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Health risk assessment procedures for endocrine disrupting compounds within different regulatory frameworks in the European Union

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ABSTRACT

In this study we have investigated how different regulatory frameworks in Europe cope with identification and risk assessment of endocrine disrupting compounds (EDCs). Four regulatory groups were selected for the investigation: existing industrial chemicals, environmental pollutants in food, pharmaceuticals and plant protection products. The legislation and guidelines for each of these groups were scrutinized and compared in detail. In addition, one recent European risk assessment document each for three identified EDCs, *i.e.* bisphenol A, dioxins and vinclozolin, were reviewed and compared. We found that the requirements for toxicity testing and availability and scope of risk assessment guidelines varied between the four regulatory frameworks. Also, the general principles regarding the human relevance of the mode of action identified in animal tests differed in the different risk assessments. In conclusion, there is little conformity in the risk assessment processes between these groups of chemicals. Because of the complicated nature of endocrine disruption, test methods, principles and criteria for data interpretation traditionally used might not be directly applicable to EDCs and further development of a transparent and reliable risk assessment process for this type of substances is needed.

1. Introduction

Concern for endocrine disrupting compounds (EDCs) in the environment is rising and there is a focus on improving the identification and assessment of the environmental and health risks posed by these compounds, which has increased during the past decade. However, risk assessment of EDCs has proven especially complicated due to many different factors, such as the complex nature of effects caused by compounds interacting with the endocrine system and potential delayed on-set of effects, as well as suggested non-monotonic dose–response relationships, potential lack of a threshold for effect and effects at very low doses (*e.g.* IPCS, 2002; NTP, 2001). Importantly, knowledge is lacking regarding mechanism of action for EDCs as well as the relationship between these molecular events, *i.e.* interactions with hormone receptors, and adverse health effects. There is also a lack of sensitive test methods and standardized test guidelines to identify and evaluate the toxicity of EDCs.

Within the European Union (EU), chemicals are risk assessed and regulated within different regulatory frameworks depending on their intended *use*; there are *e.g.* separate rules for industrial chemicals, plant protection products and pharmaceuticals. However, there are currently no generally agreed upon regulatory procedures that direct how to specifically identify or risk assess compounds with endocrine disrupting properties within the EU or internationally. Consideration of toxicological mode of action, other than genotoxicity, is generally not well established in regulatory risk assessment.

The purpose of this study was to investigate the lack of regulatory coordination for EDCs by comparing the risk assessment processes within legislative frameworks for different regulatory groups of chemicals. This analysis was conducted in two parts. In the first part four different EU legislative frameworks, for existing industrial chemicals, environmental pollutants in food, existing active substances in plant protection products and pharmaceuticals, were scrutinized and compared. In the second part of the analysis risk assessment documents for bisphenol A (BPA), dioxins and vinclozolin were critically reviewed. These model compounds represent existing industrial chemicals, environmental pollutants in food, existing active substances in plant protection products, respectively. The aim of the analysis was especially to investigate the following general issues:

- the scope and requirements of the different regulatory frameworks, including criteria for data selection,

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- the scope of available guidelines within the regulatory frameworks investigated concerning how effects assessment is carried out,
- the toxicological data on which the risk assessments for the three model compounds were primarily based,
- if different conclusions regarding the critical effect, threshold and endocrine disrupting properties would have been expected had only data specifically required by regulation been available for each model compound,
- what general toxicological principles and assumptions were applied in determining the human relevance of the mode of action identified in animal studies for each model compound.

2. Methods

We have investigated how different regulatory frameworks in Europe cope with the task of identifying and risk assessing chemicals with EDC properties. The analysis was divided into two parts. In the first part the regulations for existing industrial chemicals, environmental pollutants in food, existing active substances in plant protection products as well as for pharmaceuticals were described and compared in terms of their scope and purpose, toxicity testing requirements and risk assessment guidelines. We also investigated how much expert judgement, as opposed to strictly defined criteria, was allowed to influence the risk assessment process in each case, *i.e.* the extent of case-by-case flexibility in the risk assessment processes for each chemical group.

The EU legislation for existing (and new) industrial chemicals was replaced by the REACH legislation on June 1, 2007. REACH stands for Registration, Evaluation, Authorization and restriction of CHemicals. Since the evaluations in this study were performed in retrospect, scrutinizing risk assessments already made, it is not possible to include an industrial chemical risk assessed within the REACH system at this point. To evaluate the actual outcome of the new legislation is important, but can only be made some years after its implementation.

By definition an EDC is "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations" (IPCS, 2002). For the second part of the analysis three model compounds were thus selected accordingly (Table 1). BPA, dioxins and vinclozolin are generally regarded as EDCs and represent three different chemical categories, namely existing industrial chemicals, environmental pollutants in food and existing active substances in plant protection products. No pharmaceutical model compound was included in this part of the analysis as it was not possible to get access to an extensive risk assessment document for an appropriate EDC from this category.

In this part of the analysis actual risk assessment documents for each model compound were investigated and compared in terms of the overall conclusions regarding risk and the principles and assumptions that underpin these conclusions, such as the human relevance of the identified mode of action and the dose–response relationships. The analyses carried out in this study were limited to EU chemicals legislation. It should be noted that other rules or agreements might also be applicable to these chemicals, including international conventions and national legislations.

This project was conducted as a retrospective literature study in the sense that one original, European risk assessment document for each model compound was reviewed. Any additional information and/or debate regarding these substances influencing the scientific opinion or later risk management strategies were not included. The reason is that the purpose of this project was to gain knowledge of European risk assessment procedures in general.

2.1. Search for information on regulatory frameworks

2.1.1. Legislative documents

EU regulations and directives regulating the risk assessment process for the four chemical groups investigated were identified by searches on Eur-Lex (http://eur-lex.europa.eu/en/index.htm), which is a web-based portal to EU law.

2.1.2. Guidance documents

Internet searches for risk assessment guidelines for each of the four chemical groups investigated were conducted. Guidelines issued to aid the risk assessor and specifically outlining the requirements for the risk assessment process according to the identified EU directives and regulations were primarily explored. Guidelines for the risk assessment process for existing industrial chemicals, pharmaceuticals and existing active substances in plant protection products were obtained from the European Chemicals Bureau (ECB), the European Medicines Agency (EMEA) and the Directorate General for Health and Consumer Protection (DG SANCO) websites, respectively. No guidance document for the risk assessment of environmental pollutants in food is available.

2.2. Description of the model compounds

2.2.1. BPA

BPA is a high production-volume chemical used mainly as an intermediate in the manufacture of polycarbonate and epoxy resins. Consumer exposure occurs primarily via food in contact with BPA-containing materials, such as polycarbonate plastic baby bottles and table ware, plastic food containers and food and beverage cans lined with epoxy resins (EFSA, 2006). BPA is an estrogen agonist that binds to and activates the estrogen receptors (ER) (Matthews et al., 2001). However, the knowledge regarding mechanism of action for BPA is incomplete. Interactions with other receptors, such as the androgen and thyroid hormone receptors as well as membrane-bound receptors, have also been suggested (reviewed in Wetherill et al., 2007). BPA causes adverse effects on reproduction at doses above 5 mg/kg bw/day (*e.g.* Tyl et al., 2002, 2008) and there is an on-going debate regarding whether or not it also may adversely affect development in offspring exposed pre-natally to doses around a few μg/kg bw/day (reviewed in Richter et al., 2007).

2.2.2. Dioxins

Dioxins are a group of planar, polyhalogenated hydrocarbons. Dioxins are not deliberately produced; they are formed as by-products of reactions such as the combustion of organics, in pulp and paper production and in other industrial processes. The dioxins are very resistant to both environmental and biological degradation. Hence they persist in the environment and may enter the food chain and bioaccumulate (SCF, 2000). As a result the main route of

Table 1
An overview of the three model compounds, their mechanism of action for toxicity and main sources of consumer exposure.

	Bisphenol-A	Dioxins	Vinclozolin
Regulatory group	Existing industrial chemical	Environmental pollutant in food	Existing active substance in plant protection products
Mechanism of action	Estrogen receptor agonist	Ah-receptor agonist	Androgen receptor antagonist
Main source of human exposure	Food in contact with plastic products	Food of animal origin	Residues in vegetables and fruit

human exposure to dioxins is via food. About 90% of human exposure comes from foods of animal origin (SCF, 2000). The dioxins bind to and activate the cellular arylhydrocarbon-receptor (Ah-R), which initiates the transcription of a number of genes and consequent cellular responses, such as the induction of cytochrome P450 enzymes. Dioxins have several endocrine disrupting properties and the best characterized to date are their potential to act as anti-estrogens and their potential to interfere with thyroid hormone and retinoid systems. The activation of the Ah-R has been shown to result in a wide array of adverse effects in experimental animals, for example effects on development of offspring (Faqi et al., 1998; Gray et al., 1997; Mably et al., 1992; Ohsako et al., 2001). However, the individual compounds in this group of chemicals differ greatly in their ability to activate the Ah-R and hence in their endocrine disrupting potency.

2.2.3. Vinclozolin

Vinclozolin has been used extensively as a fungicide on crops such as grapes, berries, stone fruits and lettuce (EC, 1997) but is prohibited in the EU since 2006. Vinclozolin is readily degraded in the environment and its two main metabolites are potent androgen antagonists (EC, 1997; Kelce et al., 1994). When administered to rats vinclozolin is quickly metabolized into these two anti-androgenic metabolites (Kelce et al., 1994). Vinclozolin may disturb the function and development of tissues that are sensitive to testosterone and causes adverse effects on fertility and development in experimental animals (Colbert et al., 2005; Veeramachaneni et al., 2006).

2.3. Identification of risk assessment reports

One recent European risk assessment report, conducted according to EU regulations and directives, for each of the three model compounds was selected. The risk assessment of BPA (ECB, 2003) was attained from the ECB website, the assessment of dioxins (SCF, 2000, 2001) was available from the website of the DG SANCO and the vinclozolin documentation (EC, 1997,1998a) was provided by the Swedish Chemicals Agency (KemI).

2.4. The database

In order to facilitate comparisons across the different regulatory frameworks or model compounds detailed information was entered into a database. The database was constructed as a table in Microsoft Word to enable systematic comparisons of different aspects of the investigated legislation, as well as the risk assessment documents for the three model substances. The first column lists all the parameters to be compared and the subsequent columns represent each regulatory framework or model compound. Each parameter/inquiry was recorded for each regulatory framework or model compound and entered into the database.

2.5. Evaluations and comparisons of the selected regulatory frameworks

2.5.1. Evaluation of the scopes of the different legislative frameworks and toxicity data requirements

The legislative documents were scrutinized, key aspects were entered into the database, and the following parameters regarding the different requirements for the four legislative frameworks were compared:

- the division of responsibilities between different European and national authorities regarding the risk assessment process,
- the data requirements for effects assessment.

2.5.2. Evaluation of the availability and scopes of guidelines

The available guidance documents were reviewed and key aspects were entered into the database. The following parameters were investigated and compared for the four chemical groups:

- what authority has issued the guidelines,
- what directive and/or regulation it is referring to,
- who the guidance is intended for,
- if guidelines for data selection are included and
- how much of the effects assessment should be done according to pre-defined criteria, and in what instances "expert judgement" is called for, *i.e.* criteria determined on a case-by-case basis.

2.6. Evaluations and comparisons of the risk assessment reports for the model compounds

2.6.1. Evaluation of the effects assessments for the three model compounds

The effects assessment from each risk assessment document was carefully reviewed. The risk assessment conclusions for each effect evaluated were entered into the database. The comparison was made to clarify:

- what effects are assessed in each case and what conclusions have been drawn about these,
- what critical effect was identified,
- what no observed adverse effect level (NOAEL), if any, was established.

2.6.2. Investigation of how data pivotal for effects assessment in each case corresponded to regulatory data requirements

The pivotal data on which conclusions regarding critical effect, threshold and endocrine disrupting properties were based for each model compound were compared to the regulatory data requirements in each case. This analysis was made to investigate whether conclusions for the effects assessments could have been expected to be different had they been based exclusively on required data.

2.6.3. Identification of toxicological assumptions and principles used to evaluate the human relevance of animal data in effects assessment

Certain assumptions and principles have to be applied when evaluating effects data and deciding on a point of departure (POD), *i.e.* the dose level from which health-based guidance values or margins of safety (MOS) are derived, such as the NOAEL. These assumptions and principles are, for example, that the mode of action and effects observed in animals are relevant for humans, that conclusions regarding toxicity at the relatively low doses to which humans are typically exposed can be made based on effects observed at the high doses used in toxicity testing, and that the dose has to reach a certain threshold before adverse effects occur. Such assumptions and principles were identified from each risk assessment document and compared for the three compounds.

3. Results

3.1. Scopes of the regulatory frameworks

The scopes and main components of the four different regulatory frameworks are summarized in Table 2.

3.1.1. Priority existing industrial substances

The industrial substances are as of 1st of June 2007 regulated within the new European legislation, REACH. REACH replaces the

previous rules for existing industrial chemicals (Council Regulation No. 793/93/EEC, Commission Regulation No. 1488/94/EC).[2] Still, for reasons explained above, the REACH system is not included in this evaluation.

The industrial chemicals were divided into "new" and "existing" substances. Existing substances were those which were marketed in Europe in September 1981, when the European Inventory of Existing Chemical Substances (EINECS) was closed. The new substances were those introduced on the market after this time, and thus not included in EINECS.

There are about 100,000 substances regulated as existing industrial chemicals and 141 of these were prioritized for risk assessment before the legislation ceased to be in force. The ECB, part of the European Commission Joint Research Centre, held the responsibility for risk assessment of the industrial substances. Risk assessment was carried out by competent authorities in one of the member states (the *rapporteur*), as appointed by the European Commission, in order to evaluate if estimated exposure levels were acceptable in light of existing hazard data, or if restrictions of manufacture and/or use were needed. Ultimately any decisions to invoke restrictions of use or manufacture based on the information in the risk assessment lay with the European Commission.

3.1.2. Environmental pollutants in food

Environmental pollutants in food have *no intended use* and *no manufacturer* and are therefore, not covered by the European chemicals legislation. However, when such a chemical has been identified as a contaminant in the food chain, other regulations will become applicable. Before 2002 risk assessment of environmental pollutants in food was carried out by the Scientific Committee on Food (SCF), under the responsibility of DG SANCO. EU Regulation 178/2002 establishes the European Food Safety Authority (EFSA), which, since 2002, has taken over this responsibility. Risk assessment of these compounds is now carried out by EFSA expert groups on a case-by-case basis. The aim is the same as before 2002, namely to estimate consumer exposure, evaluate effects and to derive guidance values, such as acceptable or tolerable daily intakes (ADI/TDI).

3.1.3. Active substances in pharmaceuticals

For pharmaceuticals the risk assessment is referred to as a *safety* assessment. The safety assessment of human pharmaceuticals intended for the EU market is regulated by Directive 2001/83/EC,[3] Commission Directive 2003/63/EC, Directive 2004/27/EC and Regulation 726/2004/EC.

The toxicological safety assessment, which makes up the preclinical evaluation, of the active substances in a pharmaceutical product is carried out by experts commissioned by the manufacturer in conjunction with the marketing authorization process for that product. Since 2004 the central authority responsible for marketing authorizations of pharmaceutical products in the EU is the European Medicines Agency (EMEA).

3.1.4. Existing active substances in plant protection products

A plant protection product is an active substance or preparation containing one or more active substances used to protect plants or plant products from animals, other plants or microorganisms (Directive 91/414/EEC). The risk assessment of plant protection products is currently regulated by Council Directive 67/548/EEC, Council Directive 91/414/EEC, Commission Directive 93/71/EEC, Commission Directive 94/79/EC, Council Directive 97/57/EC and Commission Regulation 3600/92/EEC. A new regulation concerning the placing of plant protection products on the market has been proposed and will replace Directive 91/414, most probably during 2009.

The active substances in plant protection products are divided into "new" and "existing" substances according to Directive 91/414. Existing substances are those that were already in use on the European market in 1993 when Directive 91/414 was implemented, and new substances are those introduced after 1993.

Evaluation of the existing active substances is made in order to decide whether their use should be allowed to continue in the EU. Approved active substances are added to Annex I of the Directive 91/414. Altogether there are 984 existing substances to be evaluated. In accordance with Regulation 3600/92 the substances to be evaluated have been prioritized based on health and/or environmental concern, the presence of residues in food, data gaps and the agricultural/economic importance of the product. Risk assessment of the existing active substances is carried out by the competent authorities in the EU Member States. Each Member State has been designated as the rapporteur for certain substances according to Regulation 3600/92. Before 2003 DG SANCO held the responsibility for the risk assessment of active substances in plant protection products. EFSA took over this responsibility in 2003. Ultimately, the European Commission makes the final decisions on the inclusion of substances to Annex I.

3.1.5. Comparison

Table 2 summarizes the scopes and main components of the four different regulatory frameworks. The purpose of risk assessment is different in the four cases: to provide a scientific basis for determining whether or not estimated exposure levels can be accepted in light of effects data, to establish guidance values, such as an ADI or TDI, to prove that the product is safe for human use, or safe to be included in plant protection products used in the EU.

In all the investigated cases, except for pharmaceuticals, the risk assessment is reactive rather than proactive, *i.e.* a decision that a risk assessment has to be made is taken after the chemical has become commercially available, as in the case of existing industrial chemicals and active substances in plant protection products[4], or concerns regarding adverse health effects have been raised, as with environmental pollutants. Pharmaceuticals, on the other hand are assessed as part of the process to approve them for marketing and use. In the case of existing industrial substances and active substances in plant protection products risk assessment is carried out by a body independent of the manufacturer of the substance, while for pharmaceuticals the toxicological safety assessment is carried out by an expert commissioned by the manufacturer themselves.

Final decisions on any restrictions on the use of existing industrial chemicals and existing active substances in plant protection products lie with the European Commission. The EMEA makes the decision to approve pharmaceuticals for the European market. In the case of environmental pollutants in food there is no intended use or manufacturer on which any restrictions could be imposed. However, up until 2002 DG SANCO held the responsibility to propose guidance values, such as TDI, for these substances as contaminants in food and feed. After 2002 this responsibility belongs to EFSA. The EU Commission may in some cases restrict exposure to environmental pollutants via food by the setting of limit values.

[2] In addition, REACH also replaces the rules for *new* industrial chemicals which are not discussed here.

[3] European legislation of medicinal products reaches back to 1965 and has been frequently and substantially amended in different directives. Directive 2001/83/EC is the result of compiling these into one single text.

[4] Note that for *new* industrial chemicals and substances in plant protection products risk assessment is carried out before the chemical is approved for use on the European market.

Table 2
The regulatory frameworks and division of responsibilities between different European authorities and member states for four categories of compounds.

	Priority existing industrial substances	Environmental pollutants in food	Active substances in pharmaceuticals	Existing active substances in Plant protection products
Legislation regulating risk assessment	Dir 67/548 Reg 793/93 Reg 1488/94	*None*	Dir 2001/83 Dir 2003/63 Dir 2004/27 Reg 726/2004	Dir 67/548 Dir 91/414 as amended by Dir 94/79 and Dir 97/57 Reg 3600/92
Purpose of the risk assessment	Scientific basis for health risk management	Derivation of a European TDI	Scientific basis for marketing authorization	Scientific basis for the authorization for use
Authority/body responsible for data collection	Manufacturer AND the rapporteur	EFSA Work group[a]	Manufacturer	Manufacturer AND the rapporteur
Main source of data	Industry and open literature	Open literature	Industry	Industry and open literature
Risk assessment report performed by	Designated member state (rapporteur)	EFSA: work group[a]	Expert commissioned by the manufacturer	Designated member state (rapporteur)
Authority/body responsible for risk assessment	ECB	EFSA[a]	EMEA	EFSA[b]
Authority responsible for approvals, bans and restrictions	European Commission	*Not applicable*	EMEA	European Commission
Nature of risk assessment	Reactive	Reactive	Proactive	Reactive

[a] Before 2002 the Scientific Committee on Food of DG SANCO was responsible for the data collection and risk assessment of environmental pollutants.
[b] Before 2003 DG SANCO was responsible for the risk assessment of active substances in plant protection products.

3.2. Toxicity data requirements

The toxicity data requirements for risk assessment established in each of the different regulatory frameworks investigated are summarized in Table 3.

3.2.1. Priority existing industrial substances

The data requirements of the 141 *priority*[5] existing substances were specified in Council Directive 67/548. For these chemicals, the manufacturer was required to provide data at least corresponding to the so called "base-set". Base-set data included the following tests: Acute toxicity by at least two routes of exposure, eye- and skin irritation, skin corrosivity, skin sensitization, a 28-day toxicity study, mutagenicity in at least two *in vitro* tests and finally a screening test for reproductive toxicity. The risk assessor should primarily consider studies based on Good Laboratory Practice (GLP) and internationally recognized guidelines, such as the OECD test guidelines. Studies not conducted according to GLP or guidelines were to be evaluated on a case-by-case basis. In addition to data supplied by the manufacturer data available from other sources, such as the open literature should also be reviewed.

According to Regulation 793/93 the manufacturer was also responsible for updating the information for existing substances with regard to any new toxicity data which was likely to be relevant for the evaluation of its potential risk to human health.

3.2.2. Environmental pollutants in food

For the chemicals defined here as environmental industrial pollutants in food *e.g.* contaminants originating from industrial processes, no "manufacturer" can be identified to be held responsible for the production of data and consequently there are no legislated test requirements.

3.2.3. Active substances in pharmaceuticals

The data requirements for active substances in pharmaceuticals are specified in Directive 2003/63 (amending Directive 2001/83), and include information on pharmacokinetics, acute toxicity in at least two mammalian species and via two routes of exposure, local tolerance, a short-term repeated dose test lasting two or four weeks and one long-term test, the duration of which depends on the conditions of clinical use. These tests should be carried out in two mammalian species, one of which has to be a non-rodent. Mutagenicity tests are also required, but requirements will depend on the state of scientific knowledge. Carcinogenicity should be evaluated if considered relevant according to available knowledge including results from initial testing, or if the product is proposed to be used for an extended period of time. All test procedures used by pharmaceutical manufacturers should be carried out according to GLP, as stated in Directive 87/18, and should be validated and correspond to the state of scientific progress. Tests for single-dose and repeat-dose toxicity must follow internationally accepted guidelines, *e.g.* guidelines issued by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) or the Committee for Medicinal Products for Human Use (CHMP) at EMEA.

Impairment of male and female reproductive function as well as adverse effects in the offspring should be investigated using "appropriate tests" (Directive 2003/63). No criteria are stated as to which tests are to be used for the evaluation of reproductive toxicity. Embryo/fetal toxicity testing is required in two mammalian species and perinatal toxicity in one. In addition to those non-clinical data, information on the efficacy and toxicity of the substance is also required from clinical trials on humans.

Directive 2001/83 (Article 10 (a) (ii)) states that if the manufacturer demonstrates that a substance has a so-called "well established use, with recognized efficacy and an acceptable level of safety" then there are no requirements to provide any non-clinical toxicological data for that substance (EMEA, 2005). Whether this is applicable for individual substances is determined on a case-by-case basis.

After the authorization of a pharmaceutical product its safety is constantly monitored by national competent authorities and the EMEA. This "pharmacovigilance" system is in place to collect information from health care professionals on suspected adverse reactions for each product on the market and to continuously re-evaluate their benefits and risks (Directive 2001/83/EC).

3.2.4. Existing active substances in plant protection products

Toxicity data requirements for plant protection products are laid down in Directive 91/414 as amended by Directives 93/71 and 94/79. The manufacturer/applicant is responsible for providing the required toxicity data for the active substances as well as for the product as a whole. Tests should be carried out according to GLP and relevant guidelines.

[5] There were no toxicity data requirements for the almost 100,000 non-prioritized existing industrial substances.

Table 3
Toxicity data required (as stated in legislation) from the manufacturer to be used as basis for risk assessment.

	Priority existing industrial substances	Environmental pollutants in food	Active substances in pharmaceuticals	Existing active substances in Plant Protection Products
Absorption, distribution, excretion and metabolism	Toxicokinetic behaviour to the extent that can be derived from base-set and other available data	*Not required*	Pharmacokinetics	Tests in one mammal (usually rat)
Acute toxicity/single-dose	At least two routes of exposure: orally and by inhalation or percutaneously	*Not required*	In at least two mammalian species and using at least two different routes of administration	Administered orally, percutaneously and via inhalation (if relevant)
Corrosiveness/irritation/ local tolerance	Eye and skin	*Not required*	All observations made in acute tests (as above). In addition local tolerance tests in sites of the body that may come in contact with the substance	Eye and skin
Skin sensitization	Required but no further criteria stated in legislation	*Not required*	Required in at least one test system for chemicals applied to the skin	Required but no further criteria stated in legislation
Repeated dose toxicity	28-day study	*Not required*	Short-term + long-term studies in at least two mammals (one non-rodent)	90-day study in rat and dog
Mutagenicity/genotoxicity	One bacterial gene mutation test and one other *in vitro* test capable of detecting chromosomal aberrations[a]	*Not required*	Obligatory for any new substance (case-by-case basis)	*In vitro* mutagenicity test battery[a]
Chronic toxicity/ carcinogenicity	*Not required*	*Not required*	If justified by chemical analogy, mutagenicity or other tox-test results, or if likely to be administered to patient over an extended period of time	One long-term and carcinogenicity study in rat (may be combined) and one carcinogenicity study in mouse
Toxicity to reproduction	Screening test	*Not required*	Embryo/fetal toxicity in two mammals (one non-rodent) and peri-/postnatal toxicity in one mammal Male and female reproductive function as well as adverse effects in the offspring should be evaluated in "appropriate" tests.	2-generation study in rat and teratogenicity in rat and rabbit
Neurotoxicity	*Not required*	*Not required*	*Not required*	Required if the substance shows similarities to organophosphates
Human data	*Not required*	*Not required*	Yes, clinical trials	*Not required*

[a] If test is positive further testing must be carried out.

Tests of absorption, tissue distribution, excretion and metabolic pathways are required, and acute toxicity should be tested at least orally and percutaneously. Furthermore, tests for eye and skin irritation, skin sensitization and *in vitro* mutagenicity, as well as 90-day repeated dose toxicity studies in both rat and dog, a long-term oral toxicity and carcinogenicity (two years) study in rat (may be combined), and one carcinogenicity study in mouse are required. One 2-generation study in rat and teratogenicity studies in rat and rabbit are required to investigate reproductive toxicity. If the substance shows structural similarities to organophosphorus compounds, then its potential to cause delayed neurotoxicity after acute exposure should also be evaluated.

Plant protection products are authorized for up to 10 years only. Authorizations may be renewed if there is no new data indicating that the product, for example, has any harmful effects on human health. Further, authorizations may be withdrawn at any time if new data becomes available which indicate potential harmful effects of the product.

3.2.5. Comparison

Table 3 summarizes the toxicity data requirements for the different regulatory frameworks investigated. There are no legislated data requirements for environmental pollutants in food. Data requirements for prioritized existing industrial substances were limited, while for pharmaceuticals and plant protection products the data requirements are extensive.

While there is a systematic review system in place for prioritized industrial chemicals, pharmaceuticals and plant protection products, there are no such systems in place for environmental pollutants.

Besides the standardized, regulatory required test data, additional information may become available via research published in the open scientific literature. Obviously, such data rarely influence the pre-marketing assessment of pharmaceuticals, while for environmental pollutants, as well as for existing industrial chemicals and existing active substances in plant protection products, such sources can in some cases be an important and even dominating part of the scientific basis for risk assessment.

When toxicity data, which were generated in experiments not performed according to currently accepted guidelines or GLP, are obtained from the open literature the quality of the data needs to be assessed on a case-by-case basis. Data considered to have sufficient quality and relevance can be used for risk assessment and the manufacturer is then not obliged to carry out new tests. Preclinical toxicological testing may also be waived for active substances in pharmaceuticals if the manufacturer can show that the substance has a "well established use, with recognized efficacy and an acceptable level of safety" (EMEA, 2005).

Human data are only required for pharmaceutical substances.

It is important to note that there are no requirements for specifically investigating the endocrine disrupting potential or, consequently, for the identification of EDCs for industrial substance, environmental pollutants or plant protection products. Even though not stated as a particular criterion in the pre-clinical testing of pharmaceuticals it is assumed that the mechanism of action for the therapeutic effect as well as for any toxic side effects must be extensively investigated in the development for new candidate drugs.

3.3. Availability and scope of risk assessment guidelines

Guidance documents for risk assessment are issued to specify the legislation, and the purpose of issuing such guidelines is to further clarify what is required of the risk assessor and to promote predictability in the risk assessment process (ECB, 2003). The availability and scope of risk assessment guidelines for the investigated chemical groups have been summarized in Table 4.

3.3.1. Priority existing industrial substances

An extensive Technical Guidance Document (ECB, 2003) has been issued by the ECB. These guidelines specify predefined criteria for conducting risk assessments of existing and new industrial chemicals and biocides, but the reliance on expert judgement is still an integral part of the risk assessment process (ECB, 2003). For example, if a study was not conducted according to GLP or relevant test guidelines it is up to the risk assessor to judge the quality and relevance of such data on a case-by-case basis, as well as if there is a need to conduct new tests. Also, a no observed adverse effect level (NOAEL) should be determined on which to base the risk characterization. Deriving the NOAEL entails determining if observed effects should be considered adverse or not and this decision is consequently dependent on the expert judgment of the risk assessor. Expert judgement is also an integral part of determining if the margin of safety (MOS), *i.e.* the margin between the NOAEL and the estimated human exposure, is adequate, since this there are no pre-determined criteria for how large the MOS should be.

3.3.2. Environmental pollutants in food

As there is no legislation stating any requirements or criteria for the risk assessment of environmental pollutants there are consequently no guidelines for the risk assessment process available to the risk assessor. As a result the decision on what data to include, how to evaluate the data in terms of quality, what effects to focus on, and what assessment factors to use when calculating TDI is subject to case-by-case expert judgement.

3.3.3. Active substances in pharmaceuticals

Guidelines for the safety assessment of pharmaceuticals are available from the EMEA (EMEA 2003a,b). These guidelines are issued in accordance with ICH in order to harmonize the application process for registration of pharmaceutical products on the European, US and Japanese markets. For the purposes of the European market, these guidelines support the requirements and criteria stated in Directive 2003/63. These guidance documents are intended for the experts commissioned by the manufacturer to conduct the safety assessment. The purpose of these guidelines is to assist the authors in the preparation of the non-clinical and clinical overviews in an "acceptable format" and they cover mainly what should be included in the reports, *e.g.* which effects and aspects of toxicity to be considered. However, the expert is responsible for highlighting the most important findings. Furthermore, these guidelines do not state any criteria about how to evaluate the toxicity data that has been provided by the manufacturer. The ICH and EMEA also provide separate guidance documents for the evaluation and interpretation of certain toxicity data, *e.g.* genotoxicity (EMEA 2008a) and reproductive toxicity (EMEA 2008b).

3.3.4. Existing active substances in plant protection products

Guidelines for carrying out the risk assessment of active substances in plant protection products have been issued by the Directorate General for Agriculture of the European Commission (EC, 1998b). These guidelines outline general criteria for the layout, subject matter, terminology, and units of measurements, but state that the authority responsible for risk assessment is "required to use expert judgement in preparing the documentation concerned" (EC, 1998b). There are thus no specific criteria for data selection. The risk assessor should, however, consider all the data provided by the manufacturer and may also, when such data is available and considered relevant, include data on toxicity from the open literature.

3.3.5. Comparison

Table 4 gives an overview of the availability and scope of guidelines for conducting risk assessments for the different regulatory groups investigated. Guidelines are available for three of the four investigated chemical groups. As there is no legislation covering the requirements for risk assessment of environmental pollutants in food there are no guidelines for this process. The guidance document for priority existing industrial substances, the TGD (ECB, 2003), is significantly more comprehensive and detailed than for any of the other compounds.

One important aspect of the guidelines is to what extent the risk assessment process should make use of pre-defined criteria and how much that is left to case-by-case judgements. Reliance on pre-defined criteria contributes to making the risk assessment process predictable, but at the same time less flexible. A system based on case-by-case judgements on the other hand, makes the process more dependent on the knowledge, views and experiences of the person(s) conducting the assessment. Expert judgement is required in all four cases, however, for risk assessment of priority existing industrial substances the criteria are relatively detailed, for environmental pollutants on the other hand, expert judgement is the only basis for effects assessment. Expert judgement has also been given significant importance in assessments of pharmaceutical substances and of active substances in plant protection products.

3.4. Assessment of effects in the investigated risk assessment reports

The effects assessments in each of the risk assessment reports for the three model compounds were scrutinized and the conclusions regarding toxicity, critical effect and NOAEL were identified. Table 5 summarizes the results of the effects assessments for the three model compounds.

3.4.1. BPA

All the effects stipulated in Regulation 1488/94 have been evaluated in this risk assessment (EU, 2003).[6] The overall conclusion of the risk assessment of BPA, is that there is mainly a concern for liver toxicity and effects on fertility. Concern for eye and respiratory tract irritation is relevant for occupational exposure during the production of products containing BPA, such as polycarbonate plastics and epoxy resins.

The assessment also indicates that developmental toxicity could be a critical effect but the risk assessors could not agree on the relevance of these findings to humans. It was concluded that further testing was needed before a robust conclusion could be drawn. Because BPA was already known, from previous research, to have estrogenic potential studies on endocrine modulating activity were also evaluated in the risk assessment, and the risk assessors concluded that BPA can act weakly estrogenic by binding to nuclear estrogen receptors.

The risk assessors excluded studies reporting low-dose effects of BPA from the final risk characterization. Because of varying and contradictive results at low doses (below 50 mg/kg bw/day), as well as study designs not following regulatory guidelines or

[6] The authors are aware that the risk assessment published by ECB in 2003 has lately been up-dated. However, this up-date should be considered as a supplement to the original risk assessment and does not contain any changes to the established NOAEL or conclusions concerning risk stated in 2003.

Table 4
The availability and scope of guidelines issued to assist the risk assessor in conducting the risk assessments.

	Priority existing industrial substances	Environmental pollutants in food	Active substances in pharmaceuticals	Existing active substances in Plant protection products
Guidelines available?	Yes	No	Yes	Yes
Guidelines issued by:	European Chemicals Bureau	*Not applicable*	European Medicines Agency (EMEA)	European Commission – DG agriculture
Legislation implemented by guidelines	Reg 793/93	*Not applicable*	Dir 2003/63	Dir 91/414
Who is the guidance for?	Risk assessor and manufacturer	*Not applicable*	Risk assessor[a]	Risk assessor
Number of pages	311[b]	*Not applicable*	113[b]	94
Effects assessment mainly subject to criteria or expert judgement?	Criteria and expert judgement	Expert judgement	Expert judgement	Expert judgement

[a] The format of the report assessing the safety of a pharmaceutical substance is a safety assessment, "risk assessor" might therefore not be a correct term for the expert conducting the report.
[b] Includes only guidelines for carrying out *human health* risk assessment.

Table 5
Conclusions regarding the effects assessed for the three model compounds in each risk assessment document.

	BPA (EU, 2003)	Dioxins (SCF, 2000, 2001)[a]	Vinclozolin (EC, 1997, 1998a)
Acute toxicity/single-dose	Low	–	Very low
Corrosiveness/irritation/ local tolerance	Irritating to eyes and respiratory tract	–	Not irritating to skin or eye
Skin sensitization	Limited (more info needed)	–	Yes
Repeated dose toxicity	Multinuclear giant hepatocytes in mice observed	–	Low
Mutagenicity/ genotoxicity	Aneugenic *in vitro*, not *in vivo*	Not genotoxic	Not genotoxic
Chronic toxicity/ carcinogenicity	No carcinogenic potential	Human carcinogen	Carcinogenic in rodents
Toxicity to reproduction	Adverse effects on fertility, no consensus regarding developmental toxicity	Feminization of male offspring and adverse effects on spermatogenesis	Feminization of male offspring
Neurotoxicity	–	–	–
Endocrine modulating activity	Estrogenic	Antiestrogenic	Anti-androgenic
Critical effect identified	Decreased litter size in rats	Perturbed development of the male rat reproductive tract	Non-neoplastic changes in liver and adrenals in male rats
NOAEL	50 mg/kg bw/day	Not established. LOAEL = 25 ng/kg (body burden)	1.2 mg/kg bw/day

–, not covered by the reviewed risk assessment documents.

GLP, these studies were not considered robust or reliable enough to serve as the basis for the assessment of human health risks. However, this data material added to the uncertainties concerning developmental toxicity expressed in the risk assessment conclusions.

The critical effect identified in the European risk assessment (EU, 2003) was reproductive toxicity with a NOAEL of 50 mg/kg/day. The NOAEL was based on a reduction in litter size observed in rats exposed to 500 mg/kg/day in a multi-generation study.

3.4.2. Dioxins

The risk assessment of dioxins made by the Scientific Committee on Food (SCF) in 2000 (SCF, 2000) and up-dated in 2001 (SCF, 2001) was based on a previous assessment carried out by the World Health Organization (WHO-ECEH/IPCS 2000). Since there is no legislation to regulate what effects should be evaluated for environmental pollutants, the focus of SCF was directed towards effects indicated as critical in the WHO assessment (WHO-ECEH/IPCS 2000) and in general scientific discussions (SCF, 2000, 2001). Therefore, the assessment was limited to carcinogenicity and toxicity to reproduction.

In the SCF assessment, reproductive toxicity was extensively evaluated and it was concluded that developmental toxicity is of high concern. The SCF assessment also includes an evaluation of data on endocrine modulating activity, concluding that dioxins are Ah-R agonists interfering with the function of estrogen receptors, often resulting in anti-estrogenic effects.

The SCF identified perturbed development of the male reproductive tract as the critical effect of dioxin exposure based on animal studies investigating the most toxic congener 2,3,7,8-TCDD. A NOAEL could, however, not be derived from the scientific material available to the risk assessors, therefore a lowest observed adverse effect level (LOAEL) of 25 ng/kg bw (body burden), corresponding to an estimated human daily intake (EHDI) of 20 pg/kg bw, was used to derive a TDI of 2 pg 2,3,7,8-TCDD/kg bw. To be able to extend the TDI to all dioxins and dioxin-like PCBs the TDI was further expressed in Toxic Equivalency (TEQ), *i.e.* 2 pg TEQ/kg bw.

3.4.3. Vinclozolin

The initial risk assessment of vinclozolin (EC, 1997) was amended in 1998 (EC, 1998a), mainly due to a re-evaluation of the reproductive toxicity of this compound. In reproductive toxicity studies adverse effects on development, in particular feminization of males, was shown in rodents. Relatively high doses were tested and maternal toxicity occurred in many cases. Since results from short-term, chronic and reproductive toxicity studies indicated and anti-androgenic effect of vinclozolin investigations of interactions with the androgen signalling pathways were conducted. The anti-androgen properties of two of the main metabolites of vinclozolin, were established in endocrine modulating tests. The evaluation of vinclozolin furthermore concluded that the substance is a non-genotoxic carcinogen in rodents.

The critical effect of vinclozolin was established in a chronic toxicity study as non-neoplastic changes in liver and adrenals in

male rats, with a NOAEL of 1.2 mg/kg/day. The NOAEL for reproductive toxicity was established at 2 mg/kg bw/day based on the results from a two-generation study in rats where effects such as reduced size and weight of male reproductive organs, hypospadias and hypoplastic penis were observed in offspring at higher doses. These effects were stated to be due to the "anti-androgen effects" of the substance.

3.4.4. Comparison

The effects assessment results for the three model compounds have been summarized in Table 5. Endocrine modulating activity was evaluated for BPA, dioxins and vinclozolin even though this was not required in the legislation for any of these compounds. The reason is that this characteristic was already known or suspected for the model compounds based on results from standard toxicity tests, as in the case of vinclozolin, or because of extensive academic interest and research, as for BPA and dioxins. It should also be noted that all the model compounds modulate steroid hormone signalling pathways and that this is compatible with the observed adverse effects of BPA, dioxins and vinclozolin.

The above comparison also shows how the scope of the risk assessments differs between these compounds. This mirrors the requirements stated in the EU Directives and Regulations for the different chemical categories they belong to. The evaluation of dioxins, for which there are no legislated requirements, is focused on the effects deemed the most sensitive in previous evaluations, such as the WHO evaluation from 1998, *i.e.* carcinogenicity and reproductive toxicity. In contrast the assessments of BPA and vinclozolin have a wider scope, determined by their respective legislation, and aimed at hazard identification.

3.5. Expected risk assessment conclusions regarding critical effect, threshold and endocrine disrupting properties based on required data

As already mentioned, there were no particular requirements for investigating the endocrine disrupting activity of compounds within any of the regulatory frameworks to which the model compounds belong. Still, endocrine disrupting properties were evaluated for each of the model compounds since they were already known. It is of interest to investigate if the critical effect and threshold now identified for the model compounds would have been the same, as well as whether or not EDC properties of the compounds would have been identified, had only the minimum required toxicity data been available for these compounds. This analysis assumes that the toxicity of the model compounds were previously unknown.

3.5.1. BPA

Reduced number of offspring was established as the critical effect of BPA (NOAEL = 50 mg/kg bw/day) based on results from a multi-generation reproductive toxicity study (ECB, 2003). However, the risk assessors did not clearly state whether they considered the endocrine disrupting activity of BPA to be the underlying mechanism of toxicity. BPA was acknowledged to be weakly estrogenic based on other data specifically investigating its estrogenic potential. These data were not included in the toxicity data requirements for risk assessment stated in regulation but was available because it was previously known that BPA had some estrogenic potential. As determined by Directive 67/548 the minimum required data for reproductive toxicity for existing industrial substances was a screening test. The corresponding OECD test guideline (421) investigates effects on gonadal function and mating behaviour in exposed adults, as well as conception, fetal development and parturition. Offspring are killed four days after birth and are only examined externally for gross abnormalities. The reproduction toxicity screening test would not provide any information indicating that BPA causes adverse effects on post-natal development and such concerns would probably not have been raised. It is possible that the effects on litter size would have been picked up in such a screening. However, similar one-generation studies on the effects of BPA in rats were included in the risk assessment but did not show an effect on fertility.

3.5.2. Dioxins

Environmental pollutants in food are only risk assessed if there is a known or suspected concern for human health effects. As there are no legislated data requirements for environmental pollutants the endocrine disrupting or other toxicity properties of dioxins would not have been investigated had they not been previously known or suspected.

3.5.3. Vinclozolin

Non-neoplastic changes in the liver of male rats was established as the critical effect of vinclozolin exposure (NOAEL = 1.2 mg/kg bw/day) in a chronic toxicity study. This study was part of the required data material provided by the manufacturer to the rapporteur member state (risk assessor). Based on the evaluation of the androgenic potential of vinclozolin included in the risk assessment the risk assessors concluded that the critical effect, as well as the effects on reproduction (with a slightly higher NOAEL of 2 mg/kg bw/day), were due to the endocrine disrupting properties of the substance. Effects such as perturbed development and reduced function in male genital and reproductive organs, as well as cell proliferation and hyperplasia observed in organs involved in the feedback control mechanisms for steroid hormones, were reported from the required short-term, chronic and reproductive toxicity studies. In the case of vinclozolin there were thus quite clear indications of hormone dysregulation from the required data material.

3.5.4. Comparison

The identification of critical effects and NOAELs for BPA and vinclozolin may have remained the same as in the investigated risk assessment documents had only the minimum required data been available. However, based exclusively on required data the endocrine disrupting potential of BPA, at least, would not have been identified.

The toxicity data requirements were higher for plant protection products than for existing industrial substances, which might increase the chances for identifying endocrine disrupting properties for these chemicals. However, the toxicological properties of the substance itself play a role in identifying these characteristics. In the case of vinclozolin the effects indicating hormone dysregulation were evident, manifesting as severe effects on the function and development of male reproductive organs at high doses. For BPA no such clear effects of endocrine disruption were reported from the required toxicity tests.

Dioxins would not have been risk assessed had they not been known from previous research to be highly toxic and to cause adverse health effects in experimental animals as well as in accidentally and occupationally exposed persons.

3.6. Risk assessment conclusions regarding human relevance of effects

The risk assessment documents for the three model compounds were further scrutinized for conclusions regarding the relevance of the identified toxicological hazards to human health and what principles and assumptions these conclusions were based on. Primarily, two important toxicological concepts, mode of action and dose–response relationships, were addressed. Table 6 summarizes our conclusions on how these concepts were used in the three cases.

Table 6
A summary of the toxicological principles and assumptions underpinning the conclusions regarding the relevance of the identified toxicological hazards to human health in the three risk assessment documents.

	BPA (EU, 2003)	Dioxins (SCF, 2000, 2001)	Vinclozolin (EC, 1997, 1998a)
Mode of action relevant to humans?	Not clearly stated	Yes – stated that humans are as sensitive or less sensitive than responsive rodent strains	Yes – stated that humans are less sensitive than test animals
Dose-range evaluated in risk assessment	1000–10,000 times estimated consumer exposure	About 10 times estimated consumer exposure	1000–100,000 times estimated consumer exposure
Assumptions regarding extrapolation from high to low doses	High doses predict the effects at low doses	Levels close to human exposure are evaluated	High doses predict the effects at low doses
Assumptions regarding the shape of the dose–response curve	There is a threshold for toxicity	There is a threshold for toxicity	There is a threshold for toxicity
	The dose–response relationship is monotonic	The dose–response relationship is monotonic	The dose–response relationship is monotonic

3.6.1. Mode of action

The relevance of the critical mode of action, *i.e.* the endocrine modulating potential, to humans has been regarded differently in the risk assessments of the three compounds. In the BPA risk assessment (EU, 2003) the estrogenic activity was evaluated based on *in vitro* and *in vivo* data. A marked strain difference in rats was indicated by the data, but no explicit comment was made in the BPA risk assessment regarding the relevance of the different results to humans. Furthermore, it is not clearly stated whether the effects on fertility observed in rodents, and on which the risk assessment is based, are considered to be due to the estrogenic activity of BPA. If that was the case it would mean that an assumption was made by the risk assessors that the mode of action identified in animals is in fact relevant for humans.

Based on results obtained from *in vitro* studies comparing the response in human and rodent cells to dioxin exposure the conclusion in the dioxin risk assessment (SCF, 2000, 2001) was that average humans are "less sensitive than responsive rodent strains" to adverse effects caused by Ah-R activation. Further, it was also concluded from *in vitro* studies that rodents and humans show "comparable sensitivity" to the induction of CYP 1A1 and 1A2. Based on these conclusions the risk assessors extrapolated the NOAEL for developmental toxicity, which was identified as the critical effect, from rats to humans without the use of uncertainty factors to account for any interspecies or interindividual variability in sensitivity (toxicodynamics). This indicates that the inter- and intraspecies variations of the critical developmental effects observed *in vivo* were not assumed to be larger than the variations observed between species *in vitro*.

In the vinclozolin assessment (EC, 1997) similarities between this chemical and the anti-androgenic commercial drug flutamide used to treat prostate cancer were discussed. The conclusion was that since this drug is efficient in man, the anti-androgenic mechanism of toxicity of the structurally similar vinclozolin should be regarded as relevant to humans. However, results from clinical trials on flutamide led the risk assessors to conclude that man is less sensitive to perturbations in hormone balance than rodents and that the mechanism causing neoplastic changes in rats is species-specific and does not pose a hazard to humans.

3.6.2. Dose–response relationships

For BPA and vinclozolin very high doses, compared to estimated consumer exposure, were tested in the studies used in the risk assessments. In contrast, in the dioxin assessment low doses, *i.e.* only about 10 times the estimated consumer exposure based on body burden comparisons, were used.

It is generally assumed in toxicology that the dose–response curve is monotonic, *i.e.* above a certain concentration (a "threshold") increasing dose leads to increasing response. This is one of the major assumptions on which risk assessments are traditionally based. However, regarding EDCs non-monotonic, *i.e.* bell-shaped or (inverted) U-shaped, dose–response relationships are often discussed (Gray et al., 1997; vom Saal et al., 1997; Gupta, 2000; Sheehan, 2000; Rubin et al., 2001; Timms et al., 2005). In addition, it may be argued that since these substances mimic endogenous hormones no threshold can be assumed, as endogenous hormones already occur at concentrations sufficient to cause an effect the threshold is already exceeded (Barlow, 1999; Sheehan et al., 1999; Sheehan, 2000).

The shape of the dose–response curve and potential thresholds for toxicity were not explicitly discussed in the risk assessment reports studied. However, both the shape of the curve and assumed thresholds can be inferred from the proposed NOAEL/LOAEL values and how these values have been used in the risk characterization. Simply, the setting of a NOAEL indicates that a threshold for effect was assumed. The application of assessment factors to derive health-based guidance values, as in the case of dioxins and vinclozolin, indicates that a monotonic dose–response relationship was assumed. The calculation of a MOS, as for BPA, also indicates that the dose–response curve was assumed to be monotonic. Extrapolation from high to low dose can only be made if the dose–response curve is assumed to be monotonic, *i.e.* effects are assumed to be qualitatively similar at high and low doses, and only change quantitatively.

3.6.3. Comparison

Table 6 summarizes some of the toxicological assumptions and principles used in the hazard assessments for the three model compounds. The relevance of the critical mode of action, *i.e.* the endocrine modulating potential, to humans has been regarded differently in the risk assessments of the three compounds. In contrast to the other two risk assessments, the assessment of BPA does not clearly state whether the adverse effects deriving from the estrogenic activity of BPA observed in rodents is believed to be relevant for humans. For dioxins and vinclozolin the risk assessors conclude that humans are less sensitive than test animals to adverse effects caused by their respective endocrine modulating potential. Some differences are noted regarding the doses tested. However, the traditional toxicological principle of extrapolating from high to low doses was made for all three model compounds.

4. Summary and conclusions

Consideration of toxicological modes of action is generally not well established in regulatory risk assessment. Further, there is no generally agreed procedure under any of the mentioned legislations for chemicals control that directly specifies how substances with EDC characteristics are to be identified or risk assessed, what end-points are crucial to investigate, or how the results of such investigations are to be interpreted. As a result the regulatory risk assessment process, as well as underlying policies, criteria and requirements may differ for different EDCs. Indeed, there is little

conformity in the risk assessment processes between the model compounds discussed in this study, even though the primary consumer exposure scenario (oral exposure) and the mode of toxicity (endocrine modulation) are similar.

Technical guidelines have been introduced to promote conformity in the risk assessment process. However, different guidelines, issued by different authorities, apply to different regulatory groups of chemicals. Analysis of the available guidelines, including criteria for data selection and interpretation, shows that expert judgement is more or less influencing the risk assessment process for the four chemical groups investigated. The risk assessment conclusions rely on the expert judgement of the risk assessors, including the toxicological principles and assumptions that are used when selecting and interpreting data.

There are no requirements for testing endpoints that specifically enables discovery of endocrine disruption included in any of the regulatory frameworks investigated here. The only standardized screening test with international regulatory acceptance is currently the uterotrophic bioassay, which was adopted by the OECD in 2007. This assay may be used to screen for estrogenic properties of chemicals. However, generally EDCs are only handled as such if their endocrine disruption potential has been previously identified via, for example, academic research, or is indicated by effects observed in required toxicity tests. It is acknowledged that EDCs can affect humans and animals at low exposure levels and that responses to EDCs are in many cases complex, activating a range of different molecular events, *e.g.* receptor-agonism/antagonism and enzyme induction, in multiple hormone systems. As a result, regulatory testing for these effects, and evaluating the results is complicated. Further, test methods, assumptions and criteria for data interpretation commonly used for general and reproductive toxicity and carcinogenicity, might not be directly applicable.

It should be noted that the purposes of use for the chemical groups investigated here, as well as the resulting exposure scenarios, differ. Plant protection products are deliberately sprayed onto fruits and vegetables in large quantities to control pest organisms and increase crop yield. Pharmaceuticals on the other hand, are deliberately administered to patients with the purpose to improve the well-being of that patient. Both pesticides and pharmaceuticals are thus produced with the purpose to interact in a specific manner with biological organisms; pesticides with the purpose to be highly toxic to particular organisms, and pharmaceuticals to exert a specific pharmacological effect in humans. This intentional use contrasts with the unintentional and wide-spread exposure of consumers to low concentrations of industrial substances and environmental pollutants that inadvertently end up in food.

The analysis of the risk assessment documents for the three model compounds reveals that endocrine disrupting potential was investigated in all three cases even though this is not required by legislation. In the case of vinclozolin investigations of anti-androgenic properties were prompted by the strong indications of anti-androgenic effects from short-term, chronic and reproductive toxicity testing. The endocrine disrupting properties of BPA and dioxins were however, investigated because they were already known previous to risk assessment and a large amount of data was available for both these substances supporting the presence of such effects. It is probable that there are other substances where endocrine disrupting characteristics may be overlooked, irrespective of which legislative framework they are regulated within, because there is no pre-existing research interest beyond what is required for regulatory risk assessment. As pointed out earlier, the main factor hampering reliable risk assessment of potential EDCs is the lack of internationally validated and accepted protocols for identifying and testing these compounds. The resulting uncertainties and data gaps in EDC risk assessment may reasonably be believed to be extensive in many cases and have to be handled by the risk assessor in a transparent fashion. This issue is highly important and requires further investigation and discussion and makes up a main part of on-going investigations.

In the new regulation for industrial chemicals, REACH, and the proposed new regulation for plant protection products efforts have been made to tighten the control on EDCs. These regulations specifically state that EDCs are compounds of concern that should not be approved for use unless it is shown that their use does not pose a threat to human health or the environment. However, the problem of identifying compounds as EDCs remains.

Work to develop and validate test methods to screen for and evaluate EDCs is currently being conducted by the OECD Endocrine Disrupter Testing and Assessment (EDTA) task force. Further development of risk assessment guidance, *i.e.* how to interpret data from experiments using doses significantly higher than the expected human exposures, which assumptions regarding the shape of the dose–response curve that can be made, and principles for extrapolating data from experimental species to humans, is also needed. This is supported by an international agreement that there is reason to move in this direction, based on a general acceptance that EDCs present a risk to humans and the environment alike. Since the risk assessment of chemicals is an interaction between experimental scientists, risk assessors, regulators and industry, the success of the work towards improving the legislation for EDCs requires continued close cooperation between these groups.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

Barlow, S.M., 1999. Dilemmas facing regulatory and advisory bodies dealing with conflicting results. Chemosphere 39, 1287–1292.

Colbert, N.K.W., Pelletier, N.C., Cote, J.M., Concannon, J.B., Jurdak, N.A., Minott, S.B., Markowski, V.P., 2005. Perinatal exposure to low levels of the environmental antiandrogen vinclozolin alters sex-differentiated social play and sexual behaviours in the rat. Environ. Health Perspect. 113, 700–707.

Commission Directive 2003/63/EC of 25 June 2003 amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use. Official Journal of the Communities L 159, 27/06/2003, pp. 0046–0094.

Commission Directive 93/71/EEC of 27 July 1993 amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market. Official Journal of the Communities L 221, 31/08/1993, pp. 0027–0036.

Commission Directive 94/79/EC of 21 December 1994 amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market. Official Journal of the Communities L 354, 31/12/1994, pp. 0016–0031.

Commission Regulation (EC) No. 1488/94 of 28 June 1994 laying down the principles for the assessment of risks to man and the environment of existing substances in accordance with Council Regulation (EEC) No. 793/93. Official Journal of the Communities L 161, 29/06/1994, pp. 0003–0011.

Commission Regulation (EEC) No. 3600/92 of 11 December 1992 laying down the detailed rules for the implementation of the first stage of the programme of work referred to in Article 8 (2) of Council Directive 91/414/EEC concerning the placing of plant protection products on the market. Official Journal of the Communities L 366, 15/12/1992, pp. 0010–0016.

Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the Communities 196, 16/08/1967, pp. 0001–0098.

Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. Official Journal L 230, 19/08/1991, pp. 0001–0032.

Council Directive 97/57/EC of 22 September 1997 establishing Annex VI to Directive 91/414/EEC concerning the placing of plant protection products on the market. Official Journal of the Communities L 265, 27/09/1997, pp. 0087–0109.

Council Regulation (EEC) No. 793/93 of 23 March 1993 on the evaluation and control of the risks of existing substances. Official Journal of the Communities L 084, 05/04/1993, pp. 0001–0075.

Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. Official Journal of the Communities L 311, 28/11/2001, pp. 0067–0128.

Directive 2004/27/EC of the European Parliament and of the Council of 31 March 2004 amending Directive 2001/83/EC on the Community code relating to medicinal products for human use. Official Journal of the Communities L 136, 30/04/2004, pp. 0034–0057.

EC, 1997. European Commission Peer Review Programme, ECCO meetings, Vinclozolin, vol. 3 Annex B.

EC, 1998a. European Commission Peer Review Programme, ECCO meetings, Vinclozolin, Addendum to EC, 1997 – toxicology and metabolism.

EC, 1998b. Guidelines and criteria for the evaluation of dossiers and for the preparation of reports to the European Commission by Rapporteur Member States relating to the proposed inclusion of active substances in Annex I of Directive 91/414/EEC. Available from: <http://europa.eu.int/comm/food/plant/protection/evaluation/>.

ECB, 2003. Technical guidance document on risk assessment, Part I. Available from: <http://ecb.jrc.it/existing-chemicals/>.

EFSA, 2006. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2,2-bis(4-hydroxyphenyl)propane (Bisphenol A). Available from: <www.efsa.europa.eu>.

EMEA, 2003a. CPMP/ICH/2887/99 Common technical document for the registration of pharmaceuticals for human use – Safety. Available from: <www.emea.europa.eu/>.

EMEA, 2003b. CPMP/ICH/2887/99 Common technical document for the registration of pharmaceuticals for human use – Efficiacy. Available from: <http://www.emea.europa.eu/>.

EMEA, 2005. CPMP/SWP/799/95 Guideline on the non-clinical documentation for mixed marketing authorisation applications. Available from: <http://www.emea.europa.eu/>.

EMEA, 2008a. CHMP/ICH/126642/2008 Note for guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use – draft. Available from: www.emea.europa.eu/.

EMEA, 2008b. CHMP/203927/2005 Guideline on risk assessment of medicinal products on human reproduction and lactation: from data to labelling. Available from: <www.emea.europa.eu/>.

EU, 2003. Bisphenol-A: European Union Risk Assessment Report (CAS No. 80-05-7). Available from: <http://ecb.jrc.it/>.

Faqi, A.S., Dalsenter, P.R., Merker, H.-J., Chahoud, I., 1998. Reproductive toxicity and tissue concentrations of low doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in male offspring rats exposed throughout pregnancy and lactation. Toxicol. Appl. Pharmacol. 150, 383–392.

Gray Jr., L.E., Ostby, J.S., Kelce, W.R., 1997. A dose–response analysis of the reproductive effects of a single gestational dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in male Long Evans hooded rat offspring. Toxicol. Appl. Pharmacol. 146, 11–20.

Gupta, C., 2000. Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. PSEBM 224, 61–68.

IPCS, 2002. Global assessment of the state-of-the-science of endocrine disruptors. In: Terri Dmastra, Sue Barlow, Aake Bergman, Robert Kavlock, Glen van der Kraak (Eds.). Available from: <http://whqlibdoc.who.int/hq/2002>.

Kelce, W.R., Monosson, E., Gamcsik, M.P., Laews, S.C., Gray Jr., L.E., 1994. Environmental hormone disruptors: evidence that vinclozolin developmental toxicity is mediated by androgenic metabolites. Toxicol. Appl. Pharmacol. 126, 276–285.

Mably, T.A., Moore, R.W., Peterson, R.E., 1992. In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 3. Effects on spermatogenesis and reproductive capability. Toxicol. Appl. Pharmacol. 114, 118–126.

Matthews, J.B., Twomey, K., Zacharewski, T.R., 2001. In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors α and β. Chem. Res. Toxicol. 14, 149–157.

NTP, 2001. The National Toxicology Program's Report of the Endocrine Disruptors Low Dose Peer Review. Available from: <http://ntp.niehs.nih.gov/>.

Ohsako, S., Miyabara, Y., Nishimura, N., Kurosawa, S., Sakaue, M., Ishimura, R., Sato, M., Takeda, K., Aoki, Y., Sone, H., Tohyama, C., Yonemoto, J., 2001. Maternal exposure to a low dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) suppressed the development of reproductive organs of male rats: dose-dependent increase of mRNA levels of 5α-reductase type 2 in contrast to decrease of androgen receptor in the pubertal ventral prostate. Toxicol. Sci. 60, 132–143.

Regulation (EC) No. 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the Communities L 31, 1/2/2002, pp. 1–24.

Regulation (EC) No. 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency. Official Journal of the Communities L 136, 30/04/2004, pp. 0001–0033.

Rubin, B.S., Murray, M.K., Damassa, D.A., King, J.C., Soto, A.M., 2001. Perinatal exposure to low doses of Bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. Environ. Health Perspect. 109, 675–680.

SCF, 2000. Opinion of the Scientific Committee on Food on the risk assessment of dioxins and dioxin-like PCBs in food. Available from: <http://europa.eu.int/comm/food/fs/sc/scf/out78_en.pdf>.

SCF, 2001. Opinion of the Scientific Committee on Food on the risk assessment of dioxins and dioxin-like PCBs in food. Up-date based on new scientific information. Available from: <http://europa.eu.int/comm/food/fs/sc/scf/out90_en.pdf>.

Sheehan, D.M., Willingham, E., Gaylor, D., Bergeron, J.M., Crews, D., 1999. No threshold for estradiol-induced sex reversal of turtle embryos: how little is too much? Environ. Health Perspect. 107, 155–159.

Sheehan, D.M., 2000. Activity of environmentally low doses of endocrine disruptors and the bisphenol-A controversy: initial results confirmed. Proc. Soc. Exp. Biol. Med. 224, 57–60.

Timms, B.G., Howdeshell, K.L., Barton, L., Bradley, S., Richter, C.A., vom Saal, F.S., 2005. Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra. PNAS 102, 7014–7019.

Tyl, R.W., Myers, C.B., Marr, M.C., Thomas, B.F., Keimowitz, A.R., Brine, D.R., Veselica, M.M., Fail, P.A., Chang, T.Y., Seely, J.C., Joiner, R.L., Butala, J.H., Dimond, S.S., Cagen, S.Z., Shiotsuka, R.N., Stropp, G.D., Waechter, J.M., 2002. Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague–Dawley rats. Toxciol. Sci. 68, 121–146.

Tyl, R.W., Myers, C.B., Marr, M.C., Sloan, C.S., Castillo, N.P., Veselica, M.M., Seely, J.C., Dimond, S.S., Van Miller, J.P., Shiotsuka, R.N., Beyer, D., Hentges, S.G., Waechter Jr., J.M., 2008. Two-generation reproductive toxicity study of dietary bisphenol A in CD-1 (Swiss) mice. Toxicol. Sci. 104, 362–384.

Richter, C.A., Birnbaum, L.S., Farabollini, F., Newbold, R.R., Rubin, B.S., Talsness, C.E., Vandenbergh, J.G., Walser-Kuntz, D.R., vom Saal, F.S., 2007. In vivo effects of bisphenol A in laboratory rodent studies. Reprod. Toxicol. 24, 199–224.

Veeramachaneni, D.N., Palmer, J.S., Amann, R.P., Kane, C.M., Higuchi, T.T., Pau, K.Y., 2006. Disruption of sexual function, FSH secretion, and spermiogenesis in rabbits following developmental exposure to vinclozolin, a fungicide. Reproduction 131, 805–816.

vom Saal, F.S., Timms, B.G., Montano, M.M., Palanza, P., Thayer, K.A., Nagel, S.C., Dhar, M.D., Ganjam, V.K., Parmigiani, S., Welshons, W.V., 1997. Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. Proc. Natl. Acad. Sci. 94, 2056–2061.

Wetherill, Y.B., Akingbemi, B.T., Kanno, J., McLachlan, J.A., Nadal, A., Sonnenschein, C., Watson, C.S., Zoeller, R.T., Belcher, S.M., 2007. *In vitro* molecular mechanisms of bisphenol A action. Reprod. Toxicol. 24, 178–198.