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Regulation of endocrine-disrupting chemicals: Critical overview and deficiencies in toxicology and risk assessment for human health

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Regulation of endocrine-disrupting chemicals is reviewed in terms of hazard assessment (regulatory toxicology) and risk assessment. The current range of regulatory general toxicology protocols can detect endocrine toxicity, but specific endocrine toxicology tests are required to confirm mechanisms (e.g. oestrogenic, anti-androgenic). Strategies for validating new endocrine toxicology protocols and approaches to data assessment are discussed, and deficiencies in regulatory toxicology testing (e.g. lack of adrenocortical function assessment) identified. Recent evidence of a role of prolactin in human breast cancer also highlights deficiencies in regulatory evaluation. Actual human exposure to chemicals and the high-exposure example of chemicals in body-care cosmetics is reviewed with reference to evidence that common ingredients (e.g. parabens, cyclosiloxanes) are oestrogenic. The hypothesis and epidemiology concerning chemical exposure from body-care cosmetics (moisturizers, lotions, sun screens, deodorants) and breast cancer in women is reviewed, applying Bradford-Hill criteria for association and causality, and research requirements are identified.

Key words: oestrogen; anti-androgen; antithyroid; adrenal; hyperprolactinaemia; EDSTAC; OECD; REACH; cosmetics; parabens; siloxanes; pesticides; endocrine disruption; development; cancer.

Regulations concerning non-pharmaceutical chemical safety have evolved over the past half-century to protect human and environmental health. The general regulatory process concerned with human health can be divided into two main

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activities, the first being regulatory toxicology, or hazard assessment, and the second is risk assessment where the results of such toxicity tests are applied to the human situation. Fundamentally, in regulatory risk assessments applied to human health, the relevance of the toxicity findings and the dose levels at which they occur are related to human exposure to the chemical, and margins of safety are calculated which are then used to set exposure limits and controls (exposure to a chemical is an important aspect and obligatory prerequisite for the induction of toxicity). Risk assessment also takes into account the nature of the toxicity (e.g. severity and type of pathology, reversibility, mechanisms of toxicity, evidence of species–specific effects, sex and age differences in sensitivity, dose-response relationships, acute versus chronic effects, evidence of tolerance, special cases of carcinogenicity, mutagenicity and teratogenicity, etc.) as well as human groups at high risk of exposure (occupational exposures, children) and subgroups with expected altered sensitivity or response (e.g. in pre-existing disease, genetic/metabolic polymorphism, chemical allergy and sensitization). Essential to this is the extrapolation of findings in laboratory species to humans, and assumptions are made on the relevance of the animal model and potential differences in human dose sensitivity.

Regulatory toxicology studies are relatively uniform in design, whether they are part of the pre-clinical toxicology package of a new pharmaceutical prior to patient clinical trials or an industrial chemical, and whilst there is a good record for predicting general toxicity in animal models, there are differences in physiology, not least endocrinology, and expression of the toxic response. In such cases, toxicology is predictive of general potential. For example, a compound shown to be oestrogenic by inducing a uterotrophic response in rodents would be considered potentially oestrogenic in humans; however, it would not necessarily be expected to also increase uterus weights in women, but could be considered a risk to embryo/foetal development and women at high risk of breast cancer. Comparative aspects of endocrine and hormonal toxicology and interpretation of the significance of findings and mechanisms of toxicity can be found in Harvey et al [1].

REGULATORY TOXICOLOGY (HAZARD ASSESSMENT)

The process of hazard assessment starts with internationally agreed lists of mammalian toxicology studies that must be conducted on a particular type of chemical, for example an agrochemical, to support registration and safe use. These toxicity tests usually cover (1) acute single high-dose exposures; (2) repeat dose administration for up to 12 months for chronic toxicity studies, which are often preceded by daily repeat dose studies of 1 month's (subacute) or 3 months' (subchronic) duration and usually conducted in rodents and non-rodents; (3) carcinogenicity studies in two rodent species; (4) reproductive toxicology (multigeneration study); (5) developmental toxicology (teratogenicity studies in two species); (6) genetic toxicology studies in vitro and in vivo; and (7) additional studies conducted on a case-by-case basis, such as neurotoxicity testing or indeed evaluation for oestrogenic and other endocrine activities.

National regulatory bodies have specific guidelines detailing what studies must be completed to support a chemical registration (e.g. the United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and

Toxic Substances (OPPTS) regulates agrochemicals, public health pesticides and high production volume industrial chemicals, and similar agencies exist in other countries) and also the specific protocols that should be followed to ensure a quality standard. Similar guidelines have been adopted internationally, and the Organization for Economic Cooperation and Development (OECD) has a standardized set of toxicology guideline protocols which specify the benchmark standard for acceptable toxicity test designs. The specific protocols from such guidelines (USEPA or OECD) give advice on dose level selection, species, number of treatment groups, number of animals per sex per group, route and frequency of exposure, in-life measurements (clinical observations, body weights, food consumption), blood clinical chemistry and haematology, urine analysis, macroscopic findings at necropsy and microscopic histopathology evaluation of all organs and tissues. Repeat dose toxicity studies (from sub-acute to chronic exposures) are capable of detecting toxicity to the endocrine system through organ weights and macroscopic and microscopic histopathology (hormone evaluation is not included in standard protocols).

The route of exposure of regulatory toxicity tests must be relevant to the human route of exposure, and the majority of tests on chemicals are conducted by the oral route, with additional studies conducted by the dermal or inhalation route as appropriate. Whilst some extrapolation between routes of administration can be made with regard to general systemic toxicity, significant human exposure to the chemical by any route other than the oral route warrants specific regulatory toxicology using this route (in addition to the oral route if necessary). A second important consideration in regulatory toxicology concerns the selection of dose levels: as toxicity is dependent on dose and time (dose is usually expressed as mg/kg body weight/day of the test chemical) the validity of a regulatory test is dependent on achieving the maximum tolerated dose (MTD). In the absence of other dose-limiting toxicity, the MTD is usually defined as the dose level shown to produce a 10% reduction of body weight gain over the duration of study (although other signs of toxicity may be considered) and is established through a progression of short-term to long-term studies each refining the high dose according to increasing exposure duration. The MTD is essentially a 'within-study' quality control criterion providing evidence that an effective dose challenge has been made (i.e. a sufficiently high dose has been evaluated), but with an emphasis on tolerance, it ensures that confounding data are not produced that will make interpretation of primary chemical effects difficult, due to excessive toxicity and major non-specific physiological perturbation. The MTD is often set for threshold effects as the highest dose that can be reasonably tolerated by the test species, since treatment at higher dose levels would not be tolerated (a typical example would be the onset of convulsions with a neurotoxic compound at dose levels above a trigger threshold).

All regulatory studies must be conducted to international standards of good laboratory practice (GLP), and the studies, raw data (including electronic and calculated data), archives and report are all subject to independent quality assurance audit and monitoring by governmental agencies. In the UK, the GLP monitoring authority is the Medicine and Healthcare Product Regulatory Agency (MHRA) who also audit laboratories conducting non-pharmaceutical industrial toxicology. The results and reports of such toxicology studies are compiled into a regulatory submission at National (e.g. USEPA) or Supranational (e.g. European commission) regulatory agencies, and the process of evaluation of the study final reports and risk assessment proceeds (at this stage inadequate guideline toxicology reports will be rejected).

Table 1. Comparison of the process of hazard and risk assessments, potential human exposure, and regulation of non-pharmaceutical chemicals with potential endocrine actions.

Type of chemical	Route of exposure	Opportunity of exposure	Hazard assessment (toxicology)	Risk assessment	Regulatory agency
Body-care cosmetic	Dermal	Very high. Repeat application of multiple products to skin. Absorption of chemical formulants	Minimal requirement for any regulatory toxicology studies	Self-certification by industry risk assessor. Prohibited materials, limits and conditions of use of certain materials imposed by regulatory bodies. IARC assessment of some ingredients	Cosmetic ingredient review expert panel (overseen by USFDA). EC Directive 76/768/EEC-compliance guidance provided by SCCNFP
Public health pesticide	Dermal, inhalation, oral	Low if label instructions followed	Extensive toxicology requirements-USEPA OPPTS and OECD-including repeat-dose chronic toxicity, carcinogenicity, genetic toxicity, teratogenicity, reproduction (these protocols would identify endocrine toxicity) and special investigations e.g. for neurotoxicity and EDSTAC for endocrine evaluations. GLP requirements	Extensive independent toxicology evaluation within USEPA, EC and National agencies. Also WHO and IARC evaluation	USEPA, EC, National Agencies e.g. Japanese MAFF, UK PSD, UK HSE (biocides under 98/8/EC). Numerous statutory instruments for toxicology and risk assessment and regulation
Agrochemicals	Oral (dermal and inhalation in operators)	Low. Food residues monitored. Label use restrictions enforced	Extensive toxicology requirements-USEPA OPPTS and OECD-including repeat-dose chronic toxicity, carcinogenicity, genetic toxicity, teratogenicity, reproduction (these protocols would identify endocrine toxicity) and special investigations e.g. for neurotoxicity and EDSTAC for endocrine evaluations. GLP requirements	Extensive independent toxicology evaluation within USEPA, EC (including EC Scientific Committee on Food) and National agencies. Also WHO/FAO, (JMPR, JECFA) and IARC evaluation	USEPA, EC, National Agencies e.g. Japanese MAFF. Numerous statutory instruments (FQPA, SDWA) ensuring thorough toxicology and risk assessment and regulation

(*continued on next page*)

Industrial chemicals high volume	Oral (dermal & inhalation in operators)	Low. Processes contained. Occupational accidents. Environmental spillage.	As for agrochemicals		
Drinking water chemicals	Oral, dermal (low inhalation potential)	Usually low in developed countries. Disinfection byproducts. Ozone removal	Toxicology databases on commonly utilized chemicals. Further evaluations as necessary	Extensive independent evaluations and permissible levels. Also WHO and IARC evaluation	Drinking water regulations, USEPA, SDWA, EC, WHO. National agencies e.g. UK Drinking Water Inspectorate

IARC, International Agency for Research on Cancer; USFDA, United States Food and Drug Administration; EC, European Commission; USEPA, United States Environmental Protection Agency; OPPTS, Office of Prevention, Pesticides and Toxic Substances (USEPA); OECD, Organization for Economic Co-Operation and Development; EDSTAC, Endocrine Disrupter Screening and Testing Advisory Committee (USEPA); GLP, Good Laboratory Practice; WHO, World Health Organization (United Nations); MAFF, Ministry of Agriculture, Fisheries and Food; SCCNFP, The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers; UK PSD, United Kingdom Pesticides Safety Directorate; UK HSE, United Kingdom Health and Safety Executive; FAO, Food and Agriculture Organization (United Nations); JMPR, Joint Meeting on Pesticide Residues (Joint WHO/FAO evaluation procedure to set Acceptable Daily Intakes [ADIs] for chemicals based on toxicological margins of safety); JECFA, Joint Expert Committee on Food Additives (WHO/FAO); FQPA, Food Quality Protection Act; SDWA, Safe Drinking Water Act.

COMPARISON OF REGULATORY APPROACHES FOR DIFFERENT CHEMICAL TYPES

Whilst a complete regulatory toxicology data package is required for new chemicals considered to result in human exposures (for example agrochemicals with a potential exposure of operators, and consumers to low level residues in food) many older chemicals have a deficient or grossly inadequate toxicology data package. Table I compares hazard and risk assessment approaches to regulation of non-pharmaceutical chemicals compared with potential human exposure; whilst not intended to be exhaustive, it does demonstrate the stringent requirements for some types of chemicals (e.g. agrochemicals) compared with the lack of independent assessment for others (e.g. cosmetics). Cosmetics represent a regulatory anomaly; on the one hand human exposure to the chemicals in these increasingly sophisticated formulations is high due to actual chemical content [2–4] and regular direct application (e.g. dermal application of deodorants, sun lotions, moisturizers-including to children), yet the system allows for self-regulation/certification by an industry risk assessor. Cosmetics toxicology testing in animals has largely been abolished in the UK and other EU states on ethical grounds, but there are in vitro techniques available which are particularly amenable to endocrine toxicology, and steroid receptor-mediated endocrine and hormonal effects can be assessed in both human cells and other in vitro systems. A significant number of chemicals used in cosmetic formulations have been shown to be oestrogenic using these techniques, and structure/activity relationships are well developed for oestrogenic activity[5,6], allowing screening of chemicals for structural alerts. Cosmetic risk assessment in the light of recent toxicology is discussed later.

Gad[7] has reviewed all the major compound categories (human pharmaceuticals, animal health products, medical devices and materials, agrochemicals and pesticides, food additives, cosmetics, over-the-counter health products, nutraceuticals, industrial chemicals etc.) and their regulation in the US, Europe and Japan, including toxicity testing requirements and legislation. However, although a complete and modern dossier is likely to detect an endocrine-disrupting chemical through repeat dose studies and microscopic histopathological examination of samples of 50 or more organs and tissues (including the glands that comprise the endocrine system, their target tissues, and the reproductive system) and specialist mammalian toxicology studies such as two-generation reproduction studies and developmental toxicity studies[8], this package of regulatory studies has been judged to be deficient by USEPA who have forwarded recommendations for specific separate studies for endocrine toxicity.

SPECIFIC REGULATORY ENDOCRINE TOXICITY TESTS

The background to the move by USEPA to instigate a programme of additional endocrine toxicity testing developed out of the US food quality protection act (FQPA) which specifically identified endocrine-disrupting chemicals as a risk to human health. USEPA convened the Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) to forward recommendations on the approaches (study designs, endpoints, validation and data interpretation) to toxicological evaluation of chemicals for endocrine-disrupting properties. EDSTAC[9] published its recommendations for such studies, and the various protocols are now in a validation stage and provide an additional level of specialist safety evaluation of a range of chemicals. The range of tests

Table 2. United States Environmental Protection Agency (USEPA) Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) recommendations for additional endocrine toxicity tests.

Tier 1 screening battery
In vitro:
Oestrogen receptor binding or reporter gene assay
Androgen receptor binding or reporter gene assay
Steroidogenesis assay with minced testis
In vivo:
Rodent 3-day uterotrophic assay (subcutaneous)
Rodent 14–20-day pubertal female assay
Rodent 5–7-day Hershberger assay (castrated males to detect androgen agonism and antagonism)
Frog metamorphosis assay (anti-thyroid assay)
Fish gonadal recrudescence assay (sexual maturation in response to lengthening days)
Other assays:
In vitro placental aromatase assay
In vivo uterotrophic assay (intraperitoneal)
In vivo adult male assay (anti-androgen effects, full thyroid and reproductive hormone screen)
Tier 2 toxicity testing
In vivo:
Two-generation mammalian reproductive toxicity study in rodents (current USEPA OPPTS protocol)[a] or one of the 'less comprehensive tests'
Less comprehensive tests
Alternative mammalian reproductive test (AMRT-smaller version of the two-generation study)
Developmental toxicity test (current USEPA OPPTS protocols for teratogenicity in mammals)[a]
One-generation test (peri/postnatal study, covers parental reproduction and offspring development)
Test for other animal taxa
Avian reproduction (current USEPA OPPTS protocol); fish life cycle (fat head minnow); mysid life cycle (Americamysis); amphibian development and reproduction

Tier 1 and 2 tests applied to all chemicals and mixtures.
[a] Standard USEPA OPPTS mammalian toxicology requirements (e.g. agrochemicals).

recommended by EDSTAC is designed to cover environmental effects as well as mammalian tests for extrapolation to human safety, and these are summarized in Table 2.

These tests are arranged in two tiers, and are specifically designed to provide a framework for upgrading deficient toxicology databases of the many thousands of chemicals that fall under the regulatory remit of the USEPA. The first tier is a screening battery and includes in vitro and short-term in vivo mammalian toxicity assays such as the rodent uterotrophic assay. This assay is becoming the standard test for oestrogenicity, where an increase in uterus weight in sexually immature/ovariectomized rats induced by a test chemical at a range of dose levels over 3 days dosing, compared with a negative control and oestradiol-17β as a positive control, constitutes a positive result and allows potency relative to oestradiol to be calculated. The second tier includes two-generation mammalian reproduction studies; this core USEPA requirement for a new agrochemical or pesticide is triggered for chemicals lacking this study if tier 1 tests were positive. The newer tests comprising the tier 1 screening battery are completing validation, but methodological refinements continue: for example, the use of immature males rather than surgically castrated males in the Hershberger assay.[10] The significance of results also continues to stimulate debate,

including the extrapolation of low-dose effects to humans[11], which is a key scientific and regulatory issue particularly relevant to endocrine disruption and is discussed later. The significance of long-term low-level exposures to xeno-oestrogens or other endocrine disrupters and human health is unknown[12–15], and there are no human data because appropriate (e.g. epidemiological) studies have not been conducted.

Nevertheless, priority setting for the large number of chemicals that must be screened has commenced within the USEPA; the USEPA's 'endocrine disruptor screening programme: early priority setting activities' can be viewed online.[16] Additionally, the new European Union Registration, Evaluation and Authorization of Chemicals (REACH) system for risk assessment will result in extensive testing of chemicals for endocrine toxicity; endocrine disruption chemical strategy within the European Commission can also be viewed online.[17] An overview of the various regulatory approaches and strategies for endocrine toxicity testing-including the US, EU and role of the OECD-can be found in Cockburn and Leist[8]; this also provides the historical, scientific and statutory background to the various developments in testing for endocrine disruption, and a list of the standard guideline general toxicity tests for both USEPA OPPTS and OECD which are also capable of detecting endocrine toxicity.

The OECD is also validating toxicity tests to incorporate endocrine endpoints, and progress has been made in developing the 28-day repeat dose toxicity study (Test guideline 407) to detect (anti)-androgenic[18,19], (anti)-oestrogenic[20,21] and (anti)-thyroid[22,23] effects of chemicals. Gelbke et al has reviewed the OECD test strategies and methods for endocrine disrupters and the status of the validation of the rodent uterotrophic assay (completed), enhanced test guideline 407 rodent 28-day toxicity study (completed) and the Hershberger assay (near completion).[24] Intra-agency collaborations on validation of a variety of assays for endocrine disruption is discussed by Worth and Balls[25], and a summary of activities in the EU incorporating a survey of stakeholder organizations on the proposed new European Chemicals Policy (REACH) involved with endocrine testing is given by Dandrea and Combes.[26] However, criticisms have been raised concerning the validation process of OECD for the uterotrophic assay.[27] The focus on oestrogenic, anti-androgenic and anti-thyroidal effects and ignorance of adrenocortical effects and the functioning of the integrated endocrine system has been considered a critical regulatory omission[28] and is discussed later. Another important point concerns adequately assessing metabolism of chemicals when employing in vitro test models for endocrine disrupters.[29] Chemicals can be metabolized to inactive forms, but equally, metabolism can result in a more potent range of metabolites. This also has a bearing on route of exposure, where compounds administered orally are generally metabolized by the liver; however, dermal application may result in local tissue absorption, deposition and accumulation in subcutaneous fat that escapes the metabolic capacity of the skin, and the dermal route of exposure is particularly important for some chemicals (the case of chemical exposures from body-care cosmetics is discussed later).

However, there are differences in general philosophy behind the various agency approaches that affect the assessment of data generated by endocrine toxicity tests and ultimately risk assessment. Whilst the USEPA has been primarily driven by legal stimuli in the form of the FQPA, the EU has been more reserved. The focus of the USEPA has been to protect vulnerable subgroups-particularly children-from exposure to endocrine-active chemicals, where the most damaging effects are considered to occur at critical periods during development. There is broad agreement with this philosophy, and Sharpe and Irvine[15], writing in the British Medical Journal, further suggest that exposure in utero poses the greatest risk to human health from endocrine

disrupters, and note that such effects may only manifest in later life; this may have serious implications for determining causal associations. Thus, the primary focus of EDSTAC-recommended endocrine tests has been to evaluate endocrine effects that may affect reproduction and development, and there has been a focus on tests for oestrogenic, anti-androgenic and thyroid effects at the expense of the integrated endocrine system. Additionally, whilst any effect on development is unarguably a serious and potentially irreversible adverse finding, there is uncertainty on the significance of general toxicity within the endocrine system as may be detected in guideline standard repeat-dose toxicity tests, and there appears to be a lack of regulatory guidance. Toxicity to the endocrine system, as with toxicity to any other vital organ, should be fully incorporated into risk assessment, and a weight-of-evidence approach applied, including consideration of the dose response, reversibility, derivation of no-observed-adverse-effect-level (NOAEL) and lowest-observed-effect-level (LOEL) doses based on histopathology (as current general toxicology guidelines do not include hormone measurement of endocrine function) in each test species and study type, from which margins of safety and be extrapolated. See Harvey and Johnson[30] for suggested approaches to assessing toxicity data with endpoints related to endocrine disruption. However, there also is a need for development of existing guideline protocols to include endocrine function of all the various constituent axes and glands of the integrated endocrine system in vivo if endocrine toxicity is to be properly evaluated[31], and this has also been noted to be a critical omission from regulatory endocrine toxicology evaluation strategies.[28,30]

DEFICIENCIES IN CURRENT REGULATORY TESTING STRATEGIES FOR ENDOCRINE DISRUPTION

Current international strategies and toxicity test validation programmes (USEPA-EDSTAC, OECD, EU-REACH) have ignored development of tests for adrenocortical function and the thorough assessment of steroidogenesis.[28] The adrenal is one of the most common toxicological targets[32] and is fundamentally involved in the expression of toxicity.[33] The cases of aminoglutethimide and etomidate in human medicine show the potential of unrecognized adrenocortical toxicity (functional suppression) resulting in deaths from a single dose.[34] Exposure to chemicals may also result in adrenal dysfunction, and this should be investigated under a regulatory framework if endocrine disruption is to be adequately tested.

Similarly, EDSTAC recommends an in vivo adult male assay (to evaluate anti-androgen effects and incorporating a full thyroid and reproductive hormone screen) under tier 1 testing and there needs to be a similar study of females. The importance of this is that conditions such as hyperprolactinaemia in the female may otherwise go undetected; hyperprolactinaemia does not necessarily interfere with reproduction (hyperprolactinaemic female rats will often mate and produce litters) and could be missed in standard reproduction studies. The significance of hyperprolactinaemia is that it can induce rat mammary carcinogenesis, but this was thought to have no relevance to humans.[35] However, recent evidence suggests that prolactin stimulates human breast cancer growth and development[36–40], and it is therefore relevant to establish whether chemicals cause secretion of prolactin as this could be a risk to women with existing or undiagnosed breast cancer. From this, there is also a need to reconsider the toxicological relevance of hyperprolactinaemia.

The following tests are suggested to cover the inadequacies in current regulatory endocrine testing strategies.

1. *In vitro evaluation of steroidogenesis in adrenocortical cells*. The H295R human cell line has a complete steroidogenic capability from steroid acute regulatory (StAR) protein through to synthesis of aldosterone, with all major steroids in the pathway available for evaluation (see discussion in Harvey and Everett[28]).
2. *In vivo evaluation of pituitary-adrenocortical function in the rat*. Corticosterone measurement in blood could be added to the current EDSTAC-recommended tier 1 in vivo adult male assay or the tier 2 two-generation reproduction study.
3. *In vivo evaluation of prolactin (and other hormones) in the adult female against stage of oestrous cycle*. This could be added to the tier 2 two-generation reproduction studies in rodents or conducted as a short stand-alone study.

Thus, in vivo data could be obtained from existing studies by extension of the range of measurements and endpoints rather than by using more animals. Due consideration of confounding factors is required, such as stress disturbance for both prolactin and corticosterone measurements in rats, and correcting hormones against cycle stage in females.

APPROACHES TO RISK ASSESSMENT: REGULATED VERSUS UNREGULATED CHEMICALS

A typical new chemical entity for example an agrochemical will have a complete toxicology data package consisting of acute, subacute, subchronic and chronic toxicity studies in rodents and non-rodents, together with carcinogenicity, reproductive and genetic toxicity studies[8]; this package is submitted to a regulatory agency such as USEPA or EU. These studies are evaluated and risk assessments conducted. Typically, the critical toxicity and the NOAEL and LOEL in each test species are identified. Once the overall NOAEL dose has been established in mg/kg/day, this can be extrapolated to human exposure; it is convention to apply a 100-fold safety factor for non-pharmaceuticals, which comprises a 10-fold factor for extrapolation from animal to human, and a further 10-fold factor designed to accommodate human variation in sensitivity. For an agrochemical this then becomes the acceptable daily intake (ADI), and total exposure to residues in food should not exceed this; maximum residue limits are also independently set, and these are monitored in all representative foodstuffs. For agrochemicals, the World Health Organization in collaboration with the United Nations Food and Agriculture Organization assess the toxicology data in the Joint Meeting on Pesticide Residues (WHO–FAO JMPR) and also confirm the ADI (this is useful worldwide as many countries lack regulatory resource).

The outstanding issue at present is whether an endocrine effect warrants further regulation. For example, in the EU the philosophy is that endocrine toxicity is another endpoint that should be treated as any other toxicological endpoint in the derivation of margins of safety. Other agencies view oestrogenic or endocrine-disrupting chemicals as being a particular risk to perinatal development and consider application of additional safety factors to protect children, particularly in the current uncertain state of science and regulation. Indeed in the US FQPA called for an additional 10-fold safety (uncertainty) factor with the aim of protecting children's health; FQPA specifically cited

endocrine-disrupting chemicals as a concern, and this was also highlighted in amendments to the US Safe Drinking Water Act (SDWA). USEPA has published a final document on the application of additional safety factors or uncertainty factors[41] to provide an additional level of risk management for children's health. Although these relate to US legislation, regulatory approaches can be adopted internationally to ensure harmonization. Recent post-EDSTAC developments have also identified other risks to children's health from environmental chemicals, including endocrine disrupters, and these include developmental immunotoxicity where a role of the endocrine system in immune system development is also known.[42,43] Of particular note is the role of the hypothalamo-pituitary-adrenocortical axis in immune function[44,45], and that glucocorticoid production is the single most important factor for the survival of an organism post infection or injury.[46] It has been previously noted that adrenocortical function is vulnerable to toxic insult and evaluation is notably lacking in regulatory endocrine toxicology strategies[28], and given the regulatory prominence of both endocrine toxicity and developmental immunotoxicity within the regulatory system (both would be priorities under FQPA and USEPA remit) the lack of test validation for adrenocortical function must be considered a major regulatory omission from both perspectives.

Whilst certain chemical types have complete, modern, regulatory compliant toxicology databases, others are deficient. Those that fall under a general regulatory remit for example USEPA and EU (REACH)-are undergoing a prioritization exercise, regulatory risk assessments (see Johnson and Harvey[47] for European Commission report on 12 priority chemicals), and regulatory requests to conduct endocrine toxicity tests as appropriate when data gaps are identified. One group of chemicals that are not as stringently regulated is the cosmetics, and the constituent chemicals that are used in increasingly sophisticated formulations; the main reason for this is that the risk assessment is largely self-regulated by the cosmetics industry. Additionally, chemical exposures to the general population are uncontrolled.

Concerns have been raised recently that a growing number of body-care formulation ingredients have been shown to be oestrogenic (both in vitro in human MCF7 and ZR-75-1 breast cancer cell lines and in vivo in rodent uterotrophic assays) and the potential this may have to adversely affect human health following repeated exposure.[14] One such group of cosmetic formulation ingredients is the esters of *p*-hydroxybenzoic acid (parabens; hydroxybenzoates) used as preservatives in cosmetics, foods and other products; Table 3 lists the recent studies showing oestrogenic activity in vitro and in vivo of seven of the esters together with the main common metabolite. Table 4 provides a rationale for an approach to a risk assessment for the parabens based on recently published data of their oestrogenic activity in vitro and in vivo, the concentration of parabens found in moisturizers, lotions and other body-care products, application rates, and dermal absorption; the absorbed dose is then corrected and related to the biological potency of oestradiol-17β derived from the numerous studies listed in Table 3. Clearly, even using the most conservative absorption factors and oestrogenic potency factors, there appears to be a significant oestrogenic challenge to the breast from parabens (Table 4). The parabens regulatory toxicology database is deficient and lacks current regulatory guideline-compliant carcinogenicity, reproductive or chronic toxicity studies by any exposure route. EC evaluation has noted deficiencies in the parabens toxicology database to support food uses[66] and recently declined to set an ADI for propylparaben due to endocrine toxicity (see below). Critical toxicology data gaps also have been noted for cosmetics uses of parabens[67], and dermal toxicity studies would be required to investigate the safe use of chemicals in body-care products. More data are also needed on the use of body-care

Table 3. Summary of in vitro and in vivo studies published on the oestrogenic activity of esters of *p*-hydroxybenzoic acid (parabens).

Ester[a]	Result	Reference
Methyl	+ve in vitro (yeast+receptor binding)	[48]
	+ve in vitro (human MCF7)	[49]
	−ve in vitro (rat uterus receptor binding)	[50]
	−ve in vivo (rat uterotrophic)	[51]
	−ve in vivo (rat dietary repeat dose reproductive toxicity study)	[52]
	+ve in vivo (rat uterotrophic)	[50]
	+ve in vivo (mouse uterotrophic)	[50]
Ethyl	+ve in vitro (yeast +receptor binding)	[48]
	+ve in vitro (human MCF7)	[49]
	+ve in vitro (human MCF7)	[53]
	+ve in vitro (rat uterus receptor binding)	[50]
	−ve in vivo (rat uterotrophic)	[51]
	−ve in vivo (rat dietary repeat dose reproductive toxicity study)	[52]
	+ve in vivo (rat uterotrophic)	[50]
	+ve in vivo (mouse uterotrophic)	[50]
Propyl	+ve in vitro (yeast+receptor binding)	[48]
	+ve in vitro (human MCF7)	[49]
	+ve in vitro (human MCF7)	[53]
	+ve in vitro (rat uterus receptor binding)	[50]
	−ve in vivo (rat uterotrophic)	[51]
	+ve in vivo (rat dietary repeat dose reproductive toxicity study)	[54]
	+ve in vivo (rat uterotrophic)	[50]
	+ve in vivo (mouse uterotrophic)	[50]
Butyl	−ve in vivo (rat teratogenicity study)	[55]
	+ve in vitro (yeast+receptor binding)	[48]
	+ve in vitro (human MCF7)	[49]
	+ve in vitro (human MCF7)	[53]
	+ve in vitro (rat uterus receptor binding)	[50]
	+ve in vivo (rat uterotrophic)	[48]
	+ve in vivo (rat uterotrophic)	[51]
	+ve in vivo (rat dietary repeat dose reproductive toxicity study)	[56]
	+ve in vivo (mouse dietary repeat dose reproductive toxicity study)	[57]
	+ve in vivo (rat development and reproductive toxicity study)	[58]
	+ve in vivo (rat uterotrophic)	[50]
	+ve in vivo (mouse uterotrophic)	[50]
Isopropyl	+ve in vitro (human MCF7)	[53]
Isobutyl	+ve in vitro (human MCF7)	[53]
	+ve in vitro (human MCF7; ZR-75-1)	[59]
	+ve in vivo (mouse uterotrophic)	[59]
Benzyl	+ve in vitro (human MCF7; ZR-75-1)	[60]
	+ve in vivo (mouse uterotrophic)	[60]
p-Hydroxy	+ve in vivo (mouse uterotrophic)	[61]
Benzoic acid	−ve in vivo (rat uterotrophic)	[50]
(metabolite)	+ve in vivo (mouse uterotrophic)	[50]

[a] Ester of *p*-hydroxybenzoic acid (common main metabolite).

Table 4. Calculated potential human dermal absorption of esters of *p*-hydroxybenzoic acid (parabens) in a body lotion related to oestrogenic potency and challenge to breast tissue.

Example: body lotion applied by women, container of 125 mL of formulated product with 0.87% of mixed parabens[2] as preservatives, used over 1 month (once daily application). This hypothetical example examines the proportion of product applied to chest and breast only (assumed 4.2 mL) and not other body areas (lotion applied elsewhere on the body is not included). Parabens are used at up to 0.8% (EU) in personal and body-care cosmetics singly (0.4% in EU) or as mixtures of methyl, ethyl, propyl, butyl, isopropyl, isobutyl and benzylparabens.

Application and exposure: lotion 125 mL containing 0.87% parabens = 1.09 g parabens. Assumed daily application over 1 month = 30 applications of c. 4.2 mL per day to the chest/breast. The application of 4.2 mL would provide a dose of 36.3 mg of parabens to breast/chest area per day.

Absorption: Of this 36.3 mg parabens esters, 10% is assumed to be absorbed intact (this is a default assumption: data for propyl paraben suggests up to 30% skin absorption)[62], the remaining 90% of esters are metabolized by skin carboxyesterases to *p*-hydroxybenzoic acid (also weakly oestrogenic; not included in this exposure example). Thus, 3.63 mg of paraben esters are assumed to be absorbed in breast/chest area per day (no data exist on persistence, but intact esters have been detected in human breast tissue).[63,64]

Potency relative to oestradiol: it is assumed that the averaged oestrogenic potency of these parabens esters absorbed intact into breast/chest area is 100 000-fold less than oestradiol-17β (in mice the uterotrophic ED50 of oestradiol-17β was 7 μg/kg compared with 18–74 mg/kg for various parabens, or 2571–10571-fold less potent[50], and in human breast cancer cells in vitro, parabens are ca. 100 000-fold less potent than oestradiol).[49,59,60] Thus, using the 100 000-fold lower potency factor to convert to oestrogenic potential, the 3.63 mg paraben esters would equate to 0.036 μg (36 ng) of oestradiol potency equivalents absorbed by the breast/chest area per day (or 1.08 μg per month, the total oestradiol equivalents in 125 mL lotion). This is significant compared to human breast tissue oestradiol concentrations (see below).

Breast/chest skin area of exposure and tissue concentrations: if it is assumed that the area of breast skin exposed is 500 cm^2, the oestradiol equivalents of absorbed paraben esters would be 72 pg/cm^2 skin/day. It can be assumed that this 72 pg is dispersed in underlying tissue (i.e. square cm surface area inputting to cubic cm of tissue assumed to be 1 cm^3 = 1 g = 1 mL) along concentration gradients to give averaged breast tissue concentration of 72 pg/g oestradiol equivalents from paraben esters. It is important to note that this is the repeated absorbed input into the breast per application (day). This calculated concentration of paraben-derived oestradiol equivalents compares with endogenous concentrations of oestradiol in breast tissue: 0.203 nM oestradiol (55.3 pg/mL = *g* tissue) has been reported in normal breast adipose and an average of 1.28 nM (348 pg/mL = g) in human breast tumours.[65]

Clearly, the daily-calculated input of oestrogenic stimulus into the breast from this example is significant even when using the lower default absorption value and 100 000-fold lower potency than oestradiol. Conversely, this xeno-oestrogenic input could increase 30+times by application of the 30% absorption factor and the 10 000 potency factor (which is actually based on in vivo data)[50]: the absorbed oestrogenic equivalents could then be 2160 pg/g/day or higher if the weakly oestrogenic metabolite *p*-hydroxybenzoic acid is also included.

General considerations, chemical persistence and implications: this calculation of paraben-derived oestrogenic impact uses estimates of data derived from a variety of sources. It is not intended to be exhaustive or definitive, but rather provide an example on which more accurate estimations can be made.

There are no data on accumulation or persistence in human tissue. Parabens are lipophilic and could accumulate in adipose tissue or act on sensitive cells. There may be exposure to a range of other oestrogenic chemicals in other body-care products, and by other routes, adding to both local and systemic body burdens, and combined/total exposure requires consideration. Application variation could produce tissue concentration hot spots. Dermal exposure to parabens is direct, and parabens can escape skin metabolism.[62] This hypothetical model shows that the breast and chest have a significant relative potential daily dermal oestrogenic chemical challenge from body-care products.

Table 4 (*continued*)

This calculated example uses conservative assumptions of paraben absorption and oestrogenic potency, but is based on real data of quantities of parabens used in products.[2] This example uses 4 mL application per day and 0.87% parabens in lotion[2]; calculation for 1 mL used per day on chest/breast, or one quarter of the formulation concentration to 0.2%, gives 72 pg/g/4 = 18 pg/g (using same low absorption and potency) and is still 33% of endogenous oestradiol in human breast adipose and a potentially significant additional mitogenic stimulus to oestrogen sensitive cells. Indeed, mean daily usage of body lotion is recently reported to be 8.70 g in a study of 360 women, which is appreciably more than the value used in this calculation.[88]

Health question: could these paraben concentrations provide direct oestrogenic or cytotoxic challenge to subcutaneous breast cells varying in sensitivity due to age, location, physiology, cell cycle stage or pre-existing (undiagnosed) disease, and is direct local dermal application of non-pharmaceutical oestrogenic chemicals a special case for risk assessment?

products and breast disease because of conflicting results in a small database.[68,69] General research into hypotheses of links between environmental chemical endocrine disrupters and human health effects[12,15], and specifically dermally applied oestrogenic chemicals and breast cancer[14], would benefit from the application of Bradford-Hill[70] criteria for association and causality (see below).

In addition to the parabens, other common cosmetics ingredients, such as the siloxanes or cyclosiloxanes, are present in high proportions in personal care products (octamethylcyclotetrasiloxane is present at 40–60% by weight in antiperspirants and other cosmetics)[71], are dermally absorbed[72], are reported to concentrate in ovaries and uterus of mice following a single subcutaneous injection[73], and are oestrogenic in the rodent uterotrophic assay.[74,75] These could also be added to the oestrogenic inputs to the breast in Table 4.

The wisdom of including oestrogenic ingredients in body-care formulations has been questioned[13,14,76,77], particularly in the light that children and high-risk groups could be exposed to multiple applications of body-care products for prolonged periods, and the lack of knowledge of the effects of long-term low-dose exposures to oestrogenic chemicals. Parabens are readily dermally absorbed and escape skin metabolism[62], have been detected in human breast tissue[63], and are oestrogenic (see Table 3); note that there is evidence of a difference in oestrogenicity between oral and parenteral routes, and parabens can induce a rodent uterotrophic response following dermal application.[60] Body-care cosmetics have been noted to be a potential exposure risk for endocrine-disrupting chemicals[13,14,76,77], and a particular concern raised is exposure of pregnant women and in turn the conceptus in utero.[15] Concerning their food uses, the parabens have received recent regulatory evaluation; the EC Scientific Committee on Food (SCF) had requested further data, noting inadequacies and uncertainties[66] in the existing database concerning developmental toxicity and other findings. Arising from this, the database has been re-evaluated by the European Food Safety Authority (EFSA), and an ADI has been set for methyl and ethyl esters (0–10 mg/kg/day), but an ADI has been declined for propylparaben. The EFSA did not set an ADI for propylparaben because of adverse reproductive and endocrine findings (reduced testosterone secretion), such that a NOAEL could not be established.[78] Studies of other parabens have produced similar results (see Table 3), and these also require incorporation into other (non-food) risk assessments; this could challenge existing regulatory positions and margins of safety depending on the use of these preservatives. The EFSA scientific panel confirmed that only the methyl, ethyl and propyl esters were used in

food, noted that other esters were oestrogenic, but considered the metabolite *p*-hydroxybenzoic acid to be non-oestrogenic[78], which may be a questionable assumption.[50,61]

Related to this is the significance of low-dose effects. Current regulatory strategy and protocols are not designed to necessarily detect ultra-low-dose effects that have been reported for endocrine-disrupting chemicals. Scientific discussion has involved the reproducibility of very low-dose effects of endocrine disrupters and extrapolation to humans.[11,30] Recent considerations of 'hormesis' and biphasic dose responses occurring outside the normally evaluated dose range, and risk assessments in the context of the precautionary principle (where a risk assessment can default to the worst case scenario and invoke regulatory controls in the absence of conclusive data on a chemical) can be found in Ricci et al [79] and Ellman and Sunstein.[80] As human exposures to endocrine-disrupting chemicals are likely to be low-level and long-term, this is a key area for future work, both in consideration of low-dose effects in regulatory toxicology/risk assessments, and the proper epidemiological investigation of long-term, low-level exposures and human health. It is also worth noting in the context of the precautionary principle that this is one strategy in dealing with the tens of thousands of chemicals that must be evaluated both for endocrine disruption and/or under the EU REACH initiative; structure/activity relationships and in vitro data could be used together with precautionary approaches to reduce animal usage in some cases, as it has been estimated that under REACH the EC will evaluate 30 000 chemicals in 15 years, which in turn will use over 10 million animals in chemical toxicity testing, including-but not exclusively-endocrine toxicology.[81]

Although there is no evidence yet that endocrine-disrupting chemicals have caused adverse human health effects, this is because there are actually no data; the necessary research work has not been conducted[12–15], and absence of evidence should not be confused with evidence of absence of an adverse effect. The hypotheses that chemical endocrine disruption may be contributing to adverse health effects in males-such as declining semen quality and disorders of the male reproductive tract[82] and, more recently, that environmental oestrogenic chemicals, particularly those from body-care products, may be associated with breast cancer in women[14] deserve rigorous testing. Epidemiology studies are required that control and record actual chemical ingredient exposure, employ longitudinal tracking and control for confounding factors.[13,14] One approach to looking at hypotheses of association and causality in environmental medicine is to apply Bradford-Hill criteria[70] to the evidence, and Table 5 provides such an analysis for the hypothesis that oestrogenic chemicals in body-care cosmetics, in this case example parabens, may be associated with breast cancer in women.

SUMMARY

In recent years, regulatory authorities have been alerted to the fact that endocrine-disrupting chemicals, often environmental oestrogens, have adversely affected wildlife. This has led to concerns over effects on human health, and mechanisms are now in place to start screening and testing the multitude of chemicals for effects on the endocrine system. Attention has been directed to mechanisms of toxicity of relevance to development, and (anti-)oestrogenic, (anti-)androgenic and (anti-)thyroidal regulatory toxicology protocols are being developed. However, there has been neglect of the function of the integrated endocrine system in vivo, and particularly of steroidogenesis and adrenocortical function; thorough assessment for endocrine

Table 5. Bradford-Hill criteria of association or causality applied to the hypothesis for an association between dermal exposure to xeno-oestrogenic chemicals and female breast cancer (parabens as an example).

Bradford-Hill criteria	Summarized current evidence
Analogy	Oestrogen is a major aetiological factor in breast cancer. Hormone replacement therapy increases risk. It is consistent that additional local dermal input of xeno-oestrogens (with analogous actions to endogenous oestradiol) in body-care formulations may add to this risk. Parabens are oestrogenic in vitro and in vivo (see Table 3)
Plausibility	As oestrogens are involved in all stages of breast cancer, and parabens are oestrogenic and cause proliferation in human breast cancer cells, the hypothesis is plausible and deserves rigorous testing. Other cosmetic ingredients-such as cyclosiloxanes-are oestrogenic, increasing oestrogenic exposure via this route
Experimental evidence	Numerous paraben esters, and the common metabolite *p*-hydroxybenzoic acid, are oestrogenic in vitro and in vivo in a variety of assays (see Table 3). Data from the million women study[83] confirms that oestrogenic inputs via hormone replacement therapy increase risk of female breast cancer and indicates dose/duration response (see below). Recent evidence of genetic instability in outer quadrants of the breast has been applied to an explanation of the disproportionately high tumour incidence in these quadrants[84,85]; such genetic instability may suggest that there may be environmental chemical exposure factors to these quadrants
Coherence	The data, studies and results from numerous sources are coherent in terms of the oestrogenicity of parabens and the major role of oestrogen in breast cancer
Biological gradient (dose response)	There are clear dose responses for parabens' oestrogenic action in a variety of human cell-based and mammalian in vivo assays. The epidemiological study by McGrath[68], although limited in scope, suggested a dose response in breast cancer rates in women using body-care cosmetics at higher rates and earlier ages
Consistency	The oestrogenic activity of the parabens has been replicated in numerous studies from different laboratories internationally (see Table 3). The data on oestrogen as an aetiological factor in human breast cancer is also consistent
Strength	The strength of an association between weak oestrogens in body-care formulations and breast cancer in women is limited by the lack of human epidemiology studies. The two studies available[68,69] are insufficient, report conflicting results, and do not specifically identify oestrogenic formulants
Specificity	Breast cancer is a multifactoral disease where risk factors include familial susceptibility, smoking, alcohol, diet and obesity. However, oestrogen is a major aetiological factor. Chemicals may cause toxicity through mechanisms other than oestrogenicity, and there may be toxicity interactions
Temporality	The rise in female breast cancer rates in the Western world in recent decades mirrors the use of body-care cosmetics and the inclusion of oestrogenic chemicals such as the parabens.[76,77] Such change in disease incidence indicates environmental/lifestyle effects, but these also include other breast cancer risk factors such as changes in age/later first pregnancy, pharmaceutical exposure (e.g. hormone replacement therapy) and obesity rates

toxicity in vivo should include these parameters and would require separate regulatory validation programmes. Additionally, the recent evidence of a role of prolactin in human breast cancer, and the fact that a variety of drugs and chemicals can induce hyperprolactinaemia, would also support the addition of prolactin to the hormone parameters assessed in vivo in regulatory toxicity studies. This is particularly relevant since prolactin-induced rat mammary carcinogenesis (historically quoted as a text-book example of a species-specific effect and a common finding in toxicology of drugs and chemicals) has been previously considered to be of no toxicological relevance to humans; a change in risk assumption is required concerning the toxicological potential of hyperprolactinaemia.[86] The human relevance of prolactin-induced non-genotoxic carcinogenesis and significance for toxicology risk assessment is discussed elsewhere.[86]

Progress in risk assessment has also been made, particularly with the addition of an extra 10-fold uncertainty factor to protect children, because of the potential for selective effects of endocrine-disrupting chemicals during development. The main area where research is now required, however, falls outside the regulatory arena, and efforts should be directed to whether there is actual evidence that long-term low-dose exposures to environmental oestrogens, and to chemicals with other endocrine actions, is harmful to human health.[14] One area of perceived high exposure to chemicals is through body-care cosmetic products, and the two existing epidemiology studies on an association between product use and breast cancer in women are inconsistent[68,69], a situation which requires urgent resolution. The body-care cosmetics scenario would provide a good area of study, because exposure to chemicals is relatively high and quantifiable. It has recently been shown in a study of 360 women aged 19–65 recruited from ten different locations in the USA that mean usage of body lotion was 8.70 g per day[88], and the population large, which together would allow the design of powerful discriminatory longitudinal epidemiology studies and rigorous testing of the adverse health effects that have been hypothesized in men[82] and women[14] to result from environmental exposures to oestrogenic chemicals. Calls for further research into the area of cosmetics and breast cancer are gathering momentum[14,87], but should not be restricted to underarm cosmetics[87] or type of product (e.g. antiperspirant, deodorant) but should also include the large range of lotions, moisturizers, sunscreens etc. that are also applied regularly to the breast or chest, with particular care being taken in recording chemical exposures.[14] Additionally, health effects detected in adulthood may be from exposures early in life, including in utero during critical periods of embryo/foetal sensitivity[15], or indeed from historical chemical accumulation and persistence in tissues, and this complication also requires consideration, together with exposure interactions/combinations and chemical priming effects. Finally, there is no evidence that exposure to endocrine-disrupting chemicals has produced human health effects because the necessary studies have not been conducted; there is in fact no evidence that exposures are safe.

Research agenda

- validation of regulatory in vitro and in vivo studies for adrenocortical function[28,30]
- validation of regulatory in vivo study of prolactin and endocrine function in females, and reconsideration of the toxicological relevance of hyperprolactinaemia to human health[86]

- epidemiology studies examining potential health effects of long-term low-level exposures to oestrogenic/endocrine active chemicals[14]
- further epidemiology on the specific use of body-care cosmetics and breast cancer[13,14,68,69,76,77,84,87]
- studies of chemical dermal absorption, persistence and accumulation[13,14,63] to accompany the recently reported 8.7 g average daily application rates for body lotions[88] and proportion applied to chest and breast areas

REFERENCES

1. Harvey PW, Rush KC & Cockburn A. *Endocrine and Hormonal Toxicology.* Chichester: Wiley; 1999.
2. Rastogi SC, Schouten A, de Kruijf N & Weijland JW. Contents of methyl-, ethyl-, propyl-, butyl- and benzylparaben in cosmetic products. *Contact Dermatitis* 1995; **32:** 28–30.
3. Rastogi SC. Analytical control of preservative labelling on skin creams. *Contact Dermatitis* 2000; **43:** 339–343.
4. Gruvberger B, Bruze M & Tammela M. Preservatives in moisturisers on the Swedish market. *Acta Dermato-Venereologica* 1998; **78:** 52–56.
5. Dodge JA. Natural and anthropogenic environmental oestrogens: the scientific basis for risk assessment. Structure/ activity relationships. *Pure and Applied Chemistry* 1998; **70:** 1725–1733.
6. Hong H, Tong W, Fang H et al. Prediction of estrogen receptor binding for 58,000 chemicals using an integrated system of a tree based model with structural alerts. *Environmental Health Perspectives* 2002; **110:** 29–36.
7. Gad SC. *Regulatory Toxicology.* 2nd edn. London: Taylor and Francis; 2001.
8. Cockburn A & Leist K-H. Current and regulatory trends in endocrine and hormonal toxicology. In Harvey PW, Rush KC & Cockburn A (eds.) *Endocrine and Hormonal Toxicology.* Chichester: Wiley, 1999, pp. 507–534.
9. EDSTAC. Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) Final Report, August 1998. United States Environmental Protection Agency. http://www.epa.gov/scipoly/oscpendo/history/finalrpt.htm; 1998.
10. Ashby J & Lefevre PA. The peripubertal male rat assay as an alternative to the Hershberger castrated male rat assay for the detection of anti-androgens, oestrogens and metabolic modulators. *Journal of Applied Toxicology* 2000; **20:** 35–47.
11. Ashby J. Testing for endocrine disruption post-EDSTAC: extrapolation of low dose rodent effects to humans. *Toxicology Letters* 2001; **120:** 233–242.
12. Harrison PTC. Endocrine disrupters and human health. Current research will establish baseline indices. *British Medical Journal* 2001; **323:** 1317–1318.
13. Harvey PW. Parabens, oestrogenicity, underarm cosmetics and breast cancer: a perspective on a hypothesis. *Journal of Applied Toxicology* 2003; **23:** 285–288.
14. Harvey PW & Darbre P. Endocrine disrupters and human health: could oestrogenic chemicals in body care cosmetics affect breast cancer incidence in women? A review of evidence and call for further research. *Journal of Applied Toxicology* 2004; **24:** 167–176.
15. Sharpe M & Irvine DS. How strong is the evidence of a link between environmental chemicals and adverse effects on human reproductive health? *British Medical Journal* 2004; **328:** 447–451.
16. USEPA. United States Environmental Protection Agency. Endocrine disruptor screening program: early priority setting activities. http://www.epa.gov/scipoly/oscpendo/prioritysetting/activities.htm..
17. European Commission. Environment Directorate General. Endocrine Disrupters Website. http://europa.eu.int/comm/environment/endocrine/strategy/substances_en.htm.

18. Wason S, Pohlmeyer-Esch G, Pallen C et al. 17 alpha-methyltestosterone: 28 day oral toxicity study in the rat based on the 'Enhanced OECD Test Guideline 407' to detect endocrine effects. *Toxicology* 2003; **192:** 119–137.
19. Kunimatsu T, Yamada T, Miyata K et al. Evaluation for reliability and feasibility of the draft protocol for the enhanced rat 28-day subacute study (OECD Guideline 407) using androgen antagonist flutamide. *Toxicology* 2004; **200:** 77–89.
20. Andrews P, Freyberger A, Hartmann E et al. Sensitive detection of the endocrine effects of the estrogen analogue ethinylestradiol using a modified enhanced subacute rat study protocol (OECD Test Guideline no. 407). *Archives of Toxicology* 2002; **76:** 194–202.
21. Kennel P, Pallen C, Barale-Thomas E et al. Tamoxifen: 28-day oral toxicity study in the rat based on the Enhanced OECD Test Guideline 407 to detect endocrine effects. *Archives of Toxicology* 2003; **77:** 487–499.
22. Yamasaki K, Tago Y, Nagai K et al. Comparison of toxicity studies based on the draft protocol for the 'Enhanced OECD test guideline no. 407' and the reasearch protocol 'Pubertal development and thyroid function in immature male rats' with 6-*n*-propyl-2-thiouracil. *Archives of Toxicology* 2002; **76:** 495–501.
23. Mellert W, Deckardt K, Walter J et al. Detection of endocrine-modulating effects of the antithyroid acting drug 6-propyl-2-thoiuracil in rats, based on the 'Enhanced OECD test guideline 407'. *Regulatory Toxicology and Pharmacology* 2003; **38:** 368–377.
24. Gelbke HP, Kayser M & Poole A. OECD test strategies and methods for endocrine disruptors. *Toxicology* 2004; **205:** 17–25.
25. Worth A & Balls M. Alternative (non-animal) methods for chemical testing: current status and future prospects. A report prepared by ECVAM and the ECVAM working group on chemicals. Endocrine disruption in humans. *Alternatives to Laboratory Animals* 2002; **30**(supplement 1)**:** 103–113 [chapter 11].
26. Dandrea J & Combes RD. A survey of stakeholder organisations on the proposed new European chemical policy. *Alternatives to Laboratory Animals* 2003; **31:** 501–528.
27. Combes RD. Peer review of validation studies: an assessment of the role of the OECD by reference to the validation of the uterotrophic assay for endocrine disruptors. *Alternatives to Laboratory Animals* 2004; **32:** 111–117.
28. Harvey PW & Everett DJ. The adrenal cortex and steroidogenesis as cellular and molecular targets for toxicity: critical omissions from regulatory endocrine disrupter screening strategies for human health? *Journal of Applied Toxicology* 2003; **23:** 81–87.
29. Combes RD. The case for taking account of metabolism when testing for potential endocrine disruptors in vitro. *ALTA* 2004; **32:** 121–135.
30. Harvey PW & Johnson I. Approaches to the assessment of toxicity data with endpoints related to endocrine disruption. *Journal of Applied Toxicology* 2002; **22:** 241–247.
31. Harvey PW, Rush KC & Cockburn A. Endocrine and hormonal toxicology: an integrated mechanistic and target system approach. In Harvey PW, Rush KC & Cockburn A (eds.) *Endocrine and Hormonal Toxicology.* Chichester: Wiley, 1999, pp. 3–11.
32. Ribelin WE. The effects of drugs and chemicals upon the structure of the adrenal gland. *Fundamental Applied Toxicology* 1984; **4:** 105–119.
33. Harvey PW. *The Adrenal in Toxicology: Target Organ and Modulator of Toxicity.* London: Taylor and Francis; 1996.
34. Raven PW, Hinson JP. Transport, action and metabolism of adrenal hormones and pathology and pharmacology of the adrenal gland. In: The Adrenal in Toxicology: Target Organ and Modulator of Toxicity. London: Taylor and Francis; 1996. p. 53–79.
35. Gopinath C. Comparative endocrine carcinogenesis. In Harvey PW, Rush KC & Cockburn A (eds.) *Endocrine and Hormonal Toxicology.* Chichester: Wiley, 1999, pp. 155–167.
36. Clevenger CV, Furth PA, Hankinson SE & Schuler LA. The role of prolactin in mammary carcinoma. *Endocrine Reviews* 2003; **24:** 1–27.
37. Tworoger SS, Eliassen AH, Rosner B et al. Plasma prolactin concentrations and risk of postmenopausal breast cancer. *Cancer Research* 2004; **64:** 6814–6819.
38. Perks CM, Keith AJ, Goodhew KL et al. Prolactin acts as a potent survival factor for human breast cancer cell lines. *British Journal of Cancer* 2004; **91:** 305–311.
39. Meng J, Tsai-Morris CH & Dufau ML. Human prolactin receptor variants in breast cancer: low ratio of short forms to the long form human prolactin receptor associated with mammary carcinoma. *Cancer Research* 2004; **64:** 5677–5682.

40. Gutzman JH, Rugowski DE, Schroeder MD et al. Multiple kinase cascades mediate prolactin signals to activate protein-1 in breast cancer cells. *Molecular Endocrinology* 2004; **18:** 3064–3075.
41. USEPA. Determination of the appropriate FQPA safety factors for use in the tolerance setting process. Office of Pesticide Programs, Office of Prevention, Pesticides and Toxic Substances. http://www.epa.gov/oppfead1/trac/science/#10_fold; 2002.
42. Kimmel CA, King MD & Makris SL. Risk assessment perspectives for developmental immunotoxicology. In Holladay SD (ed.) *Developmental Immunotoxicology.* Boca Raton: CRC Press, 2005, pp. 45–63.
43. Holladay SD. *Developmental Immunotoxicology.* Boca Raton: CRC Press; 2005.
44. Philip JC, John CD, Gillies GE & Buckingham JC. Neuroendocrine and pituitary toxicology: targets for drug action and potential consequences on immune function. In Harvey PW, Rush KC & Cockburn A (eds.) *Endocrine and Hormonal Toxicology.* Chichester: Wiley, 1999, pp. 171–208.
45. Spinedi E, Perez NB & Gaillard RC. Neuroendocrine–immune interaction: impact of gender, negative energetic balance condition, and developmental stage on the mechanisms of the acute phase response of the inflammatory process in mammals. In Holladay SD (ed.) *Developmental Immunotoxicology.* Boca Raton: CRC Press, 2005, pp. 303–353.
46. Munck A, Guyre PM & Holbrook NJ. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocrine Reviews* 1984; **5:** 25–44.
47. Johnson I, Harvey PW. Study on the scientific evaluation of 12 substances in the context of endocrine disrupter priority list of actions. Report for the European Commission, Environment Directorate General, 2002, 613 pp. Available at http://europa.eu.int/comm/environment/endocrine/documents/wrc_report.pdf; 2002.
48. Routledge EJ, Parker J, Odum J et al. Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. *Toxicology and Applied Pharmacology* 1998; **153:** 12–19.
49. Byford JR, Shaw LE, Drew MG et al. Oestrogenic activity of parabens in MCF7 human breast cancer cells. *The Journal of Steroid Biochemistry and Molecular Biology* 2002; **80:** 49–60.
50. Lemini C, Jaimez R, Avila ME et al. In vivo and in vitro estrogen bioactivities of alkyl parabens. *Toxicology and Industrial Health* 2003; **19:** 69–79.
51. Hossaini A, Larsen JJ & Larsen JC. Lack of oestrogenic effects of food preservatives (parabens) in uterotrophic assays. *Food and Chemical Toxicology* 2000; **38:** 319–323.
52. Oishi S. Lack of spermatotoxic effects of methyl and ethyl esters of p-hydroxybenzoic acid in rats. *Food and Chemical Toxicology* 2004; **42:** 1845–1849.
53. Okubo T, Yokoyama Y, Kano K & Kano I. ER-dependent estrogenic activity of parabens assessed by proliferation of human breast cancer MCF-7 cells and expression of ER alpha and PR. *Food and Chemical Toxicology* 2001; **39:** 1225–1232.
54. Oishi S. Effects of propylparaben on the male reproductive system. *Food and Chemical Toxicology* 2002; **40:** 1807–1813.
55. Daston GP. Developmental toxicity evaluation of butylparaben in Sprague–Dawley rats. *Birth Defects Research Part B Developmental and Reproductive Toxicology* 2004; **71:** 296–302.
56. Oishi S. Effects of butylparaben on the male reproductive system in rats. *Toxicology and Industrial Health* 2001; **17:** 31–39.
57. Oishi S. Effects of butylparaben on the male reproductive system in mice. *Archives of Toxicology* 2002; **76:** 423–429.
58. Kang KS, Che JH, Ryu DY et al. Decreased sperm number and motile activity on the F_1 offspring maternally exposed to butyl *p*-hydroxybenzoic acid (butyl paraben). *The Journal of Veterinary Medical Science* 2002; **64:** 227–235.
59. Darbre PD, Byford JR, Shaw LE et al. Oestrogenic activity of isobutylparaben in vitro and in vivo. *Journal of Applied Toxicology* 2002; **22:** 219–226.
60. Darbre PD, Byford JR, Shaw LE et al. Oestrogenic activity of benzylparaben. *Journal of Applied Toxicology* 2003; **23:** 43–51.
61. Lemini C, Silva G, Timossi C et al. Estrogenic effects of *p*-hydroxybenzoic acid in CD1 mice. *Environmental Research* 1997; **75:** 130–134.
62. Bando H, Mohri S, Yamashita F et al. Effects of skin metabolism on percutaneous penetration of lipophilic drugs. *Journal of Pharmaceutical Sciences* 1997; **86:** 759–761.
63. Darbre PD, Aljarrah A, Miller WR et al. Concentrations of parabens in human breast tumours. *Journal of Applied Toxicology* 2004; **24:** 5–13.

64. Harvey PW & Everett DJ. Significance of the detection of esters of p-hydroxybenzoic acid (parabens) in human breast tumours. *Journal of Applied Toxicology* 2004; **24:** 1–4.
65. Clarke R, Leonessa F, Welch JN & Skaar TC. Cellular and molecular pharmacology of antiestrogen action and resistance. *Pharmacological Reviews* 2001; **53:** 25–72.
66. SCF. Statement of the Scientific Committee on Food on the Parabens. European Commission, Health and Consumer Protection Directorate, Scientific Committee on Food SCF/CS/ADD/CONS/53 Final 2 April 2003. http://europa.eu.int/comm/food/fs/sc/scf; 2003.
67. Willis L. Final report on the safety assessment of isobutylparaben and isopropylparaben. *Journal of the American College of Toxicology* 1995; **14:** 364–372.
68. McGrath KG. An earlier age of breast cancer diagnosis related to more frequent use of antiperspirants/deodorants and underarm shaving. *European Journal of Cancer Prevention* 2003; **12:** 479–485.
69. Mirick DK, Davis S & Thomas DB. Antiperspirant use and the risk of breast cancer. *Journal of the National Cancer Institute* 2002; **94**(20)**:** 1578–1580.
70. Bradford-Hill A. The environment and disease: association or causation. *Proceedings of the Royal Society of Medicine* 1966; **58:** 295.
71. Luu HMD & Hutter JC. Bioavailability of octamethylcyclotetrasiloxane (D4) after exposure to silicones by inhalation and implantation. *Environmental Health Perspectives* 2001; **109:** 1095–1101.
72. Zareba G, Galein R, Morrow PE & Utell MJ. Percutaneous absorption studies of octamethylcyclotetrasiloxane using the human skin/nude mouse model. *Skin Pharmacology and Applied Skin Physiology* 2002; **15:** 184–194.
73. Kala SV, Lykissa ED, Neely MW & Lieberman MW. Low molecular weight silicones are widely distributed after a single subcutaneous injection in mice. *The American Journal of Pathology* 1998; **152:** 645–649.
74. He B, Rhodes-Brower S, Miller MR et al. Octamethylcyclotetrasiloxane exhibits estrogenic activity in mice via ER alpha. *Toxicology and Applied Pharmacology* 2003; **192:** 254–261.
75. McKim JM, Wilga PC, Breslin WJ et al. Potential estrogenic and antiestrogenic activity of the cyclic siloxane octamethylcyclotetrasiloxane (D4) and the linear siloxane hexamethyldisiloxane (HMDS) in immature rats using the uterotrophic assay. *Toxicological Sciences* 2001; **63:** 37–46.
76. Darbre PD. Underarm cosmetics are a cause of breast cancer. *European Journal of Cancer Prevention* 2001; **10:** 389–393.
77. Darbre PD. Underarm cosmetics and breast cancer. *Journal of Applied Toxicology* 2003; **23:** 89–95.
78. EFSA. Opinion of the scientific panel on food additives, flavouring, processing aids and materials in contact with food on a request from the commission related to para hydroxybenzoates (E 214-219). Question number EFSA-Q-2004-063. *The EFSA Journal* 2004; **83:** 1–26.
79. Ricci PF, Cox LA & MacDonald TR. Precautionary principles: a jurisdiction-free framework for decision making under risk. *Human Experimental Toxicology* 2004; **23:** 579–600.
80. Ellman LM & Sunstein CR. Hormesis, the precautionary principle and legal regulation. *Human Experimental Toxicology* 2004; **23:** 601–611.
81. Hofer T, Gerner I, Gundert-Remy U et al. Animal testing and alternative approaches for the human health risk assessment under the proposed new European regulation. *Archives of Toxicology* 2004; **78:** 549–564.
82. Sharpe RM & Skakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet* 1993; **341:** 1392–1395.
83. Beral V. Million women study collaborators. Breast cancer and hormone replacement in the million women study. *Lancet* 2003; **362:** 419–427.
84. Ellsworth DL, Ellswoth RE, Love B et al. Outer breast quadrants demonstrate increased levels of genomic instability. *Annals of Surgical Oncology* 2004; **11:** 861–868.
85. Ellsworth DL, Ellsworth RE, Liebman MN et al. Genomic instability in histologically normal breast tissues: implications for carcinogenesis. *Lancet Oncology* 2004; **5:** 753–758.
86. Harvey PW. Human relevance of rodent prolactin-induced non-genotoxic mammary carcinogenesis: prolactin involvement in human breast cancer and significance for toxicology risk assessments. *Journal of Applied Toxicology* 2005; **25:** 179–183.
87. Gikas PD, Mansfield L & Mokbel K. Do underarm cosmetics cause breast cancer? *International Journal of Fertility* 2004; **49:** 212–214.
88. Loretz LJ, Api AM, Barraj LM et al. Exposure data for cosmetic products: lipstick, body lotion, and face cream. *Food and Chemical Toxicology* 2005; **43:** 279–291.