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Metabolites in human and environmental risk assessment

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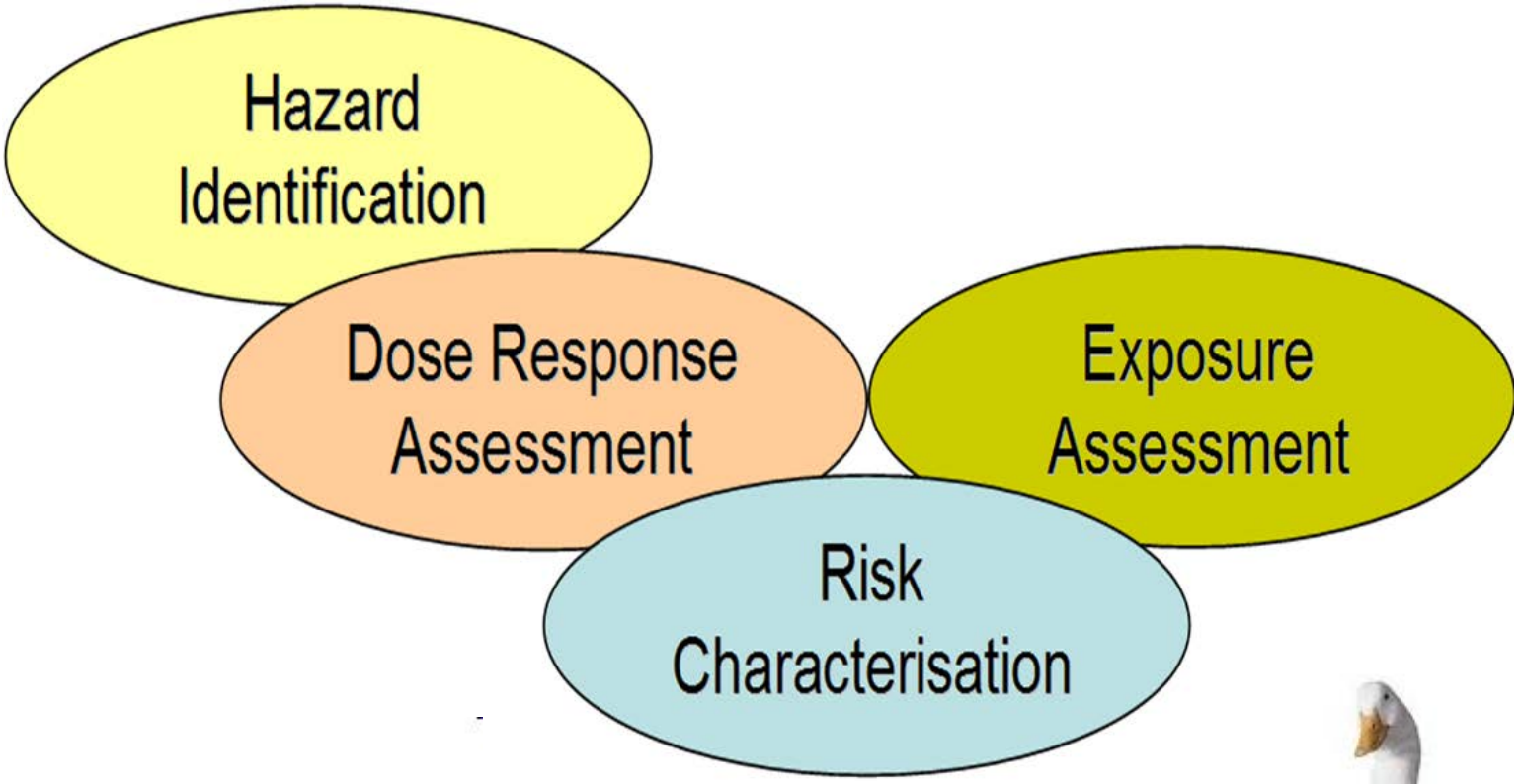
Summary of presentation

- **Basic principles…**
  - Human risk assessment
  - Environmental risk assessment
- **Metabolite risk assessment by substance class (EU only)**
  - Human pharmaceuticals
  - Veterinary pharmaceuticals
  - Pestidices
  - Biocides
  - Chemicals
  - Cosmetics
- **Summary**
- **Conclusions - metabolite risk assessment in EU**

Risk assessment components



Hazard x Exposure = Risk

Human risk assessment

Hazard identification
Toxicity target by species/strain/sex/life-stage/individual

Hazard characterization (dose-response assessment)
NOAEL, BMD → 'safe' dose (RfD, ADI, TDI, VSD, TTC)

Exposure assessment
Estimated or measured human exposure by sub-population and toxicity target

Risk characterization
Compare estimated or measured exposure with 'safe' dose

Dose-response assessment (hazard characterization)

- **A maximum 'safe' dose is defined**

$$\frac{\text{Dose at which no adverse effect is seen (NOAEL, BMD)}}{\text{Uncertainty and modifying factors}}$$

- **The size of the uncertainty factor varies, taking into account the severity of the toxicological effect and individual susceptibility**
- **A commonly used factor is 100 (10 for intraspecies and 10 for interspecies variation)**
- **Used for standard setting (e.g. ADI, AOEL, TDI)**

http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf

Human risk assessment

'Safe' doses

RfD = Reference dose (EPA pesticides, chemical)
Estimate of the amount of a chemical that a person can be exposed to on a daily basis that is not anticipated to cause adverse health effects over a person's lifetime.
Sensitive subgroups are included, and uncertainty may span an order of magnitude.

AOEL = Acceptable Operator Exposure Level (EPA, EU pesticides)
The maximum amount of active substance to which the operator may be exposed without any adverse health effects (mg/kg operator body weight)

ADI = Acceptable Daily Intake (WHO food additives)
Estimate of the amount of a substance in food or drinking water which can be ingested daily over a lifetime by humans without appreciable health risk.
Usually expressed in mg/kg body weight/day.

TDI/TWI/TMI = Tolerable Daily/Weekly/Monthly Intake (EU, WHO contaminants)
Acceptable daily intake of food contaminants; epressed in mg/person, assuming a body weight of 60 kg.

VSD = Virtually safe dose (EPA carcinogens, drinking water)
Estimated lifetime cancer risk <10E-6 based on low-dose mathematical extrapolation

TTC = Threshold of toxicological concern (special case if there are no tox data)
Generic limit based on structure class read-across from existing data

'Safe' doses - Threshold of Toxicological Concern TTC

- Developed by U.S. FDA (1995) as a carcinogenicity “threshold of regulation” for substances in food contact material, when no carcinogenicity data is available for a given substance, now also used for impurities in drugs and drinking water
- Originally based on database with >700 carcinogens; probability distribution of carcinogenic potencies was used to estimate daily exposure level (µg/person) of most carcinogens which would give rise to less than a one in a million (1 x 10E-6) upper bound lifetime risk of cancer (“virtually safe dose”).
- Individual potency calculated by simple linear extrapolation from the dose inducing 50% tumour incidence in the most sensitive species and most sensitive site (TD50) to a 1 in 10E-6 incidence (several “worst case” assumptions).
- Standard TTC value = 1.5 µg/person/day.
- For substances with structural alerts that raise concern for potential genotoxicity, a 10-fold lower TTC (0.15 µg/day) is used, except in pharmaceuticals with benefit, for which a 10-5 lifetime risk of cancer can be justified
- For drug impurities, a TTC higher than 1.5 µg/day may be acceptable under certain conditions, e.g. short-term exposure, for treatment of a life-threatening condition, when life expectancy is less than 5 years, or where the impurity is a known substance and human exposure will be much greater from other sources (e.g. food).
- Some very high potency genotoxic carcinogens are excluded from the TTC approach (aflatoxins, N-nitroso and azoxy compounds); substance-specific toxicity data are required for such substances

http//www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002903.pdf

Risk characterization

- **The margin of exposure (MoE) between the 'safe' dose and the measured or estimated exposure**

$$\text{MoE} = \frac{\text{'Safe' dose (RfD, AOEL, ADI, TDI, TTC)}}{\text{Predicted or estimated exposure}}$$

- **The acceptability of the MoE is defined independently of the risk characterization but takes into account the severity of the toxicological effect and individual susceptibility**
- **A commonly accepted MoE for many toxicological effects is 100 (may be lower for pharmaceuticals)**

Environmental risk assessment

Environmental Risk = PEC/PNEC

- PEC: **p**redicted **e**nvironmental **c**oncentration
- PNEC: **p**redicted **n**o **e**ffect **c**oncentration

Environmental risk assessment

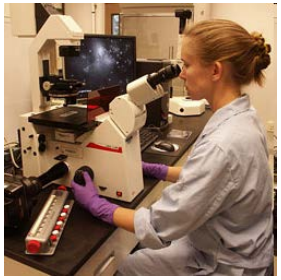
Deriving the predicted no effect concentration (PNEC) from ecotoxicological data

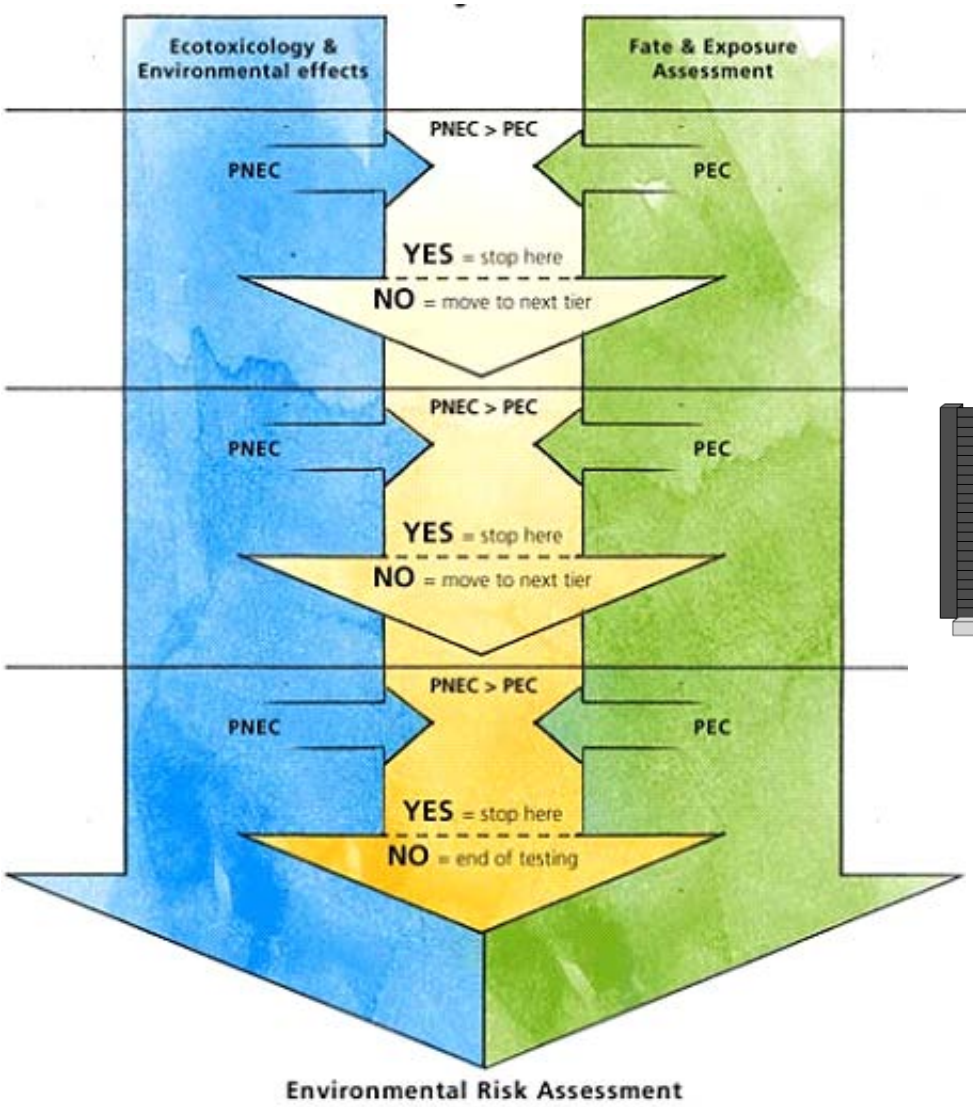
$$PNEC = NOEC / SF$$

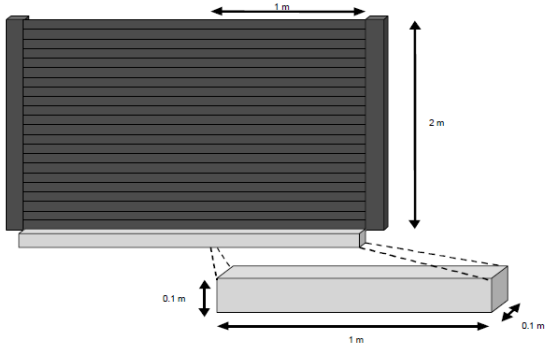
Available ecotoxicological data (Endpoint)	*SF* (safety factor)
At least one LC50 (acute) each for three trophic levels	1000
One NOEC from long term assays (chronic)	100
2 NOEC from long term assays (chronic) at 2 trophic levels	50
3 NOEC from long term assays (chronic) at 3 trophic levels	10
Field studies or mesocosm studies	Case by case

Credit: Bettina Hitzfeld, Swiss Federal Office for the Environment

Environmental risk assessment







Credit: Bettina Hitzfeld, Swiss Federal Office for the Environment

Risk assessment by substance class - EU only

- **Pharmaceuticals**
  - Human (ICH)
  - Veterinary (VICH)
- **Pesticides**
  - Plant protection products (EU Regulation 1107/2009)
  - Pesticide residues
    - in food (EFSA Opinion)
    - in groundwater (Directive 2006/118/EC)
    - in drinking water (Directive 98/83/EC)
  - Biocides (EU Regulation 528/2012)
- **Other**
  - Chemicals - REACH (EU Regulation 1907/2006)
  - Cosmetics (EU Regulation 1223/2009)

Human pharmaceuticals

Guidance = ICH (USA, EU, Japan) + regional notes

http://www.ich.org/

Human pharmaceuticals

Human Risk Assessment

- Metabolites are identified and quantified in nonclinical toxicity species (usually mouse, rat, dog and/or monkey) in plasma, urine, bile and feces.
- Metabolite toxicity is considered to be adequately characterized when animal exposure is at least 50% of human exposure in at least one species in repeat-dose toxicity, carcinogenicity , and embryo-fetal toxicity studies.
- If a human metabolite is >10% of total drug-related exposure, and is significantly greater than maximum exposure in nonclinical toxicity studies, then it may need nonclinical toxicity testing.

ICH M3(R2) (2009) + Q&A (2012).

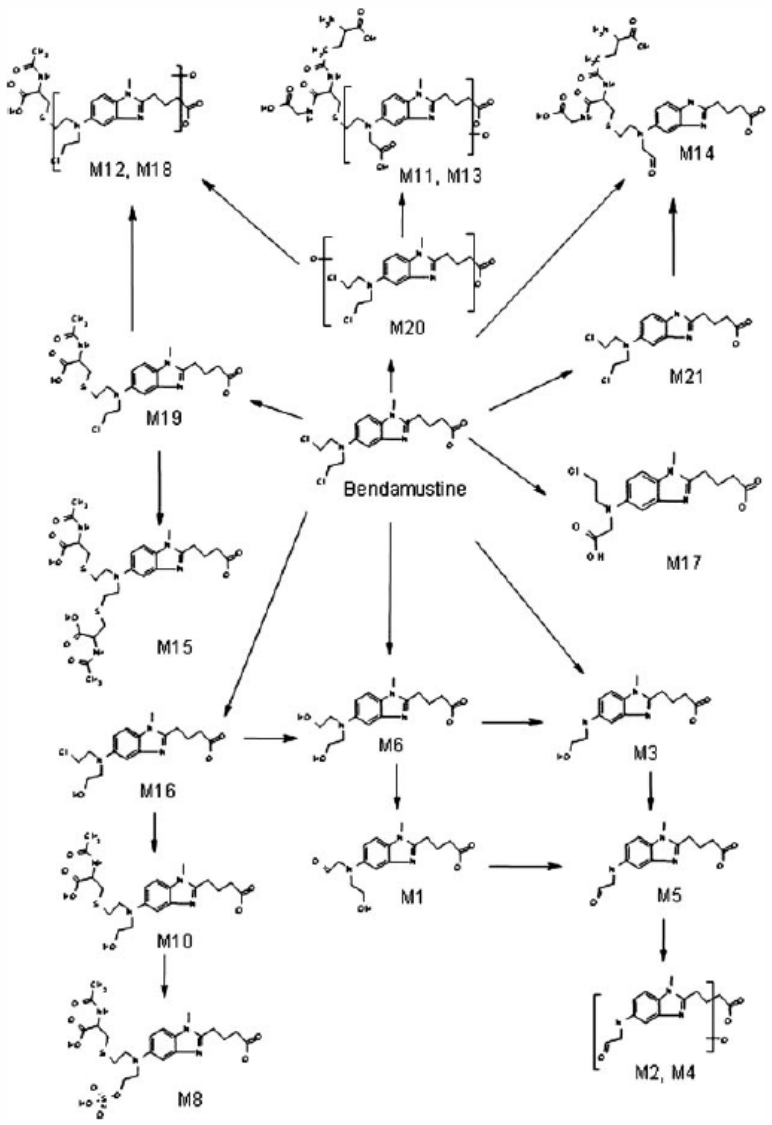
Human pharmaceuticals

Human Risk Assessment

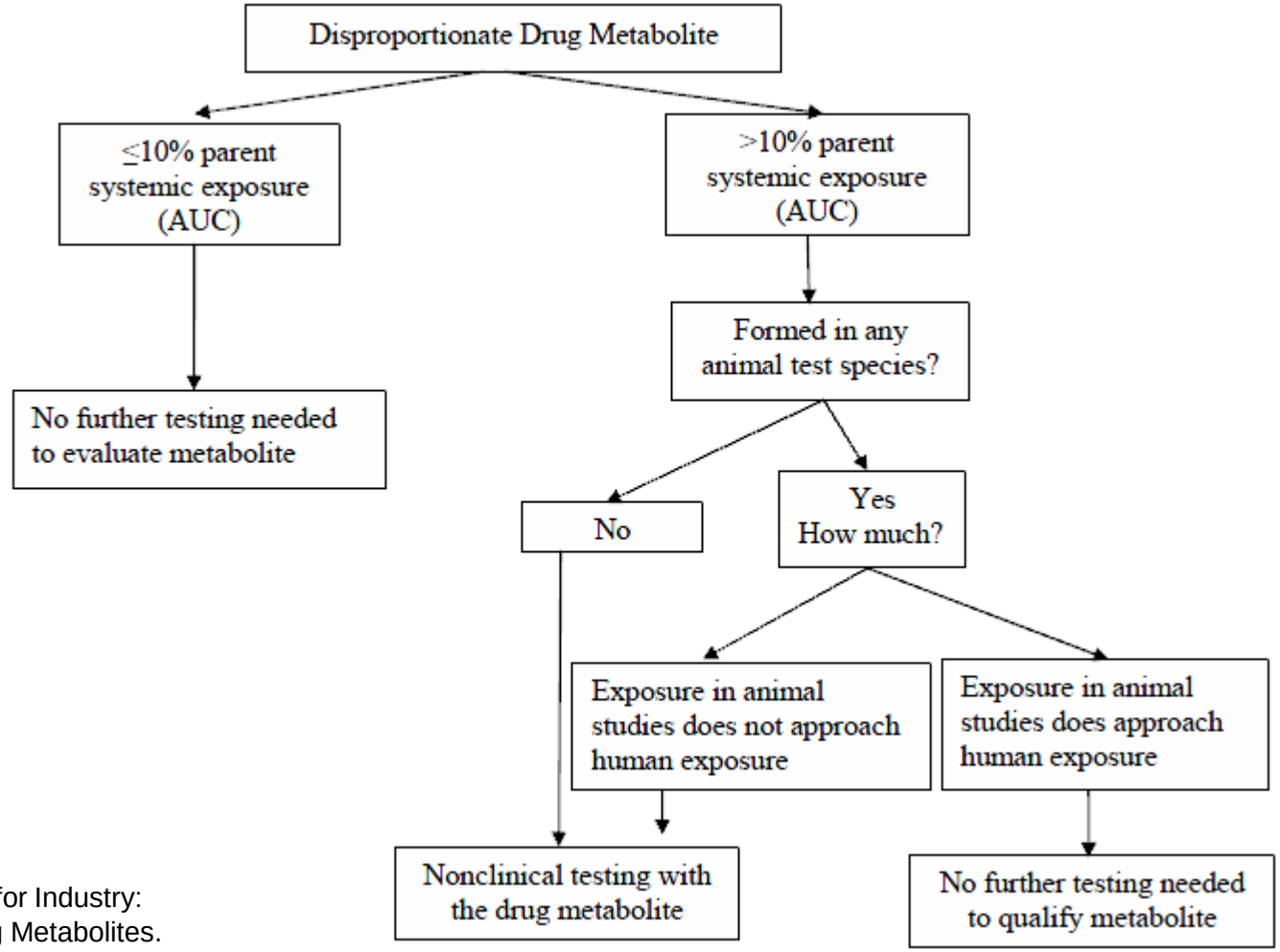
Pharma metabolite example:

Proposed metabolic pathways (M1-M21) for [14C]-bendamustine in rat urine and bile.

(Chovan et al 2007)



Human Risk Assessment



FDA 2008. Guidance for Industry: Safety Testing of Drug Metabolites.

Human pharmaceuticals

Environmental Risk Assessment

Is region-specific!

EU guidance:

- Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use (CPMP/SWP/4447/00), Dec 2006
- Q&A on the Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use (EMA/CHMP/SWP/44609/2010), Mar 2011

Human pharmaceuticals

Environmental Risk Assessment (EU specific)

- In Phase I, PEC estimates are based only on the predicted amount of drug substance used per year, irrespective of metabolism and excretion by the patient, i.e. a 'total residue approach', in which environmental fate and toxicity of metabolites are assumed to be covered by that of the parent compound (drug substance).
- In Phase II (if PEC>PNEC), relevant metabolites should be tested as for the parent. Relevant metabolites are those that are excreted in ≥10% of the administered dose.

(EMA CHMP 2006, 2011)

Veterinary pharmaceuticals

Guidance = ICH (USA, EU, Japan) + regional notes

WHAT IS VICH ?

VICH is a trilateral (EU-Japan-USA) programme aimed at harmonising technical requirements for veterinary product registration. Its full title is the **International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products**. VICH was officially launched in April 1996.

http://www.vichsec.org/en/

Veterinary pharmaceuticals

Human Risk Assessment

Metabolism & residue kinetics studies in food-producing animals

- "Residue" = veterinary drug (parent) and/or its metabolites.
- "Metabolites of interest" are those found in edible tissues of the food-producing animal which have relevance to the toxicological ADI established for the veterinary drug.
- "Major metabolites" are those comprising ≥100 µg/kg or ≥10% of the total residue in a sample collected at the earliest slaughter interval (or following attainment of steady-state or at or near the end of treatment for continuous-use drug products).
- Ordinarily, no differentiation of the radioactivity below these levels (i.e., of the minor metabolites) would be required unless there are toxicological concerns over residues occurring at the lower levels.

(VICH GL46, 2011)

Veterinary pharmaceuticals

Human Risk Assessment

Comparative metabolism in food-producing vs laboratory animals

- If the laboratory animals in the toxicology studies produce substantially similar metabolites to those produced by the food-producing animal, this will ordinarily be taken as evidence that the safety of metabolites that humans will consume has been adequately assessed in the toxicology studies.

(VICH GL47, 2011)

Veterinary pharmaceuticals

Environmental Risk Assessment

Environmental Impact Assessment (EIA) - Phase I

- For calculating the aquatic environmental introduction concentration (EICaquatic) and PECsoil, a total residue concept is adopted. This involves summing the parent drug with all related metabolites excreted by the treated animal. This assumes that 100% of the dose is excreted unless residue depletion data support a value less than 100%. The total residue approach is considered to be conservative in assessing effects in that it combines parent plus metabolites in calculating environmental concentrations, and metabolites generally have less biological activity than the parent compound.
- It is assumed that VPs that are extensively metabolized in the treated animal do not enter the environment. Demonstration of extensive metabolism may be accomplished through a radiolabeled residue depletion and excretion study. A VP may be defined as “extensively metabolized” when analysis of excreta shows that it is converted into metabolites which have lost structural resemblance with the parent drug, are common to basic biochemical pathways or when no single metabolite or the parent drug exceeds 5% of the total radioactivity excreted.

VICH GL6 (2000)

Veterinary pharmaceuticals

Environmental Risk Assessment

Environmental Impact Assessment (EIA) - Phase II

- In triggering a Phase II assessment, the exposure is based on the total residue approach; PEC is defined as the predicted concentration of parent and metabolites in soil, water and sediment compartments.
- Phase II assessment has separate decision trees for (1) aquaculture, (2) intensively reared terrestrial animals and (3) pasture animals.
- In Tier A (initial approach), a total residue approach is used; a PECinitial is calculated assuming that the VP is excreted 100% as parent.
- In Tier B (refined; if RQ≥1 for one or more tested taxonomic levels), then metabolism/excretion data from the residues and ADME part of the dossier should be considered as part of the PEC refinement. Excreted metabolites representing 10% or more of the administered dose and which do not form part of biochemical pathways should be added to the active substance to allow the PEC to be recalculated.

VICH GL38 (2004)

Pesticides

Human Risk Assessment

Regulation (EC) No 1107/2009 (plant protection products regulation)

- Requires risk assessment for different population subgroups (professional or non-professional users, bystanders, workers, residents, specific vulnerable groups or consumers) exposed directly or indirectly through food, feed, drinking water or the environment to active substance and its metabolites, impurities, breakdown and reaction products.
- Data must be sufficient to establish Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).

See also
Commission Regulation (EU) No 283/2013 (active substance data requirements)
Commission Regulation (EU) No 284/2013 (product data requirements)
Commission Regulation (EU) No 546/2011 (principles for evaluation and authorisation)

Pesticides

Human Risk Assessment

- As defined for risk assessment , 'residue' includes active substance, metabolites, breakdown and reaction products.
- Radio-labels shall be positioned at sites (one or more as necessary), to facilitate elucidation of metabolic and transformation pathways and to facilitate investigation of the distribution of active substance and residues.
- A metabolism study shall be submitted for each type of crop group for which use is proposed.
- Metabolites in plants or animal products, soil, groundwater, and air which differ from or are detected in low[er] proportions than those in animals used for the toxicology studies shall be further tested on a case by case basis, taking into account the amount and chemical structure of the metabolite compared to the parent.
- If the terminal residue to which humans will be exposed (from residues in or on treated plants, livestock, soil, ground water, air, or processed treated products) is a metabolite which is not identified as a significant metabolite in mammals, toxicity studies shall, where technically possible, be carried out on that substance unless it can be demonstrated that human exposure to that substance does not constitute a relevant risk to health.

Pesticides

Human Risk Assessment

Example: human metabolic pathways, carbaryl insecticide

http://www.inchem.org/documents/ehc/ehc/ehc153.htm

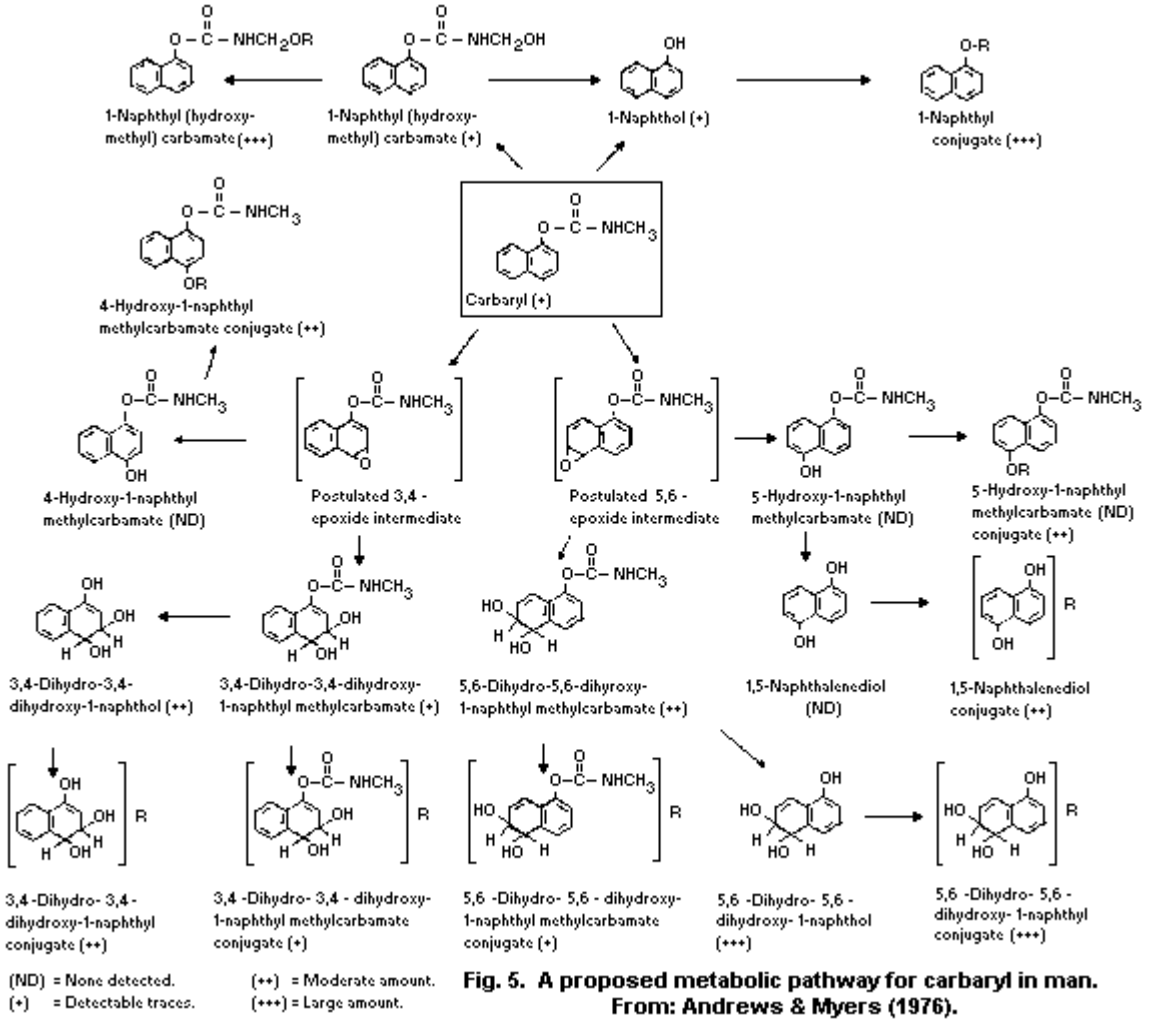


Fig. 5. A proposed metabolic pathway for carbaryl in man. From: Andrews & Myers (1976).

Pesticides

Human Risk Assessment

Pesticide residues in drinking water

Drinking Water Directive (98/83/EC)

- concentrations of pesticides and their relevant metabolites in drinking water must not exceed 0.1 µg/L.

Pesticides

Human Risk Assessment

Pesticide residues in food (EFSA)

- The TTC concept is the most appropriate tool for evaluating the toxicological relevance of pesticide metabolites.
- Ad hoc acute TTC values of 0.3 µg/kg bw/d for substances with a neurotoxicity alert and 5 µg/kg bw/d for substances allocated in Cramer class II and III were derived.
- Where exposure to a metabolite exceeds the respective TTC value, acute and chronic toxicity testing strategies are proposed.

EFSA 2012

Pesticides

Environmental Risk Assessment

EU requirements (PPP Regulation 1107/2009)

- Required for active substance and metabolites, breakdown and reaction products, where they are of toxicological or environmental significance
- PEC values calculated
  - for soil, surface water, sediment, ground water and air
  - for all residues (metabolites, breakdown and reaction products) which account for >10% of the amount of active substance added or for >5% of the amount of active substance added in at least two sequential measurements
- These PECs are compared with the endpoints derived from data from ecotoxicological study data

Pesticides

Environmental Risk Assessment

Residues in groundwater - Groundwater Directive (2006/118/EC)

- Active substances in pesticides, including their relevant metabolites*, degradation products and reaction products, must not exceed 0.1 µg/L (0.5 µg/L for the sum of all of these detected in groundwater).
- A metabolite is ‘relevant’ if it has ≥50% of the biological activity of the active substance against the target organism) or if it has certain toxicological properties that are considered severe and unacceptable.* If a metabolite has any of these characteristics it is treated like the parent active substance.
- If the environmental fate assessment predicts contamination of groundwater by any metabolite at > 0.1 µg/l, it must be further assessed by (i) biological activity screening, (ii) genotoxicity hazard screening, and (iii) toxicity hazard screening. Any metabolite that does not pass all three assessment stages is ‘relevant’ and thus unacceptable at groundwater contamination levels > 0.1 µg/l.

(SANCO 2003, UK CRD 2010)

* Consultation on the revision of Annexes I and II of the Groundwater Directive (2006/118/EC) (30-Jul-2013 to 22-Oct-2013): "It would be useful to clarify the definition of “relevant metabolite”, but it was recognized that "relevant metabolite" might have different meanings in the context of the different pieces of European legislation and the scientific community; therefore this issue cannot be tackled in this review only."…

Biocides

Human Risk Assessment

Biocides Regulation (EU) No 528-2012

- The biocidal product cannot be authorized if it contains any substance of concern or relevant metabolites or breakdown or reaction products which are PBT or vPvB, or if it has endocrine-disrupting properties, unless it is scientifically demonstrated that under relevant field conditions there is no unacceptable effect.
  'Substance of concern' is any substance, other than the active substance, which has an inherent capacity to cause an adverse effect, immediately or in the more distant future, on humans, in particular vulnerable groups, animals or the environment and is present or is produced in a biocidal product in sufficient concentration to present risks of such an effect.
- It is important to establish whether the metabolites found in food (from animals or plants) are the same as those tested in toxicity studies. Otherwise values for risk assessment (e.g. ADI) are not valid for the residues found.
- For biocides active against plants including algae, tests are needed to assess toxicity of any metabolites from treated plants, if these differ from those identified in animals.
- Must comply with the Drinking Water Directive (98/83/EC)

Biocides

Human Risk Assessment

Biocides Regulation (EU) No 528-2012

- Exposure assessments required for active substance, relevant metabolites and degradation products for
  - professional users
  - non-professional users
  - humans exposed directly or indirectly via the environment
- Hazard assessed include acute toxicity, irritancy, corrosivity, sensitisation, repeated dose toxicity, mutagenicity, carcinogenicity, neurotoxicity, immunotoxicity, reproductive toxicity, disruption of the endocrine system, other effects due to physico-chemical properties and any other adverse properties of the active substance or substance of concern, or of their relevant metabolites or degradation products
- Typically, the margin of exposure (MOE ref) - the ratio between the dose descriptor and the exposure concentration - is in the region of 100, but a MOE ref that is higher or lower than this may also be appropriate depending on, among other things, the nature of the critical effects and the sensitivity of the population.

Biocides

Environmental Risk Assessment

Biocides Regulation (EU) No 528-2012

- Risk assessments required for all compartments:
  air, soil and water (aquatic, marine and/or estuarine, including sediment)
- Identify adverse effects of active substance and any substances of concern. Calculate PNEC by applying an assessment factor to the hazard reference values (LD50, LC50, IC50, NOEC, etc).
- Calculate PECs for all compartments for active substance and all "major metabolites", defined as ≥10% of active substance at any time in the relevant degradation studies, or ≥5% at two consecutive sampling points, or ≥5% and increasing at the final time point, and for "relevant metabolites", defined as any minor or major metabolite which e.g. poses a comparable or higher hazard than the active substance
- PEC/PNEC ≤1: no further information and/or testing is necessary.
  PEC/PNEC >1: further testing, risk reduction measures, or product withdrawal
- The biocidal product cannot be authorized if it contains any substance of concern or relevant metabolites or breakdown or reaction products which are PBT or vPvB, or if it has endocrine-disrupting properties, unless it is scientifically demonstrated that under relevant field conditions there is no unacceptable effect.

Human Risk Assessment

REACH (Regulation 1907/2006)

- The human health hazard assessment shall consider the toxicokinetic profile (i.e. absorption, metabolism, distribution and elimination) of the substance
- The toxicity of metabolites formed within the duration of laboratory tests will be reflected by their parent compound, with the exception of delayed effects which are only evident after the observation time of the tests.

(ECHA 2012)

Chemicals

Environmental Risk Assessment

REACH (Regulation 1907/2006)

- Sediment organisms: The need to conduct testing may be triggered by... information on degradation of the parent compound in the water column showing formation of relevant metabolites.
  [NB "relevant metabolites" not defined]
- Aquatic toxicity; long-term toxicity to sediment organisms information on possible (fast) primary degradation would lead to inclusion of metabolites in hazard assessment of the parent compound.

Cosmetics

Cosmetic Product Regulation (EC) No 1223/2009

- No reference to metabolites for either human or environmental risk assessment; refers to REACH (Regulation 1907/2006).

Summary by substance class

Human pharmaceuticals

- Metabolites are exhaustively identified and quantified in animal toxicity tests and human clinical trials.
- Any human metabolites not present in adequate amounts in animal toxicity tests are subject to further testing.
- For environmental RA, exposure is based on amount of product used (sold), and metabolites are considered to be parent active substance.

Veterinary pharmaceuticals

- Metabolites are exhaustively identified and quantified in animals toxicity tests, and major metabolites in edible tissues of target species.
- Any edible tissue metabolites not present in adequate amounts in animal toxicity tests are subject to further testing.
- Environmental RA assumes that 100% of the dose is excreted as parent by target species.

Summary by substance class

Pesticides

- Metabolites are exhaustively identified and quantified in animal toxicity tests and for residues in or on treated plants, livestock, soil, air, ground water, drinking water and processed treated products.
- Any residue metabolites not present in adequate amounts in animal toxicity tests are subject to further testing.
- Exposure assessment required for professional and non-professional users and humans exposed directly or indirectly through the environment or work.
- For metabolites in food, ad hoc acute TTC values are used (0.3-5 µg/kg bw/d).
- For metabolites in drinking water, concentrations must be ≤0.1 µg/L.
- Environmental RA is required for all residue metabolites which account for >10% of the amount of active substance

Summary by substance class

Biocides

- Metabolites should be identified via in vitro/in vivo metabolic studies.
- "Relevant metabolites" (activity > active substance) are subject to further testing.
- Exposure assessment is required for professional and non-professional users and humans exposed directly or indirectly via the environment.
- Environmental RA is required for all metabolites which account for >10% of the amount of active substance and relevant metabolites with higher hazard than active substance (how detected?)

Summary by substance class

Chemicals

- The human health hazard assessment shall consider …metabolism. Metabolites may be identified in metabolic studies.
- The toxicity of metabolites formed within the duration of laboratory tests will be reflected by their parent compound, with the exception of delayed effects which are only evident after the observation time of the tests. [?...]
- Presence of "relevant metabolites" identified by degradation of the parent compound in the water column (otherwise not defined) may require testing is sediment organisms

Cosmetic Product Regulation (EC) No 1223/2009

- No reference to metabolites for either human or environmental risk assessment; refers to REACH (Regulation 1907/2006).

Conclusions - metabolite risk assessment (EU)

Human pharmaceuticals

- Human metabolites must be adequately tested in animals, but are effectively disregarded in environmental risk assessment.

Veterinary pharmaceuticals

- Edible product metabolites must be adequately tested in animals, but are effectively disregarded in environmental risk assessment.

Pesticides

- Comprehensive metabolite risk assessment for both humans and environment.

Biocides

- Inadequate definition of "relevant metabolites" in both human and environmental risk assessment.

Chemicals

- No clear strategy or coherent guidance on metabolites.

Cosmetics

- No guidance on metabolites.

Metabolites in risk assessment