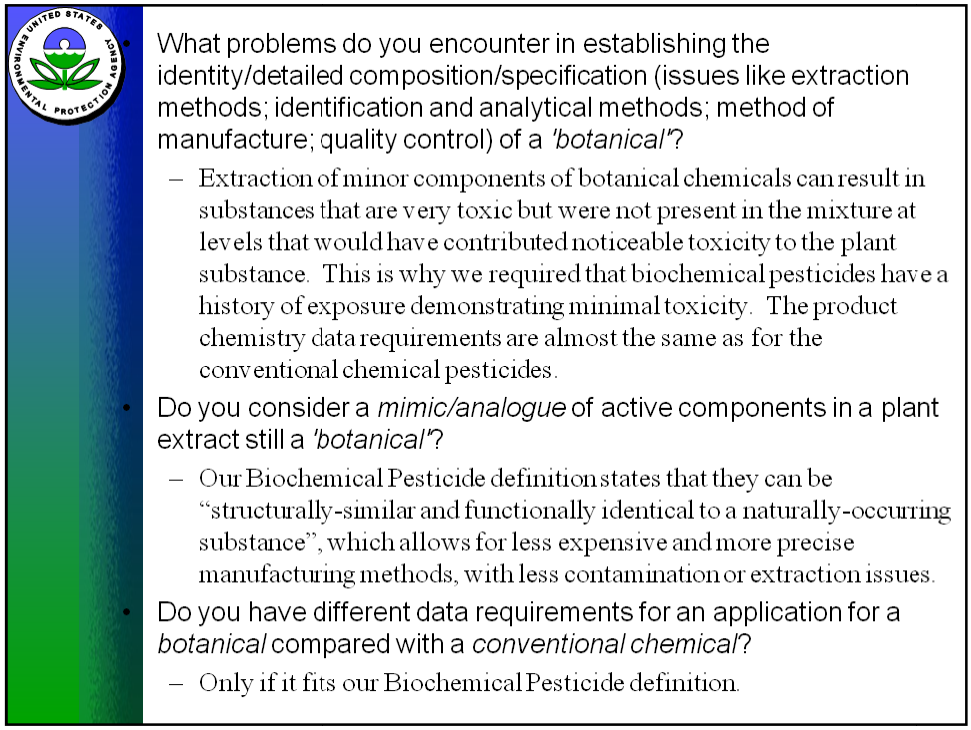
Questions
16

Presentation 10
US experiences with the biochemical/botanical pesticide system, including the "biochemical classification" system
By William Schneider (Biopesticides and Pollution Prevention Division, EPA, Arlington; USA)





- Do you use a different approach in the way you assess a *botanical* (e.g. use of different models) compared with a *conventional chemical*? In general how do you address *fate in the environment*, *residues* and –if relevant- a *metabolite issue*?
  - For the biochemical pesticides, we used our Tiered Data Requirement system but the studies reference the conventional chemical pesticide guidelines for conducting the studies. Because of the low toxicity, Residue and Fate studies are almost never required. Botanical pesticides that do not fit the Biochemical Pesticide definition are registered by the conventional pesticide divisions and are handled the same way as any conventional pesticide. We do use the standard exposure screening models, T-REX (Terrestrial Residue Exposure), and GENEEC (Generic Estimated Environmental Concentration)
- What is your opinion/experience in the approach of using groupings for plant constituents (e.g. according to their chemical family) and/or markers for botanicals or using one botanical as a model for a group of botanicals?
  - This would be done on a case-by-case basis, depending on how similar the chemical components are. This is useful in identifying potential toxicity problems if those are seen with similar chemicals and might trigger additional specific testing.

US Regulatory History

- "Biorational" pesticide class included
  - Microbial & Biochemical pesticides
- Policy announced in 1979 (44FR28093)
- 1982 Proposed data requirements and guidelines
- 1984 Final data requirements 49FR42855
- Biochemical pesticides:
  - Natural occurrence
  - Unique non-toxic mode of action
  - Low use volume
  - Target species specificity
  - Include pheromones, hormones, growth regulators, and enzymes

2007 Revised Data Requirements

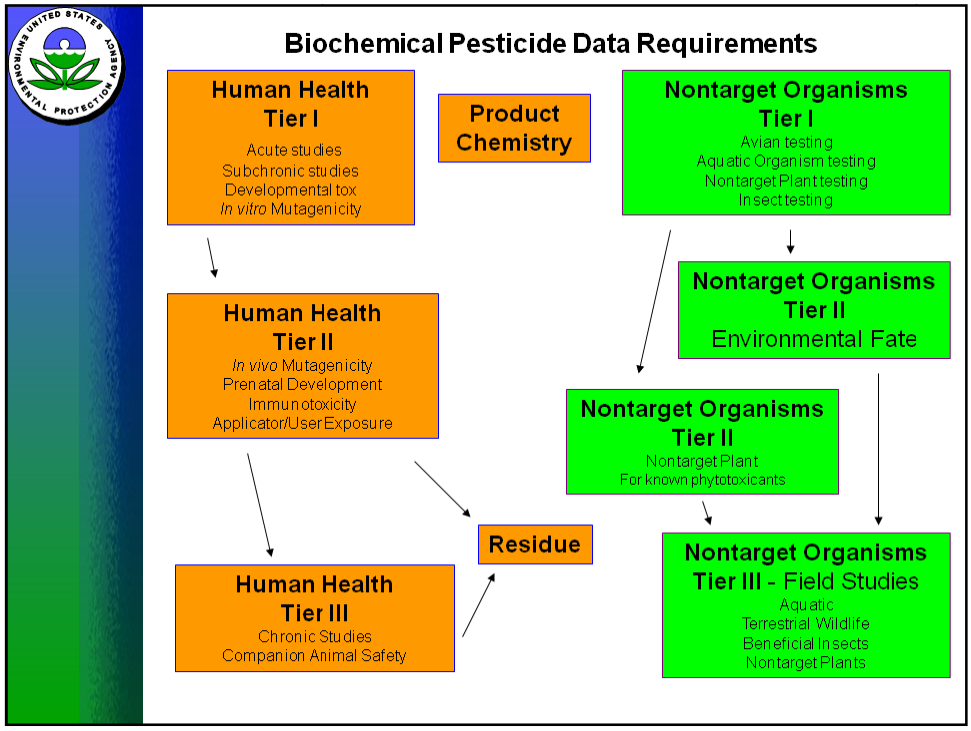
72 FR 61002 & 40 CFR 158, Subpart U

- Biochemical Pesticides
  - Naturally occurring
  - History of exposure to humans and the environment demonstrating minimal toxicity
  - Non-toxic mode of action, e.g.
    - attraction, repellency (including irritants)
    - growth regulation
    - induction of SAR (Systemic Acquired Resistance)
    - physical modes of action, e.g. suffocation, desiccation, smothering, coatings

Biochemical Data Requirements

Data Requirement Regulations 40 CFR 158

- Footnotes in data requirement tables indicate when that data is not required
- Organized into Tiers to indicate when a class of data is not required
  - e.g. exposure studies are not needed if no toxicity is seen in the Tier 1 nontarget tests
- Information from the literature may adequately satisfy the data requirement
- A formal data waiver request may be granted if a valid scientific argument is provided



Biochemical Pesticides Human Health Assessment Data Requirements

"The data requirements are organized into a tier-testing system with specified additional studies at higher tiers being required if warranted by adverse effects observed in lower tier studies"

Guideline Number	Data Requirement
Tier I	
Acute Testing	
870.1100	Acute oral toxicity - rat
870.1200	Acute dermal toxicity
870.1300	Acute inhalation toxicity - rat
870.2400	Primary eye irritation - rabbit
870.2500	Primary dermal irritation
870.2600	Dermal sensitization
none	Hypersensitivity incidents
Subchronic Testing	
870.3100	90-day oral (one species)
870.3250	90-day dermal - rat
870.3465	90-day inhalation - rat
Developmental Toxicity	
870.3700	Prenatal developmental - rat preferably
Mutagenicity Testing	
870.5100	Bacterial reverse mutation test
870.5300 870.5375	*In vitro* mammalian cell assay

Tier II	
Mutagenicity Testing (*In vivo* cytogenetics)	
870.5385 870.5895	*In vivo* Mammalian Cytogenetics
Developmental Toxicity	
870.3700	Prenatal developmental
Special Tests	
880.3550	Immunotoxicity
Applicator/User Exposure	
875.1100	Dermal outdoor exposure
875.1200	Dermal indoor exposure
875.1300	Inhalation outdoor exposure
875.1400	Inhalation indoor exposure
875.1500	Biological monitoring

Tier III	
Chronic Testing/Special Testing	
880.3800	Immune response
870.3800	Reproduction and fertility effects
870.4100	Chronic oral - rodent and nonrodent
870.4200	Carcinogenicity - two species - rat and mouse preferred
870.5380	Mammalian spermatogonial chromosome aberration test
Special Testing	
870.7200	Companion animal safety

RESIDUE DATA
Required only when Tier II or Tier III toxicology data are required

Nontarget Organisms and Environmental Fate

Not required for arthropod pheromones when applied at up to a maximum use rate of 150 grams active ingredient/acre/year except when the product is expected to be available to avian species (*i.e.* , granular formulation)

Guideline Number	Data Requirement
Tier I	
Avian Testing	
850.2100	Avian acute oral toxicity
850.2200	Avian dietary toxicity
Aquatic Organism Testing	
850.1075	Fish acute toxicity, freshwater
850.1010	Aquatic invertebrate acute toxicity, freshwater
Nontarget Plant Testing	
850.4100	Terrestrial Plant Toxicity, Seedling emergence
850.4150	Terrestrial Plant Toxicity, Vegetative vigor
Insect Testing	
880.4350	Nontarget Insect Testing

Tier II - Required on a case-by-case basis when results from Tier I Studies indicate adverse effects

Tier II	
Environmental Fate Testing	
163-1 (835.1230)	Sediment and soil adsorption/desorption for parent and degradates
163-1 (835.1240)	Soil column leaching
163-2 (835.1410)	Laboratory volatilization from soil
161-1 (835.2120)	Hydrolysis
161-1 (835.4100)	Aerobic soil metabolism
161-2 (835.2240)	Photodegradation in water
161-3 (835.2410)	Photodegradation on soil
162-2 (835.4200)	Anaerobic soil metabolism
162-4 (835.4300)	Aerobic aquatic metabolism
162-3 (835.4400)	Anaerobic aquatic metabolism
880.4425	Dispenser - water leaching
Nontarget Plant	
850.4225	Seedling emergence
850.4250	Vegetative vigor

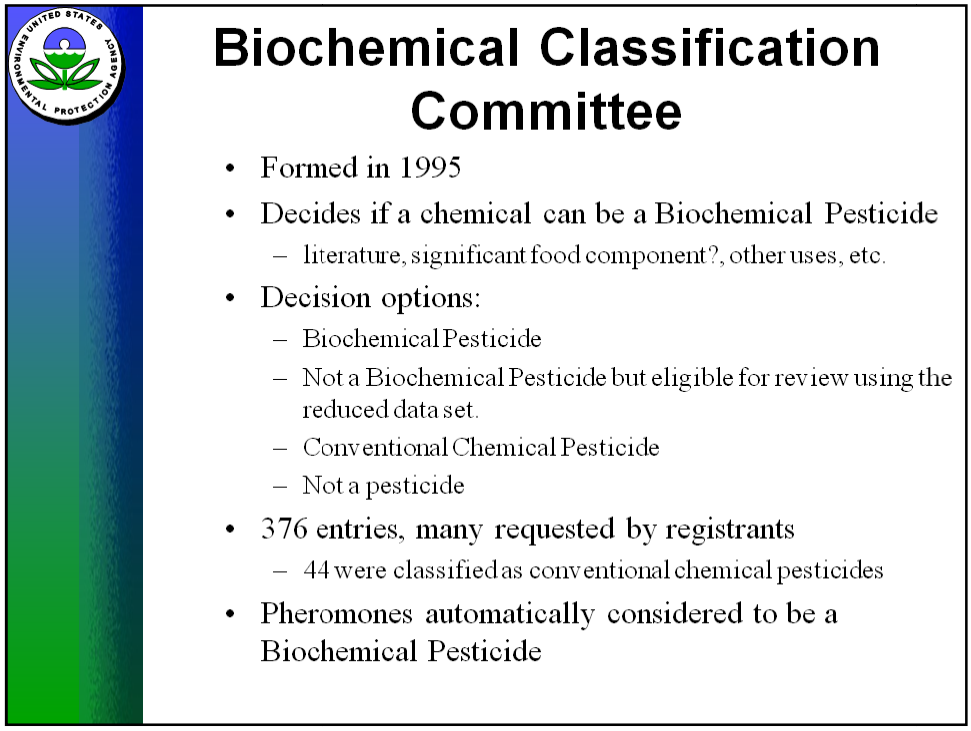
Tier III – Specific tests may be required as described in the table footnotes.

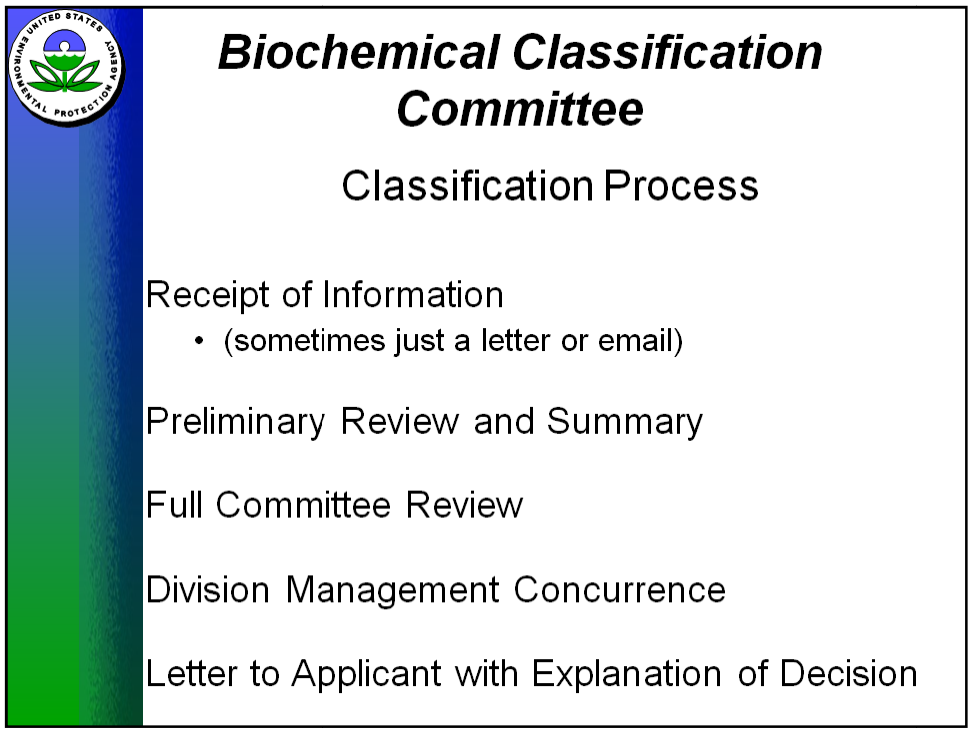
Tier III		
Aquatic Fauna Chronic, Life Cycle, and Field Studies		
850.1300 850.1400 850.1500	Freshwater fish/invertebrate testing	CR
850.1025 850.1035 850.1045 850.1055 850.1350 850.1400 850.1500	Marine/Estuarine fish/invertebrate animal testing	CR
850.1950	Aquatic field fish/invertebrate testing	CR
Terrestrial Wildlife		
850.2300	Avian Reproduction	CR
850.2400	Wild mammal acute toxicity	CR
850.2500	Terrestrial field testing	CR
Beneficial Insects		
850.3040	Field testing for Pollinators	CR
Nontarget Plants		
850.4225 850.4250 850.4300 850.4450	Nontarget plant	CR

Biochemical Classification

40 CFR 180.2000(c)

"The Agency may review, on a case-by-case basis, naturally-occurring pesticides that do not clearly meet the definition of a biochemical pesticide in a effort to ensure, to the greatest extent possible, that only the minimum testing sufficient to make scientifically sound regulatory decision would be conducted."


Biochemical Classification Committee
• Formed in 1995
• Decides if a chemical can be a Biochemical Pesticide
– literature, significant food component?, other uses, etc.
• Decision options:
– Biochemical Pesticide
– Not a Biochemical Pesticide but eligible for review using the reduced data set.
– Conventional Chemical Pesticide
– Not a pesticide
• 376 entries, many requested by registrants
– 44 were classified as conventional chemical pesticides
• Pheromones automatically considered to be a Biochemical Pesticide


Biochemical Classification Committee
Classification Process
Receipt of Information
• (sometimes just a letter or email)
Preliminary Review and Summary
Full Committee Review
Division Management Concurrence
Letter to Applicant with Explanation of Decision

Biochemical Classification Criteria

- Generally are naturally-occurring substances
  - **A synthetic active ingredient can be classified as a biochemical if it is structurally similar, and functionally identical to, a naturally-occurring active ingredient**
- Non-toxic mode of action against the target pest

Biochemical Classification Criteria – additional considerations

- Is the abbreviated set of data requirements appropriate?
  - Would it need chronic tox studies?
- Are there potential effects on non-target organisms?
- Is the exposure low?
  - No or low Persistence in Environment
  - Low application rates/volumes
- Efficacy

Information/Data Needed for a Successful Classification

- **Product Chemistry**
  - -Identify the active ingredient(s)
  - -structure
  - -CAS No. (if available)
  - -Any other physical/chemical data
- **Evidence for Natural Occurrence**
- **Evidence for non-toxic mode of action**

Information/Data Needed for a Successful Classification

- **Target Pest**
- **Method, Rate, Time of Application**
- **Human Health Data/Information**
  - -Publicly-available technical literature
  - -MSDS
  - -FDA GRAS Status
- **Ecological Effects**

Examples of Classification

- Naturally-occurring Pesticides that would not qualify for the Biochemical Pesticide Data Set
  - Rodenticides:
    - Strychnine (is a registered conventional pesticide)
    - Scilliroside (red squill) (registered 1985-1992)
    - Ricin (a bioterrorism extract from castor beans)
    - Cicutoxin (no registered uses – found in Water Hemlock)
    - Curare (no registered uses – South American poison darts from jungle vines)
  - Fish control:
    - Rotenone – from *Lonchocarpus* or *Derris* plant roots
  - Insecticides:
    - Ryanodine from *Ryania speciosa* (registered 1993 – 1997)
    - Nicotine (registered with restricted uses)
    - Juglone (from black walnut – no registered uses)

Examples of Classification

- Naturally-occurring Pesticides that were not "Biochemical Pesticides" but were reviewed using the Biochemical Pesticide Data requirements
  - Formic acid
    - Registered as a miticide
  - Phosphorous acid
    - Registered as a fungicide
  - Dodecanoic acid, monoester with 1,2,3-propanetriol
    - Registered as a fungicide
  - Saponin from Quinoa
    - Registered as a fungicide
  - Sodium metasilicate
    - Registered as a fungicide and insecticide
  - Monopotassium Phosphate
    - Registered as a fungicide

Biochemical Pesticides

over 200 registered

Semiochemicals (Pheromones)
- n-Tetradecyl acetate – a mating disruptor for codling moth

Insect growth regulators
- Azadirachtin – insect growth regulator

Herbicides
- Vinegar (acetic acid)

Repellents
- Coyote and fox urine - repel deer and other animals

Floral attractants and Plant Volatiles
- 1-Octen-3-ol attractant in electric bug traps

Insect & nematode control
- Soybean oil

Plant pathogen & microbial control
- Potassium silicate (induces Systemic Acquired Resistance)
- Laminarin (induces Systemic Acquired Resistance)

Biochemical

US Environmental Protection Agency
Office of Pesticide Programs
Biopesticides & Pollution Prevention Division

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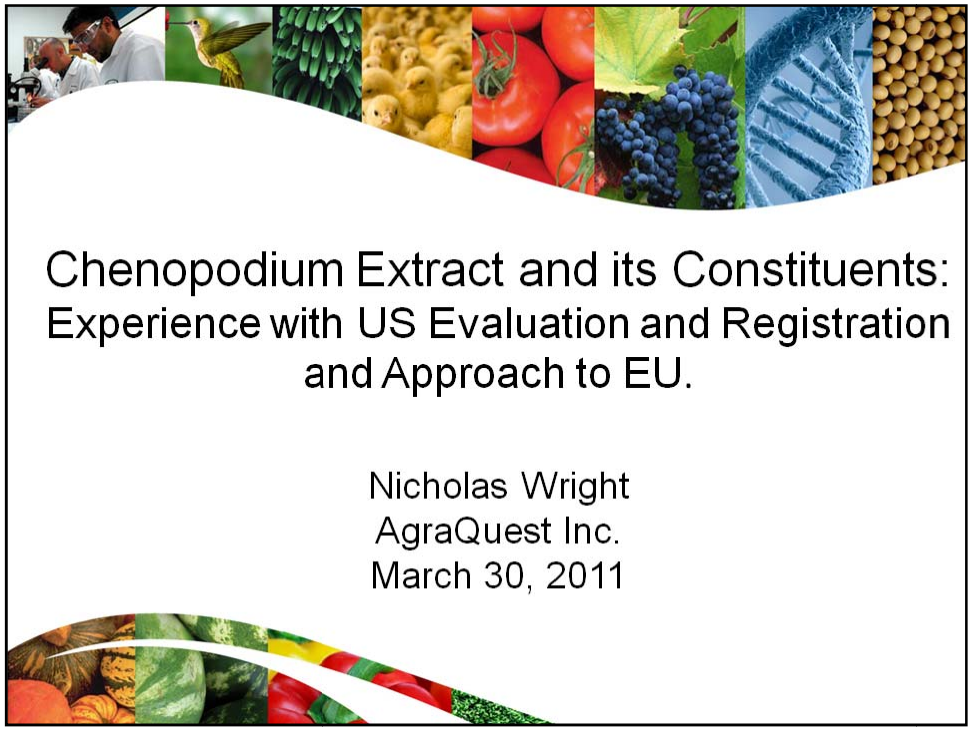
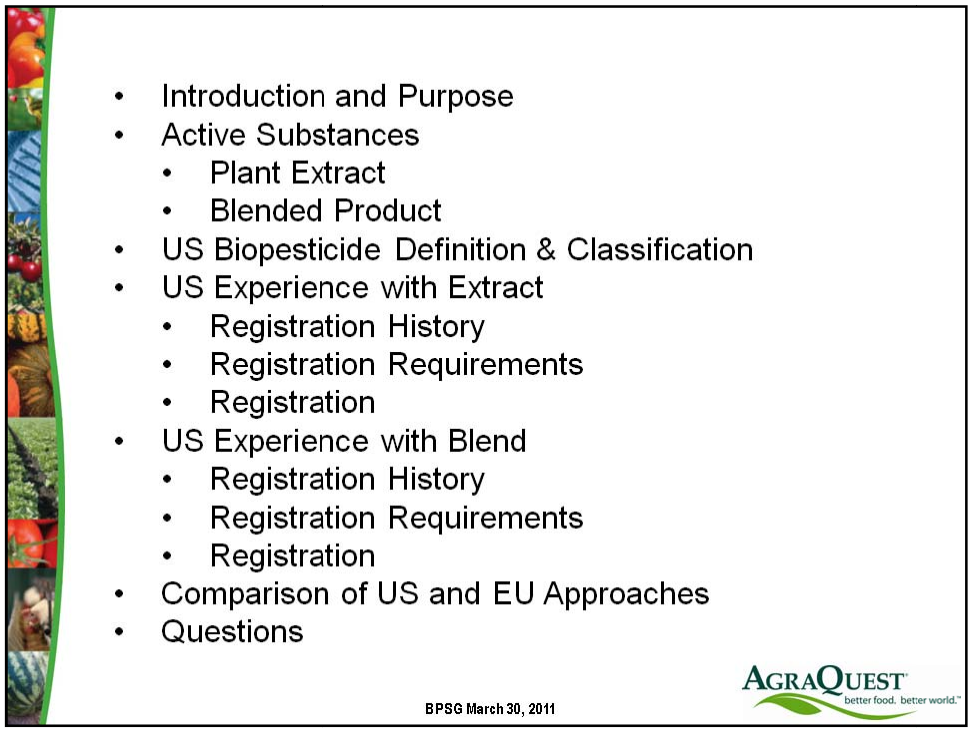
Pesticides

Presentation 11
Chenopodium extract and its constituents
Experience with US evaluation and registration and approach to EU
By Nicholas Wright (AgraQuest Inc.; USA)





Chenopodium-based Active Substances

Plant source

- *Chenopodium ambrosioides* near *ambrosioides* (commonly known as Epazote, Mexican Tea, or American Wormseed)
- Widespread global distribution → invasive weed
- Grows to a height of ~40cm, has serrated leaves, a strong odor, small green flowers, and very small, green seeds
- Plant used as a spice/flavoring and folk medicine
- Essential oil contains numerous terpenoid compounds
- Composition varies greatly depending on plant variety, growing conditions, and growth stage
- Essential oil harvested from the plant biomass using steam distillation
- Further processing required to obtain US registered active ingredient

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Chenopodium ambrosioides

(now known as ***Dysphania ambrosioides***)

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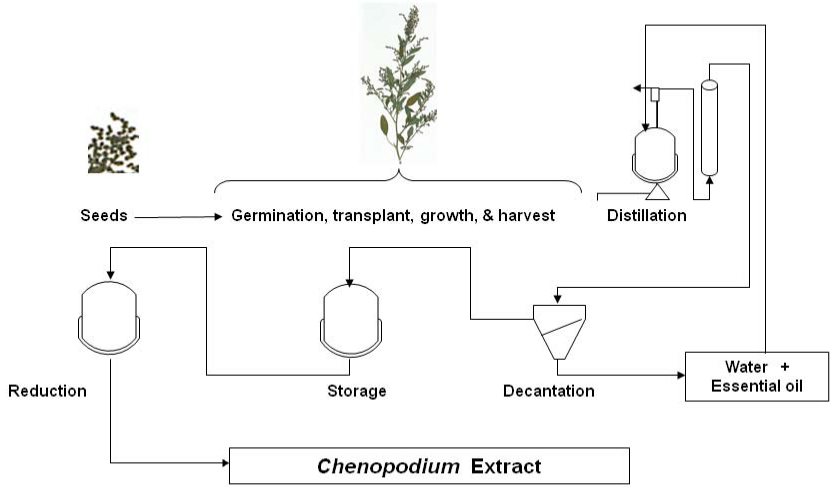
Chenopodium ambrosioides Production Process



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Chenopodium-based Active Substances cont'd

Optimized Blend

- Developed to overcome commercial production issues associated with the plant-derived extract, which include:
  - Variable composition
  - High production costs
  - Removal of ascaridole (EPA considers a toxic compound)
- Active ingredient resulting from development and screening process is "optimized" in that it is a simplified blend intended to mimic the insecticidally active component of the plant extract
- Active substance is a proportional blend of the three main terpene components - alpha-terpinene, p-cymene, and d-limonene
- Activity is based on not one chemical, but the interaction of all three

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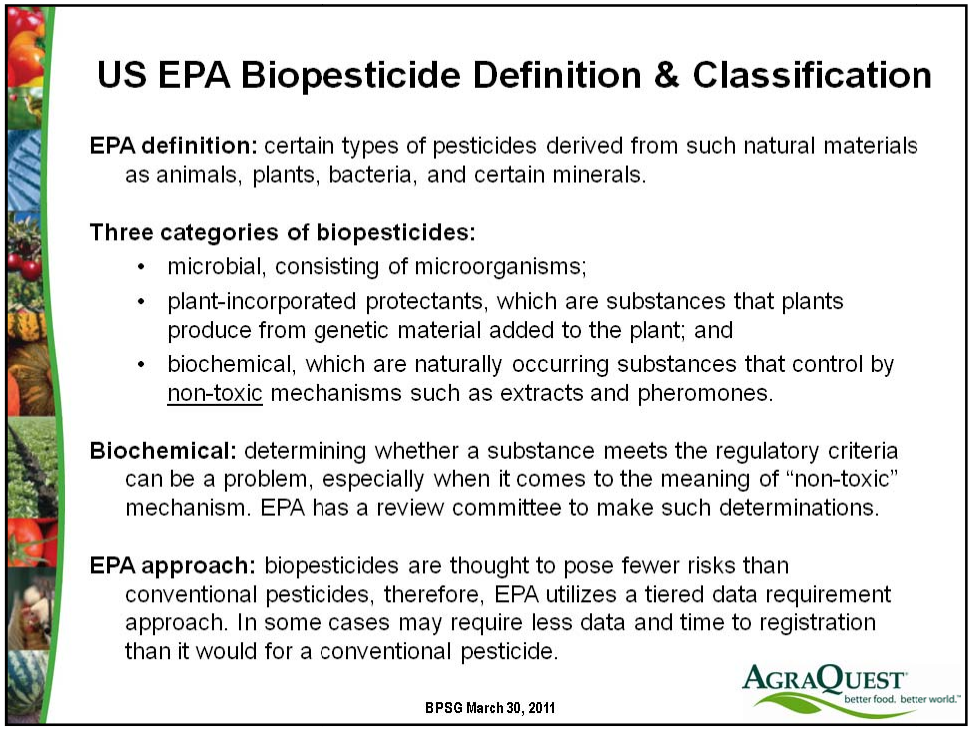
US EPA Biopesticide Definition & Classification
EPA definition: certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals.
Three categories of biopesticides:
• microbial, consisting of microorganisms;
• plant-incorporated protectants, which are substances that plants produce from genetic material added to the plant; and
• biochemical, which are naturally occurring substances that control by non-toxic mechanisms such as extracts and pheromones.
Biochemical: determining whether a substance meets the regulatory criteria can be a problem, especially when it comes to the meaning of "non-toxic" mechanism. EPA has a review committee to make such determinations.
EPA approach: biopesticides are thought to pose fewer risks than conventional pesticides, therefore, EPA utilizes a tiered data requirement approach. In some cases may require less data and time to registration than it would for a conventional pesticide.
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US Experience with Extract
Registration History
• 1999 through 2004 – several failed attempts by original product developer to get the extract classified as a biochemical pesticide
• Rejection based on insufficient characterization and the presence of ascaridole (a purported toxic compound)
• January 2004 – demonstrated ascaridole could be effectively removed, leading to EPA granting biochemical status
• November 2004 – registration package submitted
• February 2005 – EPA completed primary review
• November 2005 – EPA completed secondary review
• January 2006 – AgraQuest acquired the rights to the technology associated with this specific variant of Chenopodium and received results of EPA review, which outlined additional registration submission needs
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US Experience with Extract cont'd

Data Requirements

Product chemistry: product identity, product analysis, manufacturing process, and physical/chemical properties

Mammalian toxicology: acute, subchronic, and other mammalian-related test requirements such as chronic, genotox, immunotox, developmental, etc.

Ecological toxicity: avian acute oral and dietary, freshwater fish LC_{50}, freshwater invertebrate LC_{50}, non-target arthropod toxicity, non-target plant toxicity, etc.

Issues :

- Characterizing the extract based on marker compound approach
- Establishing achievable limits for each marker to account for plant variability
- Refining the analytical method
- Manufacturing methods related to ascaridole removal
- Conducting tox/ecotox testing and developing waivers

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US Experience with Extract cont'd

- January 2006 – August 2007 – additional data collection
- February 2007 – meeting with EPA to discuss data gaps and propose the blended active substance concept
- August 2007 – revised submission addressing all major outstanding issues
- April 2008 – active substance and end-use product registered for non-food uses (e.g. ornamentals)
  - Active ingredient registered in the US as: Extract of *Chenopodium ambrosioides* near *ambrosioides* (ECANA)
- December 2008 – registration amended for use on food crops
- EPA grants the exemption from the requirement of a tolerance for the residues of ECANA when used as an insecticide / acaricide on all food commodities

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US Experience with Blend

Registration History

- February 2007 – met with EPA regarding proposed blended active substance concept and to discuss:
  - Acceptance of concept
  - Composition
  - Additional data requirements and bridging to plant-extract data
  - Classification as a biochemical pesticide
- December 2008 – Submitted registration application and data package for blended active ingredient and end use product for food use applications
  - Extract and blended end use products have same composition in terms of terpenes and other formulants
- June 2010 – active substance and end-use product registered
  - Active ingredient registered in the US as: Terpene Constituents of Extract of *Chenopodium ambrosioides* near *ambrosioides* (ECANA) as Synthetically Manufactured
- EPA grants tolerance exemption for the blended product

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Comparison of US and EU approaches

Similarities:

- Need to meet with regulators early in the process - concept, concerns, and registration strategy.
- Acceptability of a specific mixture of three chemicals as a single active substance rather than as individual actives.
- Acceptability of data bridging back to extract as applicable.
- No clear guidance on how to define a plant extract chemically.
- Both have a defined review process and timeline to registration.
- Exempt from Tolerance in US and propose MRL exempt in EU.

Differences:

- Classification – EPA biopesticide category compared to EU regulatory framework (plant extract or conventional chemical).
- Data Requirements – clearly defined biochemical data requirements vs. hybrid approach where data requirements were negotiated.
- Significantly more data required to address EU dossier.
- Registration submission process and interaction with regulators.
- In this case, the active substance name in EU and US will be different.

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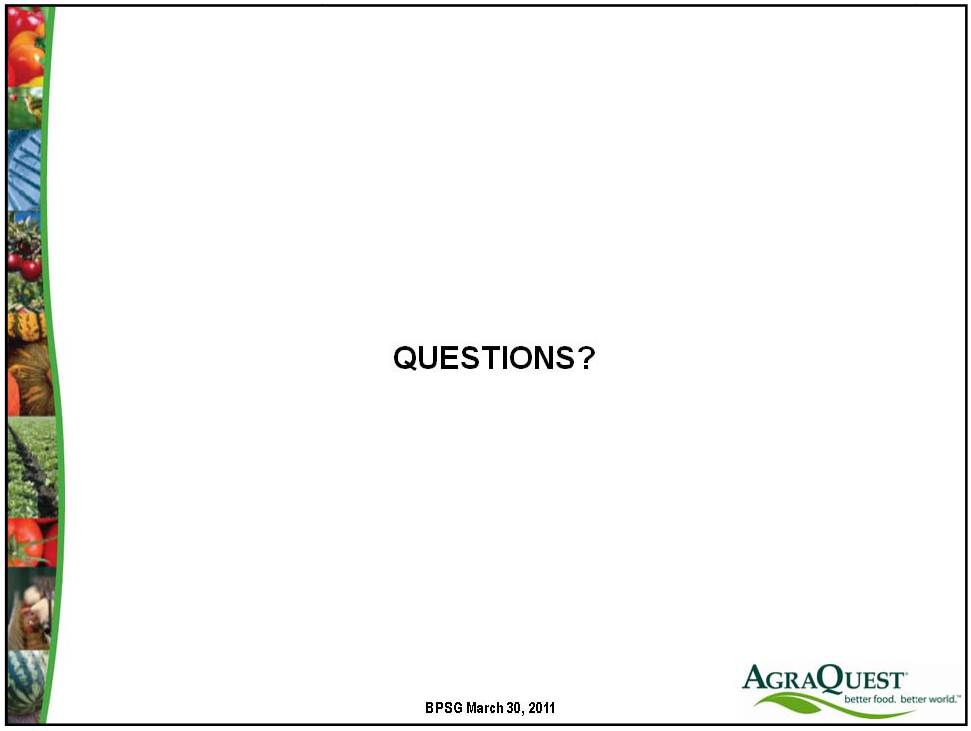
QUESTIONS?
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