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Topic 2.8

Critical factors in exposure modeling of endocrine active substances*

Pim de Voogt[1,‡] and Bert van Hattum[2]

[1]Environmental and Toxicological Chemistry, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands; [2]Institute for Environmental Studies, Vrije Universiteit, De Boelelaan 1087, 1081 HV, Amsterdam, The Netherlands

Abstract: Multimedia transport, partitioning, and degradation pathways are key processes in the probability of a substance to interact with target organisms. Biotic factors such as toxicokinetics, biotransformation capacity, and behavioral and life-cycle aspects of the organisms are determinants for final concentrations at target organs. The role of metabolites in endocrine disruption can be quite different from those of the parent compounds, and often this requires separate toxicokinetic evaluation.

The exposure assessment of endocrine active substances (EASs) suffers from a huge lack of reliable data, of both values that are used as input parameters in exposure models, and field data that are needed for validation purposes. In general, for the more classic EASs, such as PCBs, *p*,*p*′-DDE, chlorinated dioxins, some pesticides, and organotins, reliable data are sufficiently available, but careful evaluation of the quality of databases is necessary. Several data quality evaluation systems have been proposed. For the "newer" compounds, only few data have been gathered so far. The latter compounds include alkylphenols, bisphenol A, brominated flame retardants, phytoestrogens, and in particular natural and synthetic hormones, which in view of their high estrogenic potency could be the most important compounds in terms of risk.

The suitability of current exposure assessment models for EASs at this moment seems to be restricted to the persistent compounds such as PCBs, PCDDs, and PCDFs. Especially for the compounds subject to biodegradation and biotransformation, the lack of experimental data to derive model-input parameters and perform validation studies at this moment is one of the main obstacles for the further application of generic exposure models to other EASs. Most of the current models do not allow life stage-specific predictions.

Although the mechanisms of endocrine disruption involve different types of action, the principle of additivity, based on the equivalent toxicity approach (using estrogen equivalent potencies relative to 17ß-estradiol) seems promising for the design of integrated exposure and effect models for EASs.

Research programs aimed at the endocrine disruption issue must focus on promoting experimental studies for generation of reliable, high-quality parameter data on the one hand, and surveys or monitoring campaigns for collection of representative field data on the other. The non-specificity of possible effects caused through endocrine mechanisms implies that in

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‡Corresponding author

order to reveal dose–response relationships all potentially active agents, or at least as many as feasible, need be included in the risk assessments. Current regulatory monitoring programs should further be evaluated and harmonized with validation requirements of models used in exposure assessment.

INTRODUCTION

Exposure is a result of the emission of a chemical into the environment and its subsequent fate. Both emission and fate depend on many factors, which have been discussed extensively by many authors (see, e.g., [1,2]).

Abiotic factors include the amount of substance involved, its persistence, mobility and availability, and the duration of exposure. Multimedia transport, partitioning, and degradation pathways are key processes in the probability of a substance to interact with target organisms. Biotic factors include toxicokinetics, physiological, behavioral and life-cycle aspects of the organisms, predator–prey relationships, and biotransformation capacity. Dietary exposure is an important aspect, in particular for the exposure assessment of birds, mammals, and humans. The role of metabolites in endocrine disruption can be quite different from those of the parent compounds, and often this requires separate toxicokinetic evaluation. While these abiotic and biotic factors by no means are specific to the biological action of EASs, organisms in their normal development travel through various distinct stages of sensitivity accompanied or even directed by hormonal control. Examples are early life stages, and more specifically development of gonads, or egg maturation; many aspects of reproduction, such as (temperature-dependent) gender determination (in amphibians); and migration. Exposure to endocrine disrupters during such stages can result in highly specific effects. Therefore, exposure characterization needs to take into account such stages, e.g., through measuring or predicting the dose in target tissues. Measuring actual levels and tissue residues has improved our understanding of the relationships between external and internal doses. Although the mechanisms of endocrine disruption involve different types of action, the principle of additivity has been shown to hold in estrogenic activity.

The purpose of the present chapter is to highlight some critical aspects in environmental exposure assessment of endocrine disruptors and to indicate which type of research is needed to fill existing gaps in this field. The focus of this contribution is on the aquatic environment.

ABIOTIC FATE PROCESSES

The principle environmental processes that govern the abiotic fate of chemicals once brought into the environment include transport, distribution, and transformation processes.

Key parameters in environmental fate modeling therefore relate to these processes and include two types of parameters. System-dependent parameters include temperature, advection rates (e.g., wind speed, air trajectories, water currents, particle deposition rates in air or water), acidity, organic carbon contents, and particle size. Substance-related parameters include partition coefficients (e.g., sorption K_d, Henry's Law coefficients), mass transfer and diffusion rates, persistence, transformation rates (photolysis, hydrolysis, etc.), and speciation.

In general the variability in system related parameter values appears to be much lower than that in substance-related parameters. This is due in part because the former are more easy to measure (e.g., acidity, temperature, wind speed, compared to K_d or H) and also because the latter may depend on system parameters themselves. In other words, the conditions under which the substance parameters may have been determined may vary from experiment to experiment, and therefore such data have a larger intrinsic variability.

BIOTIC FACTORS

Residue levels of contaminants in organisms are a result of a variety of processes, the most important of which are the partitioning between and within biotic and abiotic compartments, and simultaneous transformation reactions such as biodegradation and biotransformation. Order of magnitude differences are present between individual compounds in both physicochemical characteristics (water solubility, volatility, hydrophobicity, photodegradation) and biological parameters and activity, e.g., microbial degradability, toxicokinetics, biotransformation, and endocrine-disrupting potency.

Uptake of compounds may take place from aqueous systems, via gills or the skin, and from dietary sources via the gastrointestinal tract. Epibenthic and sediment-inhabiting invertebrates may have additional uptake from ingested sediments or from porewater.

In aquatic environments, direct aqueous uptake of compounds (bioconcentration) seems to be dominant in most invertebrates and fish for compounds with a log $K_{ow} < 4$ [3].

The fraction of a contaminant concentration that is available for uptake by aquatic organisms (i.e., the bioavailable fraction) varies between species and depends on the relative significance of different uptake pathways [4]. Following uptake, compounds may be subject to biotransformation, to internal distribution among tissues and organs, and to elimination of parent compounds or biotransformation products. Within organisms, probably the role of metabolism is the most essential in homeostasis [2]. Although generally metabolism leads to degradation, detoxification, and elimination, products may be generated with enhanced endocrine potential. Especially in vertebrates the biotransformation route is more predominant in comparison to invertebrates and phytoplankton [5]. Elimination may be the result of redistribution at respiratory surfaces or in the gastrointestinal tract or via excretion products. Especially the ability of biotransformation of EASs seems to be of prime importance in explaining variability in tissue residues among different species in a specific habitat. For natural and synthetic hormones, alkylphenols and phthalates, only limited information is available on biotransformation in aquatic food chains [6].

DATA AVAILABILITY FOR ENVIRONMENTAL MODELING

When considering the availability of reliable data on physicochemical and environmental properties for EASs, there is a large discrepancy between the compounds previously included in priority lists, such as, e.g., PCBs, PCDDs, PCDFs, phthalates and organotin compounds, and compounds that were identified after the problem of endocrine disruption was acknowledged as an environmental issue. For many of the "old" compounds experimentally determined values are available, while for the "new" environmental agents, such as alkylphenol ethoxylates (APEOs), natural and synthetic hormones, phytoestrogens, and brominated compounds, only limited data are available.

For a preliminary exposure assessment of these compounds, quantitative structure activity relationship (QSAR)-based predictions (see, e.g., [7–9]) of the main environmental properties usually are applied, in order to allow a first screening of chemical fate and hazards with risk-assessment models, such as, e.g., EUSES [10] in the European regulatory context or the EPIWIN suite of models applied and distributed by the U.S. EPA [11].

Examples of important databases of environmental properties of compounds are the well-known publications of Mackay et al. [12], the Environmental Fate database of Syracuse [13], or the EINECS database of the European Commission [14]. These databases contain evaluated data from experimental studies and predicted values from QSAR studies. When possible, preference should be given to validated data from experimental studies.

As an example to illustrate to which extent experimental data are available, a summary is given in Tables 1 and 2 of available experimental data for 31 EASs in the Syracuse EFDB database and predicted values with the software in the EPIWIN program [11]. As can be seen for the physicochemical data, experimental data are available for 28 % (BP) to 59 % (solubility) of the compounds. Measured

Table 1 Summary of measured (bold) physicochemical data and environmental properties of EASs in the EFDB database and QSAR-based predictions with EPIWIN.*

Substance	CAS no.	Mol wt	BP °C	MP °C	VP mm Hg	Log K_{ow}	S mg/l	HLC P.m^3/mol	Log K_{oc}	Log BCF experimental	Log BCF predicted
4-Nonylphenol (branched)	84852-15-3	220.36	**295**	89.94	**9.42E-05**	5.92	**5000**	**4.22E-01**	4.708	**1.1**	3.85
Nonylphenol (linear)	25154-52-3	220.36	**293**	**42**	**9.42E-05**	**5.76**	**6.35**	**3.33E+00**	4.785		2.74
4-Nonylphenol (linear)	104-40-5	220.36	**293**	**42**	**9.42E-05**	**5.76**	**6.35**	**3.33E+00**	4.785		2.74
Bisphenol A	80-05-7	228.29	363.54	**153**	2.27E-07	**3.32**	**120**	8.98E-07	4.876	**1.0–2.0**	1.86
Tributyltin	56573-85-4	325.51	273.72	**–19**	1.30E-02	**4.76**	0.7478		4.176		3.37
Tributylstannane	688-73-3	291.07	250	28.89	3.99E-02	7.35	0.007302	1.49E+05	3.998		4.23
Tributyltin hydroxide	1067-97-6	307.07	318.06	70.99	1.55E-05	**4.09**	3.589	8.74E+01	4.176		2.85
Triphenyltin	668-34-8	351.04	358.52	94.01	1.78E-05	6.58	0.01464	7.69E+00	5.648		5.77
Estradiol (beta)	50-28-2	272.39	395.47	**221.5**	1.99E-09	**4.01**	**3.9**	3.57E-06	4.205		2.39
Estradiol (alpha)	57-91-0	272.39	395.47	**221.5**	1.99E-09	**4.01**	**3.9**	3.57E-06	4.205		2.39
Estrone	53-16-7	270.37	**154**	**260.2**	5.09E-03	**3.13**	**30**	3.72E-05	4.477		1.71
Ethynyl estradiol	57-63-6	296.41	411.21	**183**	1.95E-09	**3.67**	**11.3**	7.78E-07	4.678		2.13
DBP Dibutyl phthalate	84-74-2	278.35	**340**	**–35**	**2.01E-05**	**4.5**	**11.2**	**1.78E-01**	3.164	**1.1–3..8**	2.76
DEHP di-*sec*-octyl phthalate	117-81-7	390.57	**384**	**–55**	**1.42E-07**	**7.6**	**0.27**	**2.65E-02**	5.219	**2.1–4.1**	2.49
Di-*n*-propyl phthalate	131-16-8	250.3	**317.5**	**18.19**	**1.32E-04**	**3.27**	**108**	**3.95E-02**	2.633		1.82
Diethyl phthalate	84-66-2	222.24	**295**	**–40.5**	**2.10E-03**	**2.42**	**1080**	**5.98E-02**	2.101	**1.1–2.1**	1.16
Genistein	446-72-0	270.24	464.36	**301.5**	5.18E-12	2.84	257.7	5.02E-12	3.815		0.65
Formononetin	485-72-3	268.27	418.34	**256.5**	1.71E-09	3.11	157.3	2.18E-08	3.24		0.85
Dihydroxyisoflavone (daidzein)	486-66-8	254.24	429.52	**323**	2.77E-11	2.55	568.4	3.83E-11	3.597		0.42
Diosgenin	512-04-9	414.63	469.45	**205.5**	1.94E-11	6.34	**0.02**	1.56E-04	4.162		4.18
ß-Sitosterol	83-46-5	414.72	448.98	**140**	4.51E-10	9.65	4.63E-05	2.89E+01	6.656		1.18
Hexabromocyclododecane	25637-99-4	641.7	462.03	180.03	1.68E-08	7.74	2.09E-05	1.68E-01	5.07		3.79
Tetrabromo diphenylether	40088-47-9	485.8	405.51	161.73	2.41E-07	6.77	0.001461	2.91E-01	4.274		4.51
Decabromodiphenyl oxide	1163-19-5	959.17	**425**	**295**	2.01E-09	12.11	**0.025**	1.16E-03	5.611		7.2
Hexabromodiphenyl ether	36483-60-0	643.59	466.91	197.14	2.87E-09	8.55	4.15E-06	4.62E-02	4.702		2.69
Pentabromodiphenyl ether	32534-81-9	564.69	436.21	182.8	2.44E-08	7.66	7.86E-05	1.16E-01	4.484		3.91
PCBs	1336-36-3	291.99	359.51	122.32	**8.63E-05**	**6.29**	**0.277**	**3.36E+01**	4.651		4.76
3,3′,4,4′-tetrachloro-1,1′-Biphenyl	32598-13-3	291.99	359.51	122.32	**1.64E-05**	**6.63**	**0.000569**	**9.22E-01**	4.651		5.03
3,3′,4,4′,5-pentachlorobiphenyl	57465-28-8	326.44	378.21	134.6	2.22E-06	6.98	0.009394	9.06E+00	4.87		5.29
3,3′,4,4′,5,5′-Hexachlorobiphenyl	32774-16-6	360.88	396.9	146.34	5.81E-07	**7.41**	**0.00051**	6.71E+00	5.088		4.87
2,3,7,8-TCDD	1746-01-6	321.98	379.17	**305**	**1.50E-09**	**6.8**	**0.0002**	**4.90E+00**	5.165		4.54
2,3,7,8-TCDF	51207-31-9	305.98	382.92	138.99	1.53E-06	**6.53**	**0.000692**	1.52E+00	4.908		4.33
Experimental values			28 %	56 %	31 %	56 %	59 %	31 %		15 %	

[a]Measured values as derived from the EFDB database [13]; predicted values derived with the EPIWIN suite [11].

Table 2 Biodegradation evaluation in the EFDB database and rating of reliability of data.

Substance	Screen test	Biol. treatment simulation	Grab sample soil	Grab sample water	Field test	Aerobic summary	Anaerobic summary	No. of references	EPIWIN predicted[a]
Nonylphenol (linear?)	BST-2	BST-3				BST-1		3	w
Bisphenol A	BS-2	BFA-3		BFA-1		BST-1		4	w-m
DBP Dibutyl phthalate	BF-1	BF-1	BFA-1	BF-1		BF-1	BF-1	21	d-w
DEHP di-*sec*-octyl phthalate	BST-1	BFA-1	BFA-1	BST-1		BFA-1	BSA-1	28	w
Diethyl phthalate	BF-1	BF-3		BFA-1		BFA-1	BST-1	9	w
2,3,7,8-TCDD	BSA-3		BSA-1			BSA-1	BSA-1	3	r

Source: EFDB database [13]; codes: BF = biodegradation at a fast rate, BFA = fast rate with acclimation, BS = slow rate, BSA = slow rate with acclimation, BST = biodegrades sometimes; reliability ratings: 1 = tested in 3 or more tests with consistent results, 2 = in two tests, or >2 interpretable tests with some confilicting data, 3 = only 1 test or uninterpretable conflicting data.
[a]Predicted with BIOWIN—ultimate survey model: w = weeks, m = months, r = recalcitrant.

BCF data derived form experimental studies are included in the EFDB database for only 15 % of the compounds. Very few data are available for the biodegradability of EDC compounds. For only 6 out of the 31 EASs, data from experimental studies are available, mainly for phthalates. Some of the studies were conflicting or uninterpretable. For none of the compounds information from field tests on biodegradation under natural conditions was available in the EFDB database.

As an illustration of the large variation in physicochemical characteristics among EASs, a frequency distribution was made of the wide range of hydrophobicity (Fig. 1). The frequency distribution shows two maximums: one at log K_{ow} = 3–5, including the natural and synthetic hormones and phytoestrogens, and a second at log K_{ow} = 7–8 including the polyhalogenated aromatic compounds (PHAHs).

The wide range in hydrophobicity (K_{ow}) values and those of several other properties, such as the Henry's law constant (H), implies large differences in environmental behavior and chemical fate. This is reflected in the results of the outcome of preliminary calculations with a screening model (level III, based on the approach of Mackay et al. [15]), included in the EPIWIN programme. In the level III approach, used for this preliminary screening, the distribution over the media water, soil, air, and sediment

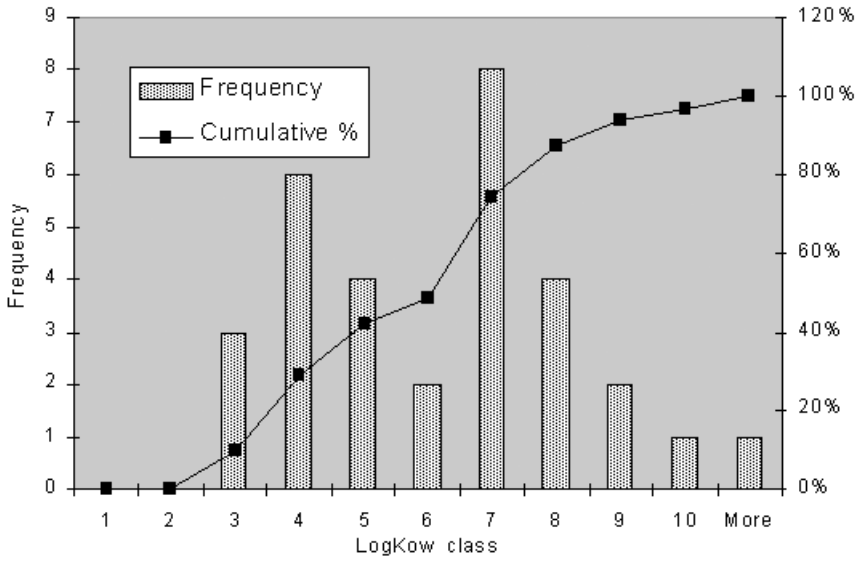

Fig. 1 Distribution of predicted log K_{ow} value classes for endocrine-disrupting compounds.

is calculated, based on equal emissions to air, water, and soil. The results are indicated in Fig. 2. Sediments and soil are identified as major sinks (total 50–95 %) in the level III model, especially for the PHAH compounds (>90 %). The water phase is an important compartment for the phenols (10–18 %), the organotins (5–30 %), natural and synthetic hormones (2–20 %), and the phthalates (10–40 %). The air compartment is only significant for some of the phthalates and TBT.

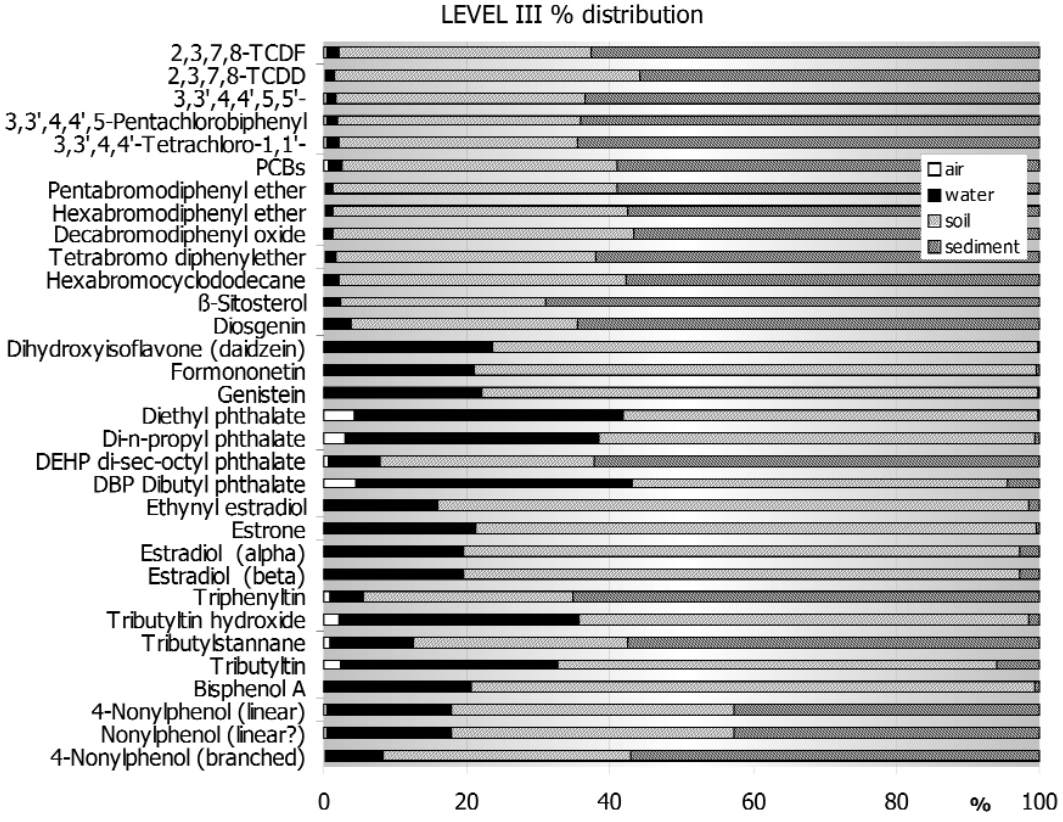

Fig. 2 Predicted environmental fate of EASs in the level III model included in EPIWIN.

DATA QUALITY

The quality and reliability of physicochemical properties data is a critical aspect in chemical fate modeling and hazard and risk assessment. Whenever a compilation of such data is made from literature reports, large discrepancies in experimental values for properties become evident. While it is obvious that different test methods used to obtain a certain physicochemical compound property will result in different outcomes, well-conducted experiments should result in values within ranges of statistical method variability. As indicated above, apart from experimental data-generating studies, (mathematical) estimation methods (e.g., QSARs) for properties can be used for obtaining data.

Kollig [16,17] and Klimisch et al. [18] have developed indicators for data quality evaluation of experimental studies. Kollig distinguished four categories of criteria: (1) analytical information; (2) experimental information; (3) statistical information; and (4) corroborative information. Each category contained subcriteria that were developed for various properties to make it possible to estimate the reliability of the measurement within one category. The data reliability indicator (DRI) consists of the relative reliability for all four categories. Klimisch et al. [18] used four reliability scores for experimental

data-generating studies: (1) reliable without restrictions; (2) reliable with restrictions; (3) not reliable; and (4) not assignable.

In a Dutch database of physicochemical properties, a modification of the Kollig approach was used to evaluate experimental as well as estimated (QSAR) data. Values were selected on the basis of three sets of general criteria [19]. For experimental studies, analytical, methodological, and statistical aspects, respectively, of the study were scored. Analogously, for QSAR studies descriptors, methodology and statistics were evaluated.

The EFDB database (which is included in the EPIWIN suite of models) contains three categories of reliability for evaluating tests: these are based on the number of times independent tests were carried out for a certain property, combined with agreement of results within that number [13]. Especially the evaluation of biodegradability data is complex, as is illustrated by the example in Table 2. The evaluation criteria used for the quality assessment of data in the European EINECS database are described in the technical guidance documents [8].

Whereas the Kollig method does not yield a final judgement of reliability, the Dutch database study has modified the Kollig procedure to provide a reliability score. The Klimisch method does provide a final judgement, but it does not give a detailed set of criteria. Finally the EFDB and EINECS categories are qualitative rather than quantitative.

MODELS

A large number of mathematical models (rate constant-, clearance-, fugacity-, physiological-, and pharmacokinetics-based) has been applied to laboratory bioconcentration and bioaccumulation studies [20,21] and food chain transfer in the field situation [22,23]. In Table 3, we have listed a selection of models and their mean features, as described in recent literature. The focus is on generic screening and aquatic models. The models range from simple screening models (EUSES, EQC level I III), which include only abiotic compartments, and are mainly used to identify the environmental compartment of concern (see example calculated with EPIWIN software shown in Fig. 2), to more elaborate multicompartment chemical fate models and biotic models including food chain transfer.

Table 4 provides an overview (noncomprehensive) of the main compounds tested or validated with the different models. With respect to compounds with ED potency, most of the information is available for di- and mono-*ortho*-substituted PCBs and dioxins. In most of the validation studies the non-*ortho*-substituted PCBs with ED potency were not addressed. In two studies, TBT was considered. Natural or synthetic estrogens were addressed only in the study of Lai and coworkers [6]. Predicted concentrations were 2 to 3 orders of magnitude below reported concentrations from experimental studies. The authors attributed this to the lack of reliable data on biodegradation rates in sediments and biotransformation rates in biota for a proper estimation of model input-parameters.

No studies could be identified, in which other than screening level models were applied to the compound categories of phthalates and brominated compounds.

With respect to the modeling of EASs, it is important to recognize that organisms in their normal development travel through various distinct stages of sensitivity accompanied or even directed by hormonal control. Examples are early life stages, and more specifically development of gonads, or egg maturation; many aspects of reproduction, such as (temperature-dependent) gender determination (in amphibians); and migration. Exposure to endocrine disruptors during such stages can result in highly specific effects. Therefore, exposure models for aquatic organisms should take into account such stages, e.g., through measuring or predicting the dose in target tissues.

Table 3 Summary and main features of recent environmental exposure models.

	C/f	Nr. of abitoic media	Dimensions	Steady state/ dynamics	Hydro dynamics	No. of trophic levels	Generic	Validated	Skills required	Availablity of model	Refs.
Screening models											
EUSES	c	4	1D	s	–		g		low	+	[10]
EQC-based models levels I-III	f	>4	1D	s	–		g		low	++	[15,24]
Simplebox 2.0	c	4	1D	s	–		g		low	+	[25]
Chemical fate models											
QWASI	f	>4	2D	s/d	+		l	+	high	+	[26]
ECOS	c	>4	3D	s/d	+		g	+	high	+	[27]
DELWAQ	c	>3	3D	s/d	+		g	+	high	+	[28]
EXAMS	c	>4	3D	s/d	+		g	+	high	++	[29]
Models including food web											
Thomann model	c	3	1D	s	–	4	g	+	high	–	[30]
TOXFATE	f	>4	2D	s/d	+	3	l	+	high	–	[31]
EMC food web model	f	3	1D	s	–	4	g	+	high	++	[6,32]
ECOFATE	f	3	1D	s/d	–	4	g	+	high	++	[33,34]
TBT freshwater	c	3	1D	s	–	4	l	+	high	–	[35]

Explanation: C/f: concentration or fugacity-based model; No. of media: number of abiotic main and subcompartments included (water column, sediment, air, soil, particulate matter); Hydrodynamics: ability to cope with more complex marine hydrodynamic features in estuarine enviornments; Generic: generic model (g) or location specific (l); Availability: ++ freeware downloadable, + commercially available, – not easily available

Although some examples exist of age specific models, e.g., for PCB biomagnification in the food web in the Seine [36], life-stage specific predictions are not possible in most of the current models.

Table 4 Overview of compounds tested or validated in chemical fate or food web models.

Model	Compounds tested or validated	Refs.
Screening models		
EQC-based models levels I–III	Chlorobenzenes, LAS	[15]
Simplebox 2.0	Triazines, BTEX, vinyl chloride, TCA, TCE	[25]
Chemical fate models		
QWASI	(v) PCBs, PAHs, antifoulants	[26,37]
ECOS	(t) Cd, PAHs, APEO, pesticides	[27,38,39]
DELWAQ	(t) Trace metals, PCBs	[28]
EXAMS	(t) PCBs, TBT, Seanine	[40]
Models including food web		
Thomann model	(v) PCBs, chlorinated pesticides, PAHs	[3,4,30]
TOXFATE	(v) PCBs, Mirex	[31]
EMC food web model	(v) PCBs, dioxins, (t) natural and synthetic estrogens	[6,32]
ECOFATE	(v) PCBs, dioxins, chlorinated pesticides	[33,34]
TBT Freshwater	(v) TBT, TPT	[35,41]

(t) Model tested; (v) model validated with reasonable match between predicted and measured values.

RISK ASSESSMENT

Environmental risk assessment of substances is based on an evaluation of exposure pathways and concentrations on the one hand and identification and selection of sensitive endpoints on the other. The concept is operationalized by comparing real or estimated (predicted) exposure concentrations (PECs) with calculated no-effect concentrations (NECs). The basic approach is similar in European and North American legislation, and has been adopted by industry [42,43]. The comparison can be implemented by calculating the quotient of exposure and NEC. If the quotient is less than, e.g., one, then the substance poses no significant risk to the environment. If the quotient is greater than, e.g. one, the substance may pose a risk, and further action is required, e.g., a more thorough analysis of probability and magnitude of effects will be carried out. Other thresholds than unity may be defined. The difference between the actual ratio and a chosen threshold is often referred to as the margin of safety. The principle outlined above assumes that threshold doses exist for endocrine-disrupting compounds, an assumption that has been questioned recently [44].

Miyamoto and Klein [2] have reviewed the risk assessment procedure and its pitfalls in particular for endocrine disruptors. Critical aspects are the assessment factors used, the poor understanding of endocrine-disrupting mechanisms, the huge differences in potencies, and the conflict of high potency even at levels below analytical detection limits (as may occur for, e.g., the synthetic hormones). Despite these pitfalls and the limited database available for EDC, current risk assessment methods are believed to be valid for estrogenic agents [43].

For the derivation of the NEC, several approaches have been proposed. Generally, these can be categorized into three distinct assessments: a conservative, a distributional, and a mixture toxicity approach. In conservative approaches, usually the most (realistic) sensitive endpoint (e.g., LC_{50}, NOEC) known is taken and divided by an uncertainty factor (e.g., 10 or 100 or 1000). The uncertainty factor value selected depends on the type of endpoint and the number of available endpoint data, and is applied to account for laboratory to field extrapolations, species differences in sensitivities, and similar uncertainties.

In distributional approaches, a series of, or all available literature data are taken and a selected cut-off value is applied to the distribution of these data. The cut-off value may be, e.g., the concentration value that will protect 95 % of the species (tested) [45]. In general, again an uncertainty factor (usually of 10) is then applied to take into account species differences.

In the mixture toxicity approach, a similar mode of action is assumed for the assessment of the combined (additive) effect of different compounds present in a medium. All relevant mixture components are scaled relative to the most potent one. This results in relative potencies for each component. The total effect of the mixture is then calculated by summing the products of concentration and relative potency for each component.

Mixture toxicities

It is obvious that endocrine disruptors can occur in the environment as complex mixtures. In risk characterization studies the toxicity of individual constituents of such mixtures, whether assayed in acute and chronic toxicity, or in estrogenicity tests, is being considered to occur—for each separate endpoint—through the same separate mode of action, and consequently to be additive.

Thus, relative potencies can be established for each individual constituent of a mixture, referring to, e.g., the toxicity of a single compound. For example, for estrogens in general, this would be 17ß-estradiol. The relative potencies are often referred to as toxic equivalent factors (TEFs), analogous to the concept that has first been applied to the toxicity of chlorinated aromatic hydrocarbons (e.g., chlorinated dioxins) that exhibit Ah-receptor mediated toxicity. A criticism of such additive approaches is that antagonistic activities are not accounted for [43].

Calculation of the estrogen equivalent concentration (EEQ) of a chemically determined mixture is based on all measured xenoestrogens with a known estradiol equivalency factor (EEF; Table 6) according to

$$EEQ_i = C_i \times EEF_i, \text{ and } EEQ_t = \Sigma\, EEQ_i$$

where i refers to compound i in the mixture with concentration C, and EEQ_t is the total EEQ. The EEFs are usually expressed on a molar basis because this is toxicologically more relevant than expressing concentrations on a weight basis.

Several studies have shown that additivity is indeed observed for estrogenic compounds (see, e.g. [46]). As an example, the assessment of nonionic surfactants of the alkylphenol ethoxylate type is discussed below. The reference compound in this case is usually nonylphenol, NP, which is one of the degradation products of NPEOs.

NPEOs are surfactants used in industrial and—formerly—household cleaning products. Commercially, they are manufactured and supplied as mixtures of oligomers and isomers.

Estrogenic activity has been observed for NP, t-octylphenol (OP), $NPEO_{1-2}$ (nonylphenol mono- and diethoxylate) and some of the carboxylated degradation products (NPECs) [47].

Until now, there is no direct evidence in the literature whether or not the mode of toxic action for NP, NPEO, and NPEC is the same. In fact, higher NPEO oligomers may well have a different mode of action than NP, because the mechanism is likely a physical surfactant effect [47]. Despite this, for, e.g., acute or chronic toxicity endpoints, NP equivalent factors have been proposed for NPEO and NPEC.

In one approach [48], NPEO and NP toxicities were collected and combined into a quantitative structure-activity relationship (QSAR). To that end, 26 available acute LC_{50} (48 or 96 h) and EC_{50} data for aquatic organisms (including fish, insects, algae) were regressed versus the number of EO units, N_{EO}.

The QSAR thus derived is shown in eq. 1:

$$\text{Ln}\,\{LC_{50} \text{ or } EC_{50}\} = 0.27\, N_{EO} + 0.17 \qquad n = 26 \qquad r^2 = 0.9331 \qquad (1)$$

The relative potency can now be derived from this equation. The risk characterization then proceeds by calculating the total concentration of the sample (expressed in equivalents of NP) as follows (eq. 2):

$$\text{Total concentration} = \text{PEC} = \Sigma\{[\text{OP}] + [\text{NP}] + [\text{NPEO}_1]/1.3 + [\text{NPEO}_2]/1.7 + [\text{NPEO}_3]/2.3 + \ldots[\text{NPEO}_{15}]/57.6\} \quad (2)$$

As eq. 2 shows, the relative potency of $NPEO_1$, as derived from eq. 1, is (1/1.3) = 0.77 times that of NP. The toxicity of octylphenol is considered equivalent to that of NP in this approach, and hence its relative potency is equal to 1. NPEC were not included in this assessment.

In the Environment Canada distributional approach to characterize risks of NP, NPEO, and NPEC [47], relative toxicities were proposed based on categorizing acute and chronic toxicities. A similar approach was used in that study to provide relative estrogenicities. Both are listed in Table 5.

Table 5 Relative potencies of alkylphenol ethoxylates and their carboxylated degradation products proposed by Environment Agency UK and Environment Canada.

Substance	Relative toxicity to NP[a]	Relative toxicity to NP[b]	Relative estrogenicity to NP[b]
NP	1	1	1
OP	1	1	4.1
$NPEO_1$	0.77	0.5	0.67
$NPEO_2$	0.59	0.5	0.67
$NPEO_{3–17}$	0.017–0.43 ($NPEO_{15}$–$NPEO_3$)	0.005	0 (0.02)
NPEC	–	0.005	0.63
$NPEC_2$	–	0.005	0.63
OPEC	–	0.005	0.63
$OPEC_2$	–	0.005	0.63

[a]From Environment Agency UK [48].
[b]From Environment Canada [47].

It can be concluded that, concerning aquatic toxicities, in the Environment Agency (UK) assessment higher relative potencies were attributed to the longer chain ethoxylates (N_{EO} = 4 to N_{EO} = 15) than in the assessment made by Environment Canada. This may be due to several reasons: the UK assessment considered only acute toxicities, whereas the Canadian considered both acute and chronic ones. Moreover, in the Canadian assessment many more data (>200) were considered than in the UK assessment, and weighing factors were applied related to the confidence in studies.

In the preceding derivation of relative potencies, aquatic toxicity was considered as an endpoint. The finding that environmental contaminants including AP and APEO, can bind to the estrogen receptor (ER) in various species and thus regulate the activity of estrogen-responsive genes has raised concern. Similarly to the relative potencies for toxicity, relative potencies for receptor binding, vitellogenin induction, and several other ER-mediated responses, such as those from the in vitro tests presented in Table 6, have been derived. In the Canadian assessment the relative estrogenic potencies shown in Table 5 have been used, which are a weighted mean of several ER-mediated responses [47]. Such relative potencies must be used with some care, as they depend of course on the endpoints considered. Moreover, it has been shown that in vitro potencies can differ substantially from in vivo data, also for APEO [49]. In Table 6, examples are given of the several relative estrogenic potencies (estradiol equivalent factors, EEFs) of different estrogens and xenoestrogens, observed in three in vitro assays.

Table 6 The mol-based estradiol equivalency factors (EEF, estrogenic potency relative to estradiol) used for calculation of the estradiol equivalents (EEQs) in mixtures of chemically analyzed xenoestrogens.

Code	Compound	ER-CALUX		YES assay	ER binding
		EEF[a]	REF	EEF[b]	EEF[b]
E2	17β-Estradiol	1	[50]	1	1
E2-17α	17α-Estradiol	0.016	[50]	0.01	0.11
E1	Estrone	0.056	[50]	0.1	0.07
EE2	17α-Ethynylestradiol	1.2	[50]	1.2	0.8
BPA	Bisphenol A	7.8E-06	[50]	1.0E-05	1.0E-03
DMP	Dimethylphthalate	1.1E-05	[50]	1.0E-06	0
DEP	Diethylphthalate	3.2E-08	[50]	5.0E-07	5.0E-07
DBP	Di-*n*-butylphthalate	1.8E-08	[50]	1.0E-07	
BBP	Butylbenzylphthalate	1.4E-06	[50]	1.0E-06	
DEHP	Di(2-ethylhexyl)phthalate	<6.0E-07	[49]		
DOP	Dioctylphthalate	<6.0E-07	[49]		
NPEO	Nonylphenol ethoxylates	3.8E-06	[50]	4.0E-06	1.0E-05
OPEO	Octylphenol ethoxylates	<6.0E-07	[49]	4.0E-06	4.0E-06
NP	4-Nonylphenol	2.3E-05	[50]	5.7E-04	5.0E-04
OP	4-t-Octylphenol	1.4E-06	[50]	1.0E-05	5.0E-05
BDE47	2,4,2′,4′-Tetrabromodiphenylether	2.0E-07	[51]		
BDE85	2,3,4,2′4′-Pentabromodiphenylether	2.0E-07	[51]		
BDE99	2,4,5,2′4′-Pentabromodiphenylether	2.0E-07	[51]		
BDE100	2,4,6,2′4′-Pentabromodiphenylether	2.0E-05	[51]		
T3-like OH-BDE	1,3,5,3′-Tetrabromo-4′-hydroxy-diphenylether	1.0E-04	[51]		
o,*p*′-DDT	2-(*o*-Chlorophenyl),2-(*p*-chlorophenyl)-1,1,1-trichloroethane	9.1E-06	[52]		
o,*p*′-DDE	2-(*o*-Chlorophenyl),2-(*p*-chlorophenyl)-1,1-dichloroethene	2.3E-06	[49]		
	Methoxychlor	1.0E-06	[52]		
	Dieldrin	2.4E-07	[52]		
	Endosulfan	1.0E-06	[52]		
	Chlordane	9.6E-07	[52]		
	Genistein	6.0E-05	[52]		

[a]Ratio of EC_{50} (17β-estradiol)/EC_{50} (compound) in the ER-CALUX in vitro test.
[b](From ref. [50]); blank values correspond to nontested chemicals.

VALIDATION

Undoubtedly, one of the weakest links in environmental fate modeling and risk assessment is validation. There are several reasons for this. First, monitoring of environmental exposure concentrations can be very expensive, certainly in the case of identification and quantification of transformation products. Such measurements require expensive analytical equipment with trained personnel. In remote areas, such as open oceans, the Arctic and Antarctic, or high-altitude locations, sampling equipment (e.g., shipping time, airplanes) becomes a limiting factor. Second, in the case of endocrine disruptors, a multitude of compound mixtures appears to be active either as estrogens, androgens, or their anti-active counterparts. Therefore, complex mixtures have to be identified and included in the assessments including congeneric and isomeric compounds (as in, e.g., halogenated aromatics, surfactants) and their transformation products.

Seemingly small differences in chemical structure may have large consequences for endocrine-disrupting potency. Fingerprint patterns of such mixtures in real field samples may be quite different from the ones for which test results are available.

Third, the sampling design and parameter choices in regulatory monitoring programs in general are not in line with requirements of model validation studies. Various organizations such as the European Chemical Industry (CEFIC) have addressed this issue [53]. With the increased use of models in risk assessment, harmonization of regulatory monitoring programs and requirements for model validation studies is of great importance. Most aquatic models use freely dissolved water concentrations as one of the major input variables. In monitoring programs, usually only total water concentrations are measured, which also include the fractions bound to suspended matter, phytoplankton, and dissolved organic carbon. For hydrophobic compounds, this overestimates the freely dissolved fraction.

In a recent study, the bioaccumulation of steroid estrogens in aquatic organisms was estimated with a food web model [6,54]. The model calculations resulted in much lower predicted bioconcentration factors than those that are actually observed in the field. The authors concluded that the models required further development and additional data, including accurate transformation rates and understanding of factors controlling uptake. Moreover, they stated that the current absence of field data on estrogens in sediments might lead to inaccurate model outputs as experimental partitioning data are being used instead [6,54].

In reviews of evidence of endocrine disruption in wildlife (e.g., [55,56]), it was concluded that reported studies on wildlife are limited to very few animal species, and that effects at the population level are scarcely observed. Recent monitoring surveys in Europe have confirmed that evidence for endocrine disruption in wildlife is probably confined to hot spots [57,58].

CONCLUSION

The exposure assessment of endocrine disruptors suffers from a huge lack of reliable data, of both values that are used as input parameters in exposure models, and field data that are needed for validation purposes. In general, for the more classic EASs, such as PCBs, *p*,*p*′-DDE, chlorinated dioxins, some pesticides, and organotins, reliable data are sufficiently available, but careful evaluation of the quality of databases is necessary. Several data quality evaluation systems have been proposed. For the "newer" compounds, only few data have been gathered so far. The latter compounds include alkylphenols, bisphenol A, brominated flame retardants, phytoestrogens, and, in particular, natural and synthetic hormones, which in view of their high estrogenic potency could be the most important compounds in terms of risk. Research programs aimed at the endocrine disruption issue must focus on filling these gaps by promoting experimental studies for generation of reliable, high-quality parameter data on the one hand, and surveys or monitoring campaigns for collection of representative field data on the other. The non-specificity of possible effects caused through endocrine mechanisms implies that in order to reveal dose–response relationships all, or at least as many as feasible, potentially active agents need be included in the risk assessments. Hence, it is obvious that comparable quality (i.e., number of data as well as reliability) of parameter and validation data for each of these agents is indispensable. EASs show a large variety in properties critical for environmental fate and therefore may elicit totally different environmental behavior. This may lead in turn to large differences not only in environmental exposure concentrations, but also in which target organisms can be potentially at risk.

The suitability of current exposure assessment models for EASs at this moment seems to be restricted to the persistent compounds such as PCBs, PCDDs, and PCDFs. Especially for the compounds subject to biodegradation and biotransformation the lack of experimental data to derive model-input parameters and perform validation studies at this moment is one of the main obstacles for the application of generic exposure models to other EASs. Current regulatory monitoring programs should be evaluated and harmonized with validation requirements of models used in exposure assessment.

Finally, most of the current models do not allow life stage-specific predictions. As EASs may affect early life stages (development of gonads, or egg maturation) there is a need to include life stage-specific predictions in exposure models.

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