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Topic 2.9

Environmental fate and metabolism: Issues and recommendations*

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Abstract: This main topic of the project and symposium includes the issue of releases of endocrine active substances (EASs) and their monitoring in the environment, food, and feed in order to provide the full set of criteria relevant for exposure assessment. Much less research has been devoted to these areas as compared to investigations on the effects. Issues of special importance regarding exposure to EASs, both from a research and risk management point of view, predominantly result from the fact that high-potency natural products are released as well as anthropogenic substances.

In order to provide reliable information for risk assessment and management, substantial research, methodological improvements, and improvements in data interpretation are needed regarding the following: releases and technologies for their mitigation; monitoring; establishment of background levels; transport, partitioning, persistence, degradation, and metabolism of EASs; dealing with "joint toxicity"; and providing reliable analytical methodology conforming to the principles of quality assurance.

Phytohormones play a special role in this whole area since they may be used as food amendments.

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BACKGROUND AND OBJECTIVES

Within risk assessment, exposure assessment is of equal importance as effects assessment. Within exposure assessment—the broader field of this article—a multitude of factors and criteria including the release of EASs into the environment, their presence in the aquatic and terrestrial environment, in food, or other consumer products, as well as issues on environmental fate and metabolism, are of crucial importance.

The challenge posed to science by the "appearance" of chemicals causing endocrine disruption in the environment has primarily led to basic research on the biochemical and biological processes explaining the effects observed. These areas are dealt with in the other topics of the current project. The studies of the fate and metabolism of endocrine substances have, however, not been as intensive. One reason is that the issue does not differ specifically for EASs as compared to other chemicals. In other words, the existing body of theory regarding the fate and metabolism of environmental chemicals is applicable for EASs. However, a number of issues are of particular importance with regard to EASs, and these will be emphasized in the current section.

Fate and metabolism depend on intrinsic substance properties, including physicochemical characteristics, but also on interacting environmental and technological (food processing) variables. In general, the major criteria can be classified into four areas:

1. Releases: industry sectors including food; agriculture; food supplements and drugs; humans and livestock; and wildlife
2. Transport and partitioning phenomena, which include mobility; bio- and geoaccumulation; bioavailability; and long-range transport in atmosphere and surface waters
3. Persistence in soil, water, and air, and either resistance to biotic or abiotic degradation
4. Metabolic pathways and metabolic patterns and their species variation. These may result in detoxification (inactivation), but also activations or reactivations. Information on metabolic pathways and metabolic patterns is the basis for the analysis of the hazard of metabolites, which may have the same or a different mode of action and potency as compared to the parent compound. Hazards of metabolites are relevant for the organisms in which they are formed, but also for other organisms, which are exposed to them.

For the purposes of risk assessment, the approaches to exposure assessment comprise a tiered system, which is governed by relevance. For generic assessments, compound properties and representative environmental variables play the major role, whereas for real situations (e.g., local or regional), exposure analyses, site-specific or regionally representative environmental variables have to be included, and especially monitoring of concentrations of the EASs in addition to the fate information plays an important role. Attempts to link these approaches have recently led to significant advances in this area resulting in stochastic and specific assessments (e.g., sensitive groups of populations) which, however, are not yet fully implemented. In all areas, especially for EASs, there is usually greater uncertainty in exposure assessment, compared with hazard characterization.

ISSUES DEALT WITH

Following the criteria and objectives on releases and the initial environmental behavior, the state of knowledge with identification of data gaps and further information needs has been discussed for the relevant groups of EASs—natural steroid hormones, phytohormones, pharmaceuticals and personal care products, industrial chemicals, and pesticides. Degradation and accumulation of EASs in the environment, metabolism in mammals, in aquatic and terrestrial systems have been addressed specifically in addition. Since integration of data and their wider use proceeds upon exposure modeling usually, special attention was given to the critical factors involved. It was not the objective of this topic to give an overview on available and missing information on the hundreds of substances, which upon hazard char-

acterization show endocrine effects, but to focus on those with high effects potential and/or having high exposure potential.

RECOMMENDATIONS RELATED TO RISK MANAGEMENT

Monitoring programs

Whereas reliable and valid information on the fate and metabolism of substances in the environment and humans is a prerequisite to perform the generic evaluation, it is also essential for the design of monitoring programs (e.g., selection of substances, sites/locations of sampling, frequency). Especially regarding the endocrine disruptor issue, the usual evaluation of monitoring programs by averaging is not optimal, but should be targeted on population groups and for the differentiation of regions. Therefore, it is essential to extend monitoring to developing countries. In this context, it should be mentioned that a further instrument, which allows for retrospective exposure assessment, is to use appropriately stored environmental and human samples (specimen cryobanking). This instrument is a valuable tool also for EASs, since it allows for the assessment of time trends, whenever a newly identified potentially high-risk substance requires assessment.

High-priority EASs, for which the likelihood of a risk for humans or the environment has been sufficiently substantiated on a scientific basis, should be included in monitoring programs. This also includes metabolites of released chemicals for which this risk has been established.

Existing monitoring programs should be adopted to the requirements of models used in exposure assessment in order to better combine the two approaches in exposure assessment and to achieve comprehensive risk assessment.

Release mitigation

Technologies are available to remove EASs from effluents and solid waste for further use. These include buffer strips, biological treatment, composting, etc. These should be used as far as possible.

Assessment concept

It seems appropriate to assess anthropogenic EASs vs. natural hormones and phytohormones. Therefore, it would be advisable to establish background levels for environment and food regarding these as reference. For the human risk assessment, these reference substances could be 17ß-estradiol and other steroid hormones. The comparison should preferably be done using "toxicity" equivalents.

Responsible use of data

The relation of exposure to effects data regarding EASs is an issue requiring special scientific responsibility, although there is a vast amount of information from in vitro studies and receptor-related investigations. These effects data cannot be directly related to environmental occurrence, external exposure, or food concentration data. Distribution and metabolism in the organisms of concern qualitatively and quantitatively change the exposure of the target. Consequently, external exposure data should be assessed reliably, only vs. corresponding in vivo effect information. This applies also for in vivo testing where still frequently nominal concentrations are used and which give rise to substantial errors

Phytohormones

In the light of the whole EAS issue, a new risk/benefit assessment of fortified food should consider other exposure routes to these EASs. This is of high priority as the uses are increasing.

Sources of endocrine potency in food and environment: TIE concept and joint toxicity

Within the issue of endocrine active substances, TIE studies with chemical analyses and bioprobe analyses have been done on a number of samples. Frequently, there is a substantial fraction of the endocrine potential, not covered by the results of chemical analyses. In sewage effluents, however, frequently 90 % of the estrogenic activity can be explained by natural hormones and 17α-ethinylestradiol from contraceptives. Nevertheless, a completion of the chemical analyses (including natural hormones) in an in vivo TIE approach will help to set priorities for management.

Considering that, apart from the different endpoints, which need to be assessed, there remains the issue of interactions (additivity, antagonism, etc.) between the EASs to which organisms are exposed simultaneously. Although it is well understood, that substantial research is still needed regarding "mixture toxicity" also with respect to exposure, a valid concept for *joint toxicity* should now be developed and become the basis for management decisions soon.

RESEARCH PRIORITIES TO IMPROVE EXPOSURE ASSESSMENT

Identification of high-potency EASs

Realizing that EASs may be formed by metabolic hydroxylation of nonendocrine active substances (e.g., from some isoflavones), and that these might become an exposure issue for a wider range of organisms through environmental occurrence, substantial research is requested to systematically investigate this type of metabolic activation. This issue includes also endocrine activity, which is not directly hormone receptor-related.

Groups of chemicals that need systematic screening for endocrine activity and risks are the anabolic agents and nonhormonal steroids used in medical treatment. For these substances, the metabolites excreted in urine and/or feces should be identified and assessed for their environmental presence along with the parent compound. An important question in this context also is the reactivation of conjugated metabolites in the environment.

Modeling exposure

In order to expand the potential of exposure modeling, high-quality parameter data and representative field data are required. Due to the specificity of possible effects within the range of EASs, a large variety of properties needs to be investigated. Especially for the compounds subject to result in endocrine active metabolites, lack of respective data is at present one of the main obstacles for the application of generic exposure models to these compounds.

As EASs may affect different life stages, there is a need to develop the information required for life stage-specific evaluations by exposure modeling.

Phytohormones

Apart from research required to investigate potential environmental risks, especially to aquatic organisms, by increased releases of phytohormones (e.g., from processing fortified food), data to develop environmental and food PNECs should be elaborated.

There are additional specific questions to be addressed in research and monitoring programs, one important issue is the in utero exposure of the fetus to phytoestrogens depending on maternal diet.

Mineralization of EASs and metabolic pathways

Considering that ED active metabolites may present an increasing problem, it would be helpful to also investigate the mineralization (total degradation) of precursors to identify EAS persistence.

Furthermore, it is suggested to establish full metabolic pathways of those chemicals that from structure–activity predictions might result in endocrine active metabolites.

To improve human safety, it has been suggested to perform metabolism studies on EASs in human volunteers with very low doses and using AMS/HPLC analytical methodology. Ethical issues in those cases need careful risk benefit evaluation. This would provide an explanation also on the occasionally reported inverted U-shape dose–response curve, which might be due to a minor metabolite, only formed in a definite concentration range.

Analytical methods

Chemical analytical methods have been developed providing adequate detection limits and precision for the analysis of the most important groups of EASs in food and the environment at the requested levels—that is, the levels of biological significance. Research to make these methods less complex and more robust includes:

- provision of more specific and sensitive biomarkers,
- better integration for diverse classes of EASs,
- more automated and selective clean-up procedures,
- increased use of LC/MS and LC/MS/MS,
- increased availability of sensitive and stable ELISA tests, and
- greater conformity to the principles of quality assurance.

SUMMARY OF RECOMMENDATIONS

Risk management

- Environmental monitoring programs should, on a global scale,
  - be focused on high priority EASs,
  - include relevant metabolites, and
  - be designed to support exposure assessment.
- Quantitative correlations for chemical analyses and bioassays (TIE) should be used to reevaluate the biological relevance of target EASs for monitoring programs.
- In addition to source control, available technologies for reducing environmental entry should also be considered.

Research priorities

- Increased reliability of detection methods
  - development of robust and economical endocrine receptor bioassays
  - greater conformity to quality-assurance principles
- Elucidation of metabolic pathways, including potential activation vs. detoxification
- Key environmental fate parameters should be generated for highly active EASs (e.g., steroid hormones, industrial chemicals, and drugs).
- Improved models for exposure assessment
  - accounting for variability over time and space
  - including less well-characterized exposure scenarios and all potential exposure pathways (e.g., wastewater treatment plant, farm animal effluent)
- Development and validation of more efficient processes for reducing environmental loading.