FINAL REPORT

A review of latest endocrine disrupting chemicals research implications for drinking water

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Executive Summary

Over recent years, the presence of Endocrine Disrupting Chemicals (EDCs) in various sewage discharges and fresh- and estuarine-waters has been extensively reported in the peer-reviewed literature. However, to date, evidence suggestive of a risk to human health from exposure to EDCs via drinking water has not been convincing. Despite this, much media and public interest continues to focus on the quality of drinking water supplies in England and Wales. It is therefore of high importance for the Drinking Water Inspectorate (DWI) to maintain an up-to-date knowledge base to assess the potential for consumers to be exposed to EDCs via this route.

The aims of this study are to comprehensively assess and summarise existing literature (both published papers and unpublished reports) on the presence in drinking waters of any substances that have, or may have, endocrine disrupting potential of relevance to human health. For substances with the potential to cause endocrine disruption in mammals, estimates of the concentrations in water bodies used for drinking water abstraction were modelled and estimates made of the resultant levels in drinking water based on the use of either conventional or advanced water treatment processes, both types commonly employed in the UK. Hazard profiles were developed to inform on the extent of risk posed by consumption of drinking water containing such substances at a 'worst-case' concentration ≥100 ng/L to characterise the nature of any risk to human health. The significance of our findings for water policy in England and Wales were also considered.

The initial literature review identified 509 articles suggesting 325 potential EDCs could be present in water bodies of potential relevance. Through application of a customised prioritisation scheme, this candidate list was reduced to 159 potential EDCs that were then subject to a multistage modelling process to estimate their environmental fate and behaviour and extent of potential removal by water treatment processes prevalent in the UK. Thirty-five of these chemicals were predicted to have highest (worst case) concentrations (i.e. following conventional treatment processes) ≥100ng/L; these modelled levels were used to estimate potential intakes (as mg/kg bw/d) via drinking water for three population subgroups: adults (>18 years), toddlers (1-2 years) and infants (0–1 year), based on standard default assumptions. The extent of the risk posed by such a worst case intake was then determined by establishing the margin of safety (MOS) between this intake and either an established authoritative health-base criteria value (e.g. tolerable daily intake) or using a study-specific exposure limit derived from the available hazard data; in either case, the value was based on what was considered to be the most sensitive endpoint irrespective to its relevance to the endocrine system. For endocrine-active pharmaceuticals considered to be of potential concern, a study-specific exposure limit was determined on the basis of the minimum therapeutic dose, using clinical judgement.

Comparison of predicted worst-case drinking water intakes against the hazard profile for the 35 chemicals subject to detailed modelling showed a very high MOS (>100) for 21 chemicals, even using worst case assumptions, and these were not considered to warrant further study. A further 8 chemicals had MOS of 10–100 and hence were considered of doubtful importance and, hence not to warrant further considerationr. For 6 chemicals (p-benzylphenol, dibutylphthalate, 4-nitrophenol, digoxin, fluticasone and salbutamol), MOS were ≤10, and hence were considered to warrant a more detailed consideration to establish the likely 'real world' situation, as opposed to the estimates derived here from the use of highly conservative 'worst case' assumptions throughout the modelling process.

Furthermore, a précis of current scientific understanding with regard to the risks posed by complex mixtures of EDCs was prepared and an indicative estimate made of the potential risk that might arise from a mixture containing those substances identified here that possess oestrogenic activity , each at their predicted worst-case level. Importantly, it was found that even such an extreme worst case combined intake, when expressed in terms of an equivalent oestradiol intake, did not raise a significant health concern.

The potential mammalian-relevant endocrine disruptive potential of several metals and inorganic metal compounds was also considered; limited concerns were identified for cadmium, chromium, cobalt and copper. However, as the exposure models used here are unsuited to inorganic substances, our initial assessment was limited to a comparison of the available data on endocrine-relevant toxicity to established UK drinking water limits.

It is suggested that the database of evidence generated through this project should be periodically reviewed, as the publically available information on a number of substances identified here is expected to increase as a result of assessment processes required by REACH and, possibly, as a result of operation of the Biocides Directive. In addition, as the various regulatory measures that have been introduced on a European basis begin to take effect, it is anticipated that, for a number of chemicals identified, further reductions in usage and, hence, in exposure levels will occur over time. Furthermore, the operation of the Water Framework Directive (WFD) can be anticipated to result in additional information being generated on levels of substances present in water and is likely to result in improvements in the general standards of catchment management and water and wastewater treatment, thus acting to reduce still further any potential exposure.

Contents

1 Introduction

1.1 Background and objectives

Although endocrine disrupting chemicals (EDCs) are known to occur in some sewage discharges and fresh- and estuarine-waters, there has never been convincing evidence of any risk to health posed by their presence in drinking water. Despite this, much media and public interest continues to focus on the quality of drinking water supplies in England and Wales and it is necessary to maintain up-to-date knowledge of the potential for EDC exposure to occur via this route.

The purpose of this desk-based study, outlined in this final report, is to comprehensively assess and summarise existing literature (both published papers and unpublished reports) on the possible presence in drinking water of any substances that have, or have been suggested to have, endocrine disrupting potential of significance to human health. In addition, for substances for which there is some evidence suggestive of possible mammalian endocrine disrupting potential, an assessment is made of at least the theoretical potential for them to reach water bodies used for drinking water abstraction. This information, along with assessment of the effects on water content of the water treatment processes used in the UK, is used here to derive estimates of the likely concentrations that might occur in water sources and, hence, ultimately in drinking water supplies.

Evaluation of the toxicological profiles of substances identified as being of potential concern in regard to their endocrine disrupting potential is combined with detailed modelled estimates of UK-relevant exposure levels in order to inform an assessment of the extent of the risks posed to human health from exposure to these substances via drinking water. The significance of the study findings to water policy in England and Wales is also considered.

A number of apparent gaps in current knowledge and suggestions for further research to address these, are also identified.

The study addresses seven distinct but inter-related objectives:

Objective 1: To review the published and grey literature on EDCs with a particular focus on those with the potential to reach water sources;

Objective 2: Produce a list of pathways by which EDCs may reach water sources, and identify which EDCs identified in Objective 1 may utilise these;

Objective 3: Produce a summary of potencies of EDCs identified as likely to reach water sources, estimates of concentrations that may arise and the potential influence of water treatment processes;

Objective 4: Identify and evaluate the potential for synergistic effects of mixtures of EDCs identified as likely to reach water;

Objective 5: Assessment of potential drinking water intake levels of EDCs;

Objective 6: Evaluation of research findings; and

Objective 7: Final Report and dissemination of research findings.

2 Methodology

This study comprised a number of inter-related and co-ordinated tasks, each contributing to particular aspects of the seven objectives identified in Section 1, as follows:

Task 1: To review the published and grey literature on EDCs with a particular focus on those with the potential to reach water sources – addresses Objective 1;

Task 2: Produce a list of pathways by which EDCs may reach water sources, and identify which EDCs may utilise these – addresses Objective 2;

Task 3: Produce a summary of the potencies of EDCs identified as likely to reach water sources, the concentrations that may arise and effects of water treatment processes – addresses Objective 3;

Task 4: Identify and evaluate potential for synergistic effects of mixtures of EDCs identified as likely to reach water – addresses Objective 4;

Task 5: Assess potential drinking water intake levels of EDCs – addresses Objective 5;

Task 6: Evaluate research findings – addresses Objective 6; and

Task 7: Produce a final report and discuss dissemination of research findings with DWI– addresses Objective 7.

2.1 Literature search strategies

2.1.1 Task 1: Identification of published and grey literature on the presence of potential EDCs in water bodies

A comprehensive search strategy was developed to identify literature relating to the presence of EDCs in drinkingwater sources. A detailed set of search terms, as detailed in Annex 1.1, were used as the basis for identifying articles and reviews through searches of SCOPUS (includes Medline & Embase) and CSA Illumina (Aqualine, Biological Sciences, Environment Abstracts, Environment Science and Pollution Management, Medline, Risk Abstracts, Toxline, Water Resources Abstracts).

The output of these searches was subjected to detailed consideration by expert risk assessors and toxicologists, as detailed in Section 2.2.1.

2.1.2 Task 3: Identification of published and grey literature for preparation of hazard profiles and derivation of EDC potency estimates

A comprehensive search strategy was developed to identify literature relating to the identification, use, toxicological profile and EDC potency of chemicals identified as being of potential concern. A detailed set of search terms, as detailed in Annex 1.2, was used as the basis for identifying articles and reviews through searches of SCOPUS (includes Medline & Embase) and CSA Illumina (Aqualine, Biological Sciences, Environment Abstracts, Environment Science and Pollution Management, Medline, Risk Abstracts, Toxline, Water Resources Abstracts).

The output of these searches was subjected to detailed consideration by expert risk assessors and toxicologists for use in risk assessment, as detailed in Section 4.1.

2.1.3 Task 4: Identification of published and grey literature on current understanding on mixtures

A comprehensive search strategy was developed to identify recent published or grey literature relating to the scientific and regulatory understanding of 'mixture effects' and 'mixture toxicity' in relation specifically to endocrine disruption potential. The search terms detailed in Annex 1.3 were used to identify reviews from year 2005 onwards, using SCOPUS (includes Medline & Embase) and CSA Illumina (Aqualine, Biological Sciences, Environment Abstracts, Environment Science and Pollution Management, Medline, Risk Abstracts, Toxline, Water Resources Abstracts).

The output of these searches was subjected to detailed consideration by expert risk assessors and toxicologists, as detailed in Section 4.3.

2.2 Approach to initial prioritisation of substances for detailed consideration

2.2.1 Data extraction and collation

Titles of all journal articles identified by the initial literature search (as detailed in Section 2.1.1) were reviewed by experienced scientists to establish their potential relevance to the project. For selected titles, abstracts and – if then judged to still be potentially relevant – full papers were obtained. Key information was then extracted on the nature of the substance, its status as a potential EDC, and on the sources and presence of the substance in relevant water bodies. These data were collated on an MSExcel spreadsheet under the following headings (as agreed with DWI):

- Potential EDC name – *the name by which the substance is generally referred to in this study*;
- CAS number – *the CAS number considered of most relevance for the scenarios being considered (i.e. generally focused on technical rather than analytical grade forms or the form most commonly used in the literature);*
- Nature/Origin – *i.e. type of chemical (e.g. pesticide, pesticide degradation product; consumer chemical etc.);*
- Activity – *Initial estimation of the principal endocrine-related biological activity identified as of concern by the source reference (e.g. oestrogenic, anti-oestrogenic; for pharmaceuticals also e.g. anti-inflammatory, anti-pyretic);*
- Additional drug activity (where appropriate) – *for pharmaceuticals only, the main therapeutic activity of the substance – if different from its endocrine-related properties);*
- Source (if suggested by the authors of the reference source) – *e.g. surface water; ground water;*
- Media analysed – *the category of media (e.g. river water, sediment) that was subject to analysis;*
- Geographical location – *the geographical region that the reference source had studied (this was used, together with other data to inform on the likely relevance of the findings of the paper to the UK situation);*
- IUPAC List – *Indication if the substance is included on the IUPAC list of substances considered to potentially have endocrine disrupting properties*;
- EU List – *Indication if the substance is included on the EU list of substances of concern with regard to potentially endocrine disrupting properties*;
- Paper ref – *Abbreviated form of the full paper citation*;

- Quantitative data (if available) – *Indication of the nature of the data included in the reference source (e.g. measured, modelled); and*
- Additional information – *any other information that was considered by the reviewing scientist to be of potential value with interpretation of the extracted summary information.*

These pre-defined criteria and entry requirements were adopted to ensure the highest possible level of consistency in data entry, thereby facilitating data retrieval and comparison throughout the project.

2.2.2 Prioritisation of substances using a weight of evidence approach

Following the initial literature search and data extraction (described in Sections 2.1.1 and 2.2.2 respectively), a number of critical assessment stages (designated codes 1-9 and codes A-C) were then undertaken for each identified chemical to determine the its potential relevance to the investigation of mammalian-relevant EDC activity in UK waters. These criteria provided the basis for a final inclusion/exclusion decision for each chemical that was used to establish if the substance should be progressed to the detailed environmental fate and behaviour and water treatment process modelling stages of the project.

Using the following 'inclusion criteria' codes, chemicals progressed through the prioritisation sequence in a step-wise manner (see Figure 2.1), as follows:

- Codes 1-3: Listing by IUPAC, EU or EUROPA as 'of concern' with regard to endocrine disruption potential;Code 4: Currently included on the UK registered pesticide listing;
- Code 5: Metal or metal-containing compound – considered further later (Section 4.2.) but not subjected to fate/behaviour and treatment modelling;
- Code 6: Identified as a conjugated hormone/EDC or phytoestrogen;
- Code 7: Clinically assessed as possessing potential EDC activity (ranked as high, medium, low potency) – criteria applied only to **pharmaceuticals**;
- Code 8: Modelling using OECD QSAR toolbox resulted in evidence of oestrogen binding activity; and
- Code 9: Modelling using OECD QSAR toolbox indicated potential protein binding or judged as of potential concern on the basis of available weight of evidence (WoE).

A number of 'exclusion criteria' codes were similarly defined as follows (substances satisfying these criteria were excluded from further considerations):

- Code A: Not listed by IUPAC, EU or EUROPA as 'of concern' and not on UK registered pesticides list;
- Code B: As Code A plus also not a metal or metal containing-compound, a conjugated hormone/EDC or phytoestrogen, or not a pharmaceutical ranked as likely to have a 'high' or 'moderate' clinical endocrine activity, or no evidence of oestrogen or protein binding using OECD QSAR toolbox; and
- Code C: As Code A and B but with some evidence of protein binding using OECD QSAR toolbox plus judged of no significant concern based on WoE judgement on endocrine activity.

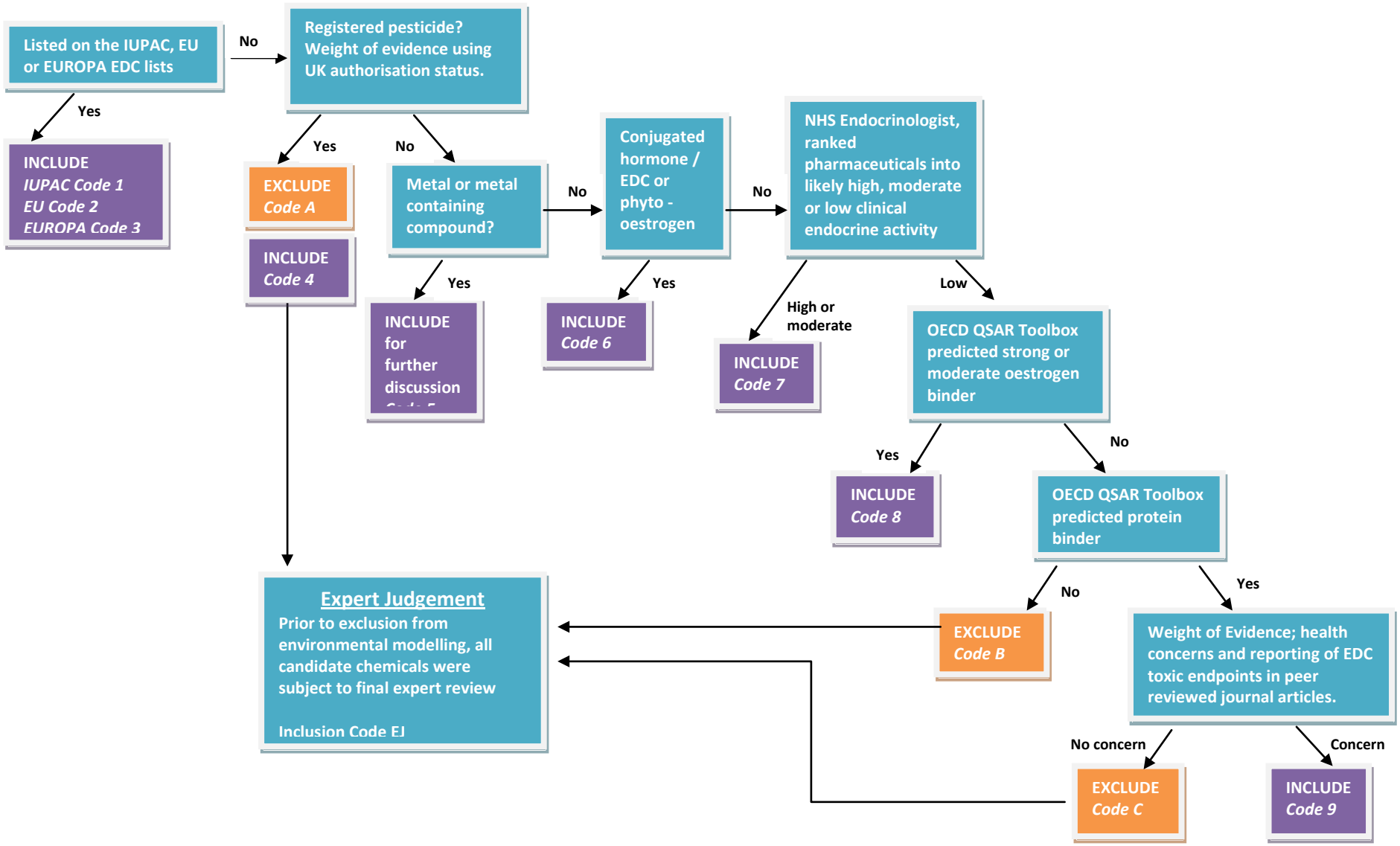

Figure 2.1: Flow chart showing the sequence for applying the initial inclusion/exclusion criteria to chemicals (code given in section 2.2.2)

2.3 Environmental fate and behaviour and water treatment modelling strategy

Modelling potential levels in drinking water was undertaken for those compounds that were judged to possess endocrine disrupting properties of relevance to mammals (and hence humans) and were identified in the literature survey as having been detected in environmental compartments of relevance to water supplies.

Following the application of the inclusion and exclusion codes, a number of metals and metal-containing compounds were identified for inclusion in the modelling exercise. However, the model used was not suitable for metals and therefore assessment of risk from these substances was considered separately (Section 4.2).

For the remaining non-metal containing prioritised substances, two principal emission scenarios were considered:

(1) Emission (release) from households and factories to the sewer system (this was applied to human pharmaceuticals, natural hormones and down-the drain chemicals) followed by discharge to surface water via a waste water treatment works before abstraction to water treatment facilities; and

(2) Emissions to agricultural land (for pesticides and veterinary pharmaceuticals that might arise from agricultural/horticultural sources) followed by modelling of leaching to shallow groundwaters subject to abstraction.

The various stages of modelling are described in greater detail in the sections below.

2.3.1 Prediction of primary emissions

2.3.1.1 Primary emissions to sewers

In the case of substances considered to be subject principally to release to sewers, a 'reasonable worst case' scenario was established in which it was assumed that drinking waters are abstracted from a lowland river receiving a source of treated waste water. Several examples of such a scenario can be identified in the UK and hence this is considered of direct relevance to the UK situation.

River concentrations at a point of abstraction for drinking water supply were estimated in two ways:

(a) using *per capita* use estimates to calculate untreated waste water concentrations, followed by predicted removal during waste water treatment before release, followed by in-stream dilution and in-stream dissipation (degradation); and

(b) adopting reported reasonable worst case measured concentrations in river water (where available) as the concentration in abstracted water (or waste calculating a river water concentration from measured data on concentrations in wastewater effluent streams).

2.3.1.2 Predicting concentrations from usage data

For general chemicals (i.e. excluding pharmaceuticals) *per capita* use rates (U_G, mg cap^{-1} d^{-1}) were calculated from the ratio of total EU tonnage (T, tonnes yr^{-1}) to relative population.

$$U_G = \frac{T.10^9}{P.365} \qquad (1)$$

For pharmaceuticals, *per capita* use (U_P) was estimated as follows

$$U_P = \frac{T.10^9}{P.365} = \frac{(N.S.D)}{P.365} \quad (2)$$

where N is the number of prescriptions issued in the UK for the active ingredient under consideration (NHS, 2008), S is the number of doses (e.g. tablets) per prescription and D is the mass (mg) of active ingredient per dose. In the absence of detailed information on doses and dose sizes, values of 24 and 200 were assigned arbitrarily to S and D, respectively. Clearly, this does not capture drug use via over-the-counter products and it was assumed that such products are not an important source for the specific chemicals considered here. To simplify the estimation process, the highly conservative assumption was also made that 100% of each drug dose administered was excreted from the body unchanged. We recognise that this will not be the case for most of the compounds considered here and, indeed, a more refined estimate could be obtained by adjusting on the basis of within body losses for appropriate population risk subgroups under consideration. As a consequence of these various assumptions there will inevitably be a considerable degree of uncertainty introduced into the calculations.

In the case of natural hormones (or their conjugates) or substances present in food, the excretion rate *per capita* (U_E, mg cap^{-1} d^{-1}) was estimated directly from the available literature sources.

For each compound thus subject to modelling, a concentration in untreated waste water was predicted from the *per capita* emission estimate using an assumed *per capita* domestic water use rate (200 L cap^{-1} d^{-1}; EC, 2003). Although this may represent a slight over-estimation of actual average water use within the UK (*ca*. 150 L cap^{-1} d^{-1}), it must be appreciated that many sewage treatment works also treat additional waste waters from industrial sources (i.e. trade effluents) and also receive drainage from combined sewers; together these sources can increase the overall effluent flow rates considerably. It is therefore still likely that this represents a relatively conservative basis for estimation.

Removal during sewage treatment was estimated using a "meta-model", *SimpleTreat* (the chemical fate model for sewage treatment employed in the EU Technical Guidance Documents for the risk assessment of chemicals: EC, 2003). These documents contain look-up tables of the fraction of a chemical which is likely to be emitted to the waste water stream, based on its hydophobicity (the octanol-water partition coefficient, K_{ow}), volatility (air-water partition coefficient) and biodegradability (performance in a ready biodegradation test). Where data were unavailable on the performance of a chemical in a biodegradation test, the likelihood of performance was estimated using the Biodegradation Probability Program (BIOWIN) which forms part of the EpiSuite estimation package (USEPA, 2011). BIOWIN incorporates a number of methods for estimating biodegradation (e.g. Howard *et al*., 1992; Boethling *et al*., 1994). It is however worth noting that the current UK Water Industry Research (UKWIR) Chemicals Investigation Programme (CIP) is monitoring throughout the UK the final effluent EDC content and removal through sewage treatment works and these data when available (*c*. 2013) could be used to further refine the modelling procedure applied.

A dilution factor of 10 was assumed when calculating the predicted environmental concentration that results from waste water emission (i.e. the PEC_{AQ}, based on the TGD: EC, 2003). In addition, a regional "background" concentration ($PEC_{regional}$) was also calculated; this represents the concentration upstream of the waste water treatment plant considered in the scenario. This was derived using a simplification of the methods recommended in EC (2003) and consider the mass (M) balance of a chemical in a regional water body. Thus, the equation was:

$$\frac{dM}{dt} = L_{IN} - L_{OUT} = \frac{r.T_{EU}.10^9}{365}.(\phi.(1-f) + (1-\phi)) - k.V.C - Q.C \quad (3)$$

where L_{IN} and L_{OUT} are the chemical loads in and out of the system, T_{EU} is the total annual tonnage released in the EU, r is the fraction of this tonnage released to a pre-defined EU "region" (0.1: EC, 2003), f is the fraction of the chemical removed from the aqueous stream during waste water treatment, ϕ is the fraction of the regional population which is connected to secondary waste water treatment (the remainder is assumed to enter the regional water environment untreated), Q is the discharge of the regional water body ($m^3\ d^{-1}$), k is an environmental degradation rate constant (d^{-1}), V is the system volume (m^3) and C (mg m^{-3}) is the regional concentration ($PEC_{regional}$). The fraction treated (ϕ) is assumed to be 100%, which is our understanding of the typical situation in the UK. At steady state, this equation reduces to:

$$PEC_{regional} = \frac{L_{IN}}{(V/\tau + (k.V))} \tag{4}$$

where τ is the residence time of the regional water body.

The combined concentration (PEC_{local}) is simply derived as a sum (of PEC_{AQ} and $PEC_{regional}$, as recommended in EC, 2003):

$$PEC_{local} = PEC_{AQ} + PEC_{regional} \tag{5}$$

Finally, in-stream losses were also adjusted (based on the estimated biodegradability) for a short distance that was assumed to exist between the point of waste water emission and the intake for the water treatment works (PEC_{WTW}), by the equation:

$$PEC_{WTW} = PEC_{local}.\exp(-k_{local}.\tau_{local}) \tag{6}$$

Where k_{local} and τ_{local} are, respectively, the in-stream rate constant and in-stream travel time between waste water emission and the drinking water intake. The value of τ_{local} was set to 9.3 hours based on an assumption of a 10 km reach and a velocity of 0.3 m s^{-1}. A different rate constant, k_{local}, was assigned to each chemical on the basis of their performance or expected performance in a ready biodegradation test. The following half lives were assumed for chemicals passing the test within a 10-day window, those passing the test, chemicals which are "inherently" biodegradable and those which are considered to not be biodegradable at all: 20, 40, 100, 10^5 hours. These values are likely to be relatively conservative based on the observed behaviour of down-the-drain chemicals in previous monitoring studies (e.g. for LAS: Whelan *et al*., 1999; and triclosan: Sabaliunas *et al*., 2003).

2.3.1.3 Estimation of emissions to land

In the case of substances identified as being most likely to be primarily applied to land (i.e. pesticides and veterinary pharmaceuticals), leaching losses were estimated using a pesticide leaching model (Whelan *et al*., 2007) which accounts for sorption to the soil solid phase, degradation and advective transfer by hydrologically effective rainfall to shallow groundwater. The pesticide is assumed to be applied to the soil surface according to manufacturers' instructions and to leach through the soil at a rate driven by monthly hydrologically effective rainfall estimated from *a priori* water balance model calculations generated for a 0.5 x 0.5 degree (lat x lon) grid for the global terrestrial surface (see Vorosmarty *et al*., 1989; Vorosmarty *et al*., 1998; Fekete *et al*., 2000). An arbitrary location in the UK (52◦N, 2◦W) is used, which is approximately consistent with the likely location of arable agriculture and groundwater abstraction for public supply. In this scenario, the predicted peak concentration in leachate at the base of the soil root zone is used as an estimate of the concentration in groundwater abstracted for drinking water supply. In reality, most groundwater supplies abstract from much deeper aquifers with long unsaturated and saturated zone travel times which are overlain by a variety of different land uses (which provide some dilution via recharge of uncontaminated water).

Thus, although preferential flow pathways have not been considered, the scenario is likely to be very conservative.

2.3.1.4 Use of measured concentration data

For some of the substances prioritised during the initial screening process, there were insufficient published data available to support the above exposure estimation modelling.

In those cases where usage data were unavailable or where the fraction of chemical likely to be emitted to sewers after use was considered highly uncertain, preference was given to using measured concentration data in appropriate environmental compartments in order to inform the modelling of exposure estimates for receiving systems and, ultimately, in drinking waters (i.e. following appropriate water treatment). Most of the concentrations that were used in this way were derived from reports in the peer-reviewed literature. Where possible, measured concentrations in river water or groundwater were adopted but if such information was unavailable, then reports of measured concentrations in either treated or untreated wastewaters were used to calculate an estimate of the anticipated river water concentration using the model assumptions outlined previously. In general worst case concentration data in the literature were adopted, ensuring any outputs would be conservative in nature. However, an exception to this selection process was made for those instances where the worst case data reported related to situations which were clearly inconsistent with the current UK situation scenario (e.g. for data relating to direct discharge situations or to arid/ semi arid climates).

Although some attempt was made to assess the relevance of literature sources to the current UK conditions, it is recognised that use rates for some substances may have changed significantly since the time of the source publication from which exposure data were extracted from, for example as a consequence of changes in product demand or intervening regulation. There may be cases, therefore, where the values assumed for concentrations in our assessment relate to substances which are either no longer on the market or for which current usage is very different from that at the time the monitoring was conducted.

It is recognised that the use of this mixture of data derivation methods to inform the estimation of exposures introduces potential inconsistencies into the assessment process. However, this approach did allow a larger number of substances to be evaluated and, in general, the assumptions made are likely to have been highly conservative in nature, particularly in the case of any substance that has been identified as being of particularly high concern with regard to its endocrine toxicity. The individual substances for which measured concentration data were used (as opposed to derivation of primary emission estimates) are indicated in the results.

2.3.2 Estimation of the extent of chemical removal during water treatment

For all substances subject to modelling, estimates were made of the likely fraction which would be removed by the various types of drinking water treatment processes used in the UK. That is, the ultimate predicted values for the concentration present in drinking waters were adjusted to allow for variations in the removal rates that would be expected to occur over the range of types of water treatment to be found in the UK. At its simplest and most inefficient, it was assumed that only very basic treatment (e.g. filtration) would be employed while to model the more sophisticated technology train more typically employed by UK water supply companies a more complex treatment cycle was assumed, giving two divergent estimates of potential drinking water concentrations (i.e. a high and low value).

It is noted that, in general, for water from sources for which there is considered to be a high probability of contamination (such as the river abstraction scenario considered here), a higher degree of treatment would be anticipated than if water was drawn from known pristine sources. It is

important to stress, therefore, that the predictions generated here relating to the levels that might occur with only a basic treatment of the water but assuming a high pollutant load is not particularly realistic of the UK situation and therefore could be argued to represent an extreme (as opposed to realistic) worst case scenario, particularly in the light of the generally conservative assumptions adopted throughout the earlier stages of the modelling.

2.3.2.1 Influence of water treatment processes

The removal of EDCs by drinking water treatment processes (DWTP) was estimated based on the physicochemical properties of the substances and the best current scientific and technical understanding of the chemical mechanisms and effectiveness of various treatment processes that are available across the UK. In particular, this stage of the modelling draws on previously published data obtained by application of a 3D-QSPR model for estimating the effectiveness for EDC-removal with ozone- and chlorine-based treatment methods (Lei and Snyder, 2007). Where the required source data were available, this model was adopted here. The following DWTPs were considered separately:

- Coagulation;
- Use of Activated carbon in the form of granules (granular activated carbon – GAC) or powder (powdered activated carbon – PAC);
- Use of Ozone (O_3); and
- Use of Chlorine (Cl_2).

In addition to determining the extent of removal achieved by each specific DWTP, two particular treatment scenarios – representing extremes of the technologies available – were considered. The first scenario represents use of only 'conventional treatment' in the UK comprising coagulation-flocculation followed by filtration and chlorination. In general terms, a coagulation-flocculation/filtration process will only remove charged colloidal species from water (Parsons and Jefferson, 2006); species that are negatively charged (anionic) at pH 7 are more amenable to removal by this approach. Filtration is not expected to give any further removal as any remaining EDC substance would be fully dissolved. The scenario considering removal by chlorine is based on the models developed and validated by Lei and Snyder (2007).

The second scenario modelled represents use of a 'advanced treatment' system consisting of pre-ozonation, followed by coagulation and powdered activated carbon and a final filtration stage. For some compounds, quantitative structure-property relationship (QSPR) models as developed and validated by Lei and Snyder (2007) were available to be used to predict percent reactivity with ozone and free chlorine (HOCl/OCl-). Molecular structures were converted from two dimensional (2D) to three-dimensional (3D) ones using LigPrep software (via Maestro). The LigPrep software uses an energy minimizational approach to calculating 3D structures on the basis of the 2D structures. Assumptions used for this conversion included an assumed pH of 7 (±2) which established the degree of ionisation of the compound to that most likely to be experienced within a water treatment scenario. Importantly, the state of ionisation affects the compound charge and, hence, its reactivity, especially in the case of consideration of the potential for chemical interactions with electrophilic reactants such as ozone and chlorine. Additionally, ionisation has a significant effect on other properties of compounds such as sorption to solids (including activated carbon) and degree of volatilisation.

2.3.2.2 Estimation of physicochemical properties

The physicochemical properties of each compound were estimated using QikProp (via Maestro). QikProp makes property predictions based on a substance's 3D-structure calculated using LigPrep. While many molecular properties were estimated, of key interest with respect to the predicted reactivity with ozone and chlorine were:

- the number of reactive functional groups that were unstable and subject to nucleophilic attack (#rtvFG);
- the number of likely reaction pathways via electrophilic pathways (#metab);

- the pi (C-H) component (PISA) of the total solvent accessible surface area (SASA) of the compound;
- the weakly polar component (WPSA) of the SASA;
- the predicted octanol/water partition coefficient (QP log Kow); and
- the calculated ionisation potential.

2.3.2.3 Estimation of reactivity

The data used in the modelling of reactivity were those published by Lei and Snyder (2007). Specifically, the correlation for percent removal via ozonation was:

$$\% \text{ ozone removal} = 67.3 + 0.0506 \bullet \text{PISA} + 5.20 \bullet \#\text{metab} - 4.34 \bullet \#\text{rtvFG} - 0.114 \bullet \text{WPSA}$$

The calculated percentage ozone removal thus developed was used to place compounds into three categories: highly; moderately; or slightly reactive to ozone.

The independent parameters in the model were PISA, the pi (carbon and attached hydrogen) component of SASA (total solvent accessible surface area) in square angstroms, using a probe with 1.4 angstrom radius); WPSA, the weakly polar component of SASA; #metab, the number of metabolites amenable to electrophilic attack; and #rtvFG, the number of unstable functional groups susceptible to nucleophilic attack.

The QSPR model for percent removal during chlorination (% chlorine removal) was developed by Lei and Synder (2007) in the same manner as the QSPR for % ozone removal.

The resulting QSPR was:

$$\% \text{ chlorine removal} = 106.8 + 0.791 \bullet (\% \text{ ozone removal}) + 7.89 \bullet \#\text{rtvFG} + 4.80 \bullet \text{QP log Kow} + 0.175 \bullet \text{FISA} - 15.0 \bullet \text{IP}$$

The calculated percentage chlorine removal was used to categorise compounds as highly, moderately, or slightly reactive with chlorine.

The independent parameters used were FISA (hydrophilic component of SASA, SASA on N, O, and H on heteroatoms); QP log Kow (predicted octanol/water partition coefficient); #rtvFG (# or reactive functional groups); IP (PM3 calculated ionisation potential), and % ozone reactivity (calculated as described above).

Where the required data were not available from Lei and Snyder (2007), ozone removal was calculated on the basis of four aspects of a substance's structure (Drewes *et al*. 2007):

1. Electron-donating groups (e.g., hydroxyl, amine, conjugated double bond, and sulphide) enhance reactivity with ozone, whereas electron-withdrawing groups (e.g., iodine, chlorine, fluorine, and nitro) reduce the reaction rate;
2. Electron-donating groups enhance the reactivity of aromatic compounds toward ozone, while electron-withdrawing groups inhibit the reactivity;
3. Phenolic compounds are highly amenable to an attack by ozone, whereas ketone groups decrease the reactivity of ozone with adjacent carbons on aromatic structures; and
4. Hydroxyl and ketone groups have an activating effect on the adjacent methylene groups of an aliphatic chain, though the oxidation rates are lower than those of corresponding aromatic structures.

A score of 1 to 10 was assigned to each substance, based on its reactivity according to the above categorisations. The dataset of substances were then separated into four nominal categories relating to their overall predicted ease of removal during water treatment:

1. Good removal (>90%);
2. Intermediate removal (90-50%);
3. Intermediate removal (50-25%); and
4. Poor removal (<25%).

The effectiveness of powdered activated carbon (PAC) was also assessed to determine the removal of each substance considered. Multiple forces are involved in activated carbon adsorption including: coulombic-unlike charges, dipole-dipole interactions, van der Waals forces, covalent bonding and hydrogen bonding (Sontheimer *et al*. 1988).

Each substance was placed in one of the following categories, based on their octanol-water coefficient (log K_{ow}) and the charge of compound at pH 7 (Drewes *et al*., 2007):

1. log K_{ow} > 4 (pH 7); uncharged
2. log K_{ow} = 0–4 (pH 7); uncharged
3. log K_{ow} < 0 (pH 7); uncharged
4. log K_{ow} = 0–1.5 (pH 7); protonated base
5. log K_{ow} < 0 (pH 7); protonated base
6. log K_{ow} = 0–2.5 (pH 7); deprotonated acid
7. log K_{ow} < 0 (pH 7); deprotonated acid.

The estimated removal for each of these was then categorised into one of seven categories, as follows:

1. >90% removal
2. 90-50% removal
3. 50-25% removal
4. 90-50% removal
5. 50-25% removal
6. 50-25% removal
7. <25% removal.

Once the predicted removal of a substance had been established under the scenario conditions defined for each treatment process stage, the data were combined to determine the total removal anticipated for an advanced treatment process.

3 Results

3.1 Prioritisation of potential EDCs in water sources (Objective 1; Task 1)

3.1.1 Initial search and data extraction

The initial literature search (as detailed in Section 2.1.1) retrieved 3052 journal citations, the titles of which were reviewed by experienced scientists to assess their potential relevance to the project. On the basis of this screening, 509 journal articles were identified as warranting a more detailed consideration. Each of these papers were therefore obtained in full and considered in detail; key data of relevance were then extracted. This review process resulted in 4393 data entries, of which 2516 were directly relevant to either the UK or Europe. A total of 325 substances for which the paper's authors stated there to be potential concerns with regard to endocrine disruption were identified. These substances had been detected or modelled in groundwaters, surface waters, freshwaters, drinking/potable waters or sewage treatment effluent and were considered of possible relevance within a European setting (individual substances detailed in Annex 2, Table A2.1).

It should be noted that no attempt to verify the validity/robustness of concerns relating to EDC-status was made at this stage of prioritisation. Rather, in order to ensure as comprehensive 'capture' of potential source papers as possible, the literature search strategy included the deliberately generic 'endocrine disrupt*' search term. Hence, it should not be inferred that the 325 identified chemicals constitute EDCs, only that they had been identified as potential candidate substances by the authors of the source papers. It was therefore recognised that, following more detailed scientific consideration a proportion of the substances prioritised would be likely to possess little robust data supporting mammalian-relevant endocrine system activities. Such a situation might, for example, arise if a substance is reported in the literature to possess endocrine properties of relevance only to invertebrate species (i.e. having no direct relevance to mammalian or human health endpoints) or if the substance was included as a potential EDC by the authors of the primary paper on a speculative basis or because of inconclusive evidence.

3.1.2 Prioritisation of substances

Following the initial data extraction, the data for 325 substances were subjected to a number of critical assessment stages to determine which substances should be included/excluded from the environmental fate/behaviour and water treatment process modelling stages (as detailed in Annex 2, Table A2.1).

Based on step-by-step application of the selection criteria, of the initial 325 chemicals identified by the literature search, 166 chemicals were excluded from further consideration and 159 were taken forward for modelling.

3.2 Modelling

3.2.1 Environmental fate and behaviour of indentified chemicals (Objective 2; Task 2)

For the 159 chemicals subject to modelling, the predicted concentration at drinking water treatment works intakes and the maximum and minimum predicted concentrations arising after treatment of the drinking water using either a basic and more modern treatment process, are presented in Table 3.1.

Most of the chemicals evaluated were predicted to occur only at relatively low concentrations, even when the conventional (essentially worst case treatment) scenario was assumed. For the basic treatment scenario (much less likely to be relevant to the UK situation), 31% of chemicals were predicted to exceed 100 ng/L and 42% were predicted to have drinking water concentrations of <10 ng/L (14% were predicted to be absent). In the case of the comprehensive treatment scenario, however, only nine chemicals were predicted as potentially exceeding a concentration in drinking water of 100 ng/L or more, these comprised: DEHP; NP2EC; NP2EO; 2,4-D; NP3EO; OP3EO; DBP; Tris(1,3-dichloro-2-propyl) phosphate; NP1E. Of the remainder (82%) were predicted to have concentrations of below 10 ng/L, of which 51% were predicted to be completely removed.

Table 3.1. Chemicals assessed for predicted environmental concentrations at the intake for drinking water treatment works (PEC_{intake}), the minimum and maximum predicted concentrations in treated water.

Chemical	PECintake (ug/L)	DW Conc (ng/L)		Concentration (meas or usage)[A]
		Min	Max	
2-Hydroxybiphenyl	0.05460	1	55	Meas
2,4-D	1.22100	396	1221	Meas
2,4-Dinitrophenol	0.12200	13	122	Meas
2-Hydroxybiphenyl	0.00036	0	0	Meas
3,4,5,6-Tetrabromo-o-cresol	1.00000	54	1000	Meas
3-t-Butyl-4-hydroxyanisole	0.00700	0	7	Meas
4-Chloro-3-methylphenol	0.00800	1	8	Meas
4-Nitrophenol	0.17400	13	131	Meas
Acipimox	0.00002	0	0	Use
Amitriptyline	0.10220	7	102	Use
Amoxicillin	0.34450	0	258	Use
Androsterone	0.54177	85	542	Use
Aspirin	0.00212	0	2	Use
Atenolol	0.37178	5	372	Use
Atorvastatin	0.08061	0	60	Use
Atrazine	0.00300	0	3	Meas
Azithromycin	0.00724	0	7	Use
BaP	0.35000	0	350	Meas
BBP	0.06565	0	66	Meas
Bentazone	0.11600	11	87	Meas
Benzo(b)fluoranthene	2.58808	7	2588	Use
Benzo(g,h,i)perylene	1.39909	0	1399	Use

Chemical	PECintake (ug/L)	DW Conc (ng/L) Min	DW Conc (ng/L) Max	Concentration (meas or usage)[A]
Benzo(k)fluroanthene	0.91740	2	917	Use
Benzophenone	0.05300	6	53	Meas
Benzylparaben	0.00030	0	0	Meas
Biochanin A	0.00197	0	1	Meas
Bisoprolol	0.17674	6	177	Use
Bisphenol A	1.92400	0	1924	Meas
Bisphenol F	0.18000	0	180	Meas
Butylparaben	0.04200	2	42	Meas
Butylphenol	0.06600	2	66	Meas
Captopril	0.00740	1	6	Use
Carazolol	0.01200	0	12	Meas
Cefuroxime	0.00070	0	1	Use
Celiprolol	0.00300	0	3	Use
Chloridazon	0.00600	1	6	Meas
Chlorophene	0.00500	0	5	Meas
Chlorotetracycline	0.00006	0	0	Use
Ciprofloxacin	0.02726	4	27	Use
Clarithromycin	0.04788	0	48	Use
Clofibrate	0.03000	3	30	Meas
Codeine	0.03400	2	34	Meas
Cortisol	0.01817	2	18	Use
Cortisone	0.00018	0	0	Use
Coumestrol	0.00160	0	1	Meas
Cyclophosphamide	0.00014	0	0	Use
Daidzein	0.85395	0	640	Use
DBP	8.80000	190	8800	Meas
DEET	0.03800	4	38	Meas
DEHP	73.13000	7898	73130	Meas
Demeclocycline	0.00018	0	0	Use
DEP	0.19000	21	190	Meas
DES	0.00064	0	1	Meas
Desethylatrazine (DEA)	0.00700	1	7	Meas
Desethylterbutylazine (DET)	0.01200	1	12	Meas
Dexamethasone	0.00458	1	5	Use
DIBP	0.01000	10	10	Meas
Digoxigenin	0.10767	4	108	Use
Digoxin	0.10767	0	108	Use
Diisodecylphthalate	0.10000	2	100	Meas
Di-isononylphthalate	1.89000	41	1890	Meas
Dilantin	0.00610	1	6	Meas
Dilantin	0.00572	1	6	Meas
Diltiazem	0.00088	0	1	Use
Dimethoate	0.00200	0	2	Meas

Chemical	PECintake (ug/L)	DW Conc (ng/L) Min	DW Conc (ng/L) Max	Concentration (meas or usage)[A]
Dimethylaminophenazone	0.05533	4	55	Meas
Diuron	0.27900	30	279	Meas
Domperidone	0.02602	1	26	Use
Doxazosin	0.24032	3	240	Use
Doxycycline	0.00015	0	0	Use
Ethynl oestradiol (EE2)	0.00010	0	0	Meas
Epi-androsterone	0.01459	2	15	Use
Erythromycin	0.06200	0	62	Meas
Estradiol-17-glucuronide	0.00033	0	0	Meas
Estrone-3-sulfate	0.00013	0	0	Meas
Ethylparaben	0.00670	0	7	Meas
Etofibrate	0.03000	4	30	Meas
Fenofibrate	0.00366	0	4	Use
Fenoterol	0.00002	0	0	Use
Flumethasone	0.00693	1	7	Use
Flunixin	0.03000	4	23	Meas
Fluticasone	0.15876	34	159	Use
Formononetin	0.00094	0	1	Meas
Genistein	0.05769	0	43	Use
Glycitein	0.01211	0	9	Use
Hydroxyacetophenone	0.00280	0	3	Meas
o-Hydroxyhippuric acid	0.07500	1	56	Meas
Indapamide	0.04299	0	43	Use
Indomethacin	0.00594	1	4	Use
Iohexol	0.00328	0	3	Meas
Iomeprol	1.89988	0	1900	Meas
Iopamidol	0.23998	0	240	Meas
Iopromide	0.69996	0	700	Meas
Kaempferol	0.00748	0	6	Use
Levonorgestrel	0.00018	0	0	Meas
Mestranol	0.00160	0	2	Meas
Methylparaben	0.01600	1	12	Meas
Metoprolol	0.04506	1	45	Use
Musk ketone	0.02600	7	26	Meas
Nadolol	0.00036	0	0	Use
Naringenin	0.25409	0	191	Use
Nonylphenol	0.26998	2	270	Meas
Nonylphenoxyacetic acid	0.55300	67	415	Meas
Norethindrone	0.00650	1	7	Meas
Norfloxacin	0.00215	1	2	Use
Nortriptyline	0.00564	0	6	Use
NP1EC	0.12700	127	127	Meas
NP1EO	0.20500	2	205	Meas

Chemical	PECintake (ug/L)	DW Conc (ng/L) Min	DW Conc (ng/L) Max	Concentration (meas or usage)[A]
NP2EC	1.70000	1700	1700	Meas
NP2EO	0.63996	640	640	Meas
NP3EO	0.22000	220	220	Meas
Octylphenol	0.09900	1	99	Meas
Oestradiol (E2)	0.02500	0	25	Meas
Oestriol (E3)	0.00310	0	3	Meas
Oestrone (E1)	0.01700	1	17	Meas
Ofloxacin	0.00190	0	2	Use
OP1EO	0.66500	9	665	Meas
OP3EO	0.22000	220	220	Meas
Oxazepam	0.00464	0	5	Use
Oxytetracycline	0.02323	0	23	Use
Paracetemol	0.16789	2	168	Use
p-Benzylphenol	25.00000	65	25000	Meas
PFDA	0.01100	11	11	Meas
Phenylphenol	0.03200	0	32	Meas
Phloretin	0.01240	0	9	Use
Piroxicam	0.00215	0	2	Use
Pravastatin	0.04747	1	47	Use
Prednisolone	0.00016	0	0	Use
Prednisone	0.01000	2	10	Meas
Progesterone	0.00080	0	1	Meas
Propoxur	0.00900	1	9	Meas
Propranolol	0.06401	0	64	Use
Propylparaben	0.00210	0	2	Meas
Quercetin	0.00924	0	7	Use
Ramipril	0.41869	16	314	Use
Roxithromycin	0.05400	0	54	Meas
Salbutamol	0.46443	0	464	Use
Sertraline	0.03658	4	37	Use
Simazine	0.12700	11	127	Meas
Simvastatin	0.54749	12	547	Use
Sotalol	0.02117	0	21	Use
Sulfadimethoxine	0.00000	0	0	Use
Sulfamethoxazole	0.00000	0	0	Use
Sulfapyridine	0.03400	0	34	Meas
Sulfasalazine	0.01579	0	16	Use
Tamoxifen	0.00482	0	5	Use
Tebuconazole	0.03000	5	30	Meas
Terbutalin	0.02332	0	23	Use
Terbutryn	0.00000	0	0	Use
Terbutylazine	0.12400	11	124	Meas

Chemical	PECintake (ug/L)	DW Conc (ng/L) Min	DW Conc (ng/L) Max	Concentration (meas or usage)[A]
Tetracyclin	0.00002	0	0	Use
Tolfenamic acid	0.00015	0	0	Use
Triamcinolone	0.00000	0	0	Use
Triamcinolone acetonide	0.00220	0	2	Use
Tributylphosphate	0.20100	32	201	Meas
Triclosan	0.00004	0	0	Use
Tris(1,3-dichloro-2-propyl) phosphate	0.28135	141	281	Meas
Warfarin	0.19618	19	147	Use
β-Sitosterol	0.17819	4	178	Meas

A: meas = data presented are measured values; usage = data presented are estimates derived from data on usage levels.

3.2.2 Influence of water treatment process (Objective 3; Task 3)

Detailed results on the removal of each substance predicted to occur as a result of conventional treatment (coagulation-flocculation/filtration), as described in Section 2.3.2, are given in Annex 3, Table A3.1. For comparison, the predicted removal of the substances when using an advanced treatment process (coagulation, ozone, activated carbon and chlorination), as described in Section 2.3, are given in Annex 3 Table A3.2.

The percent removal by ozonation showed a significant correlation to that with chlorination; ozonation removal rates were therefore used to estimate the extent of chlorine removal for those substances for which no relevant published data was available. It was clear that ozonation provided a greater removal than chlorination for all the substances considered here. The removal rates were subsequently applied to fate and behaviour modelled data (Section 3.2) to calculate predicted maximum and minimum drinking water concentrations following conventional or advanced treatments respectively.

4 Risk Assessment

4.1 Organic chemicals of anthropogenic or natural origin (Objectives 3 and 5; Tasks 3 and 5)

The potential risk posed to the general population from indirect exposure to each of the chemicals selected on the basis of exposure modelling (Chapter 3) is assessed below, based on consideration of the following data:

- the estimated daily intake of chemical by various population subgroups through consumption of drinking waters produced by either conventional (powdered activated charcoal + $C1_2$) or advanced (Adams O_3 + Cl_2) water treatments;
- estimated daily intake of the chemical from food sources (where appropriate);
- the potential toxic hazard posed by the chemical; and
- the source-specific apportionment in terms of the proportion of the tolerable daily intake (TDI) (or, where no authoritative value is available a study-specific exposure limit) arising from the predicted intake via drinking water for specific population groups of potential concern (i.e. adult population, toddlers or infants).

The average daily intake of each chemical was estimated separately for each of the two water treatment regimens considered for adults, toddlers and infants; the derivation was based on standard default estimates of daily water consumption for these population sub-groups (2L, 1L and 0.75L, respectively) and body weights of 60 kg, 10 kg and 5 kg, respectively (WHO, 1996).

The resultant estimated daily intakes for adults, toddlers and infants from drinking waters obtained from conventional or advanced treatments were compared with the established TDI or, if not available, a study-specific exposure limit [1]for each chemical. For pharmaceuticals, the study-specific exposure limit was determined by applying an additional uncertainty factor to the minimum therapeutic dose; the additional uncertainty factor was derived from expert consideration of the margin of safety required to prevent potential ED effects following exposure of infants to the pharmaceutical. The percentage contribution from drinking water to the TDI (or study-specific exposure limit) was determined and a margin of safety calculated, as detailed in Table 4.1. In developing risk characterisations, comparison of the estimated exposure with the nature and dose-response characteristics of the principal toxic hazard and (if different) the endocrine-disruptive properties of each substance was made.

To inform on the nature of the hazard that the substance might pose, hazard characterisation profiles were prepared for each substance, with a particular focus on establishing the strength of the evidence base relating to the endocrine-disrupting potential of the substance and contrasting this with its other established hazardous properties; the detailed hazard characterisation profiles are presented in Annex 4. Wherever possible the profiles were developed by reference to the most recent evaluation(s) published by authoritative organisations, with particular emphasis given to UK or European regulatory opinions, where available. In some cases, a complete dataset for a substance was not available from relevant evaluations or published papers.

The following criteria were applied when characterising the nature and acceptability of the level of risk posed by each substance in drinking water:

[1] study-specific exposure limits were derived *de novo* during the study

- Where the total predicted daily intake for infants (highest intake and greatest susceptibility) from drinking water following conventional treatment (i.e. the technology providing the lowest removal rate of chemicals) afforded a margin of safety (MOS) of greater than 100 times when compared to the TDI or study-specific exposure limit, it was considered that there was no appreciable risk posed;

- Where the MOS lay between 10 and 100 times, some further consideration was given within the study resource restraints, to characterise the relevance or otherwise of this level of expsoure; and

- Where the MOS was less than 10 times when compared to the TDI or study-specific exposure limit, detailed consideration was undertaken of the significance of this worst case estimate in the light of the nature of any underlying hazard concern.

Table 4.1 Estimated safety margin calculated using 'worst case' predicted intake from drinking water based on conventional treatment processes and conservative assumptions for the general adult population, toddlers (1-2 yrs) and infants (0–1 yrs)

Chemical Name	Conventional Treatment[a]		
	Margin of Safety (fold higher)[b]		
	Adult[c]	Toddler[d]	Infant[e]
Industrial Chemicals			
Benzo[a]pyrene (BaP)[2]	1	nc	nc
p-Benzylphenol	4.8	1.6	0
Bisphenol A (BPA)	78	26	17
DiButylphthalate (DBP)	19	6	4
Di(2-ethylhexyl)phthalate (DEHP)	>10,000	5000	3300
Diethylphthalate (DEP)	>10,000	>10,000	>10,000
4-nitrophenol	25	8	5
Nonylphenol[3]	178	56	36
4-Octylphenol[4]	>10,000	5000	3300
Tributylphosphate	>10,000	>10,000	>10,000
Tris(1,3-dichloro-2-propyl)phosphate	290	97	65
Pesticides			
Diuron	770	250	160
2,4-Dichlorophenoxyacetic acid	770	250	170
2,4-Dinitrophenol	500	167	111
Simazine	130	40	27
Tetrabutylazine	2200	666	440
Consumer Chemical			
3,4,5,6,-Tetrabromo-o-cresol	150	50	33
Natural Chemicals (plant)			
Daidzein[5]	800	nc	nc
Naringenin	>10,000	>10,000	>10,000
B-Sitosterol	>10,000	>10,000	>10,000
Natural Chemical (hormone)			
Androsterone	1380	460	300
Pharmaceuticals[6]			
Amitryptiline	660	200	113
Amoxicillin	>10,000	>10,000	>10,000
Atenolol	416	135	90
Bisoprolol[7]	42	14	9

[2] Margin of safety calculated against the UK Drinking Water Standard (DWS=0.01 µg/L) for adults only. NB: predicted concentrations for BaP are equivalent to DWS providing clear evidence that the regulatory limit is adhered to across the UK. It is considered that risks from exposure to BaP via drinking water are not of concern in the UK.

[3] For NPEs (NP1EC, NP2EC, NP3EO, NP2EO and NP1EO) there is no basis for establishing a safety factor as the substance of concern with regard to endocrine activity and other toxicities is NP.

[4] For OPEs (OP1EO and OP3EO) there is no basis for establishing a safety factor as the substance of concern with regards to endocrine activity and other toxicities is 4-OP.

[5]Margin of safety calculated against normal dietary intake – adult only (maximum of 1 mg/d = 0.017 mg/kg/bw/d for adult).

[6] Margin of safety calculated against study-specific exposure limit for all pharmaceuticals.

Chemical Name	Conventional Treatment[a] Margin of Safety (fold higher)[b]		
	Adult[c]	Toddler[d]	Infant[e]
Digoxin	15	5	4
Doxazosin	625	208	138
Fluticasone	20	6	4
Iopamidol	>10,000	>10,000	>10,000
Iopromide	>10,000	>10,000	>10,000
Paracetamol	>10,000	>10,000	>10,000
Ramipril	250	80	53
Salbutamol	13	4	3
Simvastatin	5550	1820	1220
Warfarin	60	20	14

a: conventional water treatment comprising powdered activated charcoal + C12; **b**:margin of safety calculated as reasonable worst case estimate of daily intake from drinking water of each chemical for adults, toddlers and infants expressed as a proportion of the TDI or study-specific exposure limit; **c:** adult > 18 years with assumed body weight of 60 kg consuming 2L of water per day; **d:**toddler 1-2 years with assumed body weight of 10 kg ; **e**: infant 0-1 years with an assumed bodyweight of 5 kg; nc – not able to be calculated; shaded boxes represent chemicals identified for detailed risk assessment.

4.1.1 Chemicals for which modelled intakes from drinking water were not considered to be of appreciable concern

Of the chemicals subjected to exposure modelling and risk characterisation, a number were found to have maximum predicted daily intakes from drinking water (based on highly conservative assumptions) that afforded for all sectors of the general population considered a MOS in excess of 100 times when compared to the TDI or a study-specific exposure limit. In the light of this high margin when even very conservative assumptions had been made, it was judged that no further consideration was warranted for these substances. The chemicals falling into this group were:

BaP; DEHP; DEP; 4-OP (including OP1EO and OP3EO); Tributylphospahte; Diuron; 2,4-Dichlorophenoxyacetic acid; 2,4-Dinitrophenol; Tetrabutylazine; Daidzein; Naringenin; β-Sitosterol; Androsterone; Amitryptiline; Amoxicillin; Bisoprolol; Doxazosin; Iopamidol; Iopromide; Paracetamol; and Simvastatin.

4.1.2 Chemicals for which estimated worst-case intakes afford a margin of safety of 10-100 times the TDI or study-specific exposure limit, for one or more of the sub-populations considered

Where the predicted reasonable worst-case daily intake afforded a MOS of between 10-100 times when compared to the TDI or study-specific exposure limit, a more detailed evaluation of the nature of the potential risk posed – based on conservative exposure assumptions – was undertaken through consideration of the chemical's hazard profile and the assumptions made in determining intake levels. Assessments of the risks posed by these individual chemicals, with a focus on their endocrine disrupting properties, are presented below.

Bisphenol A (BPA)

BPA is used mainly as a monomer in manufacture of polycarbonate and epoxy resins for food contact materials and consumer products such as tableware, reusable drinking bottles and infant feeding

bottles (EC, 2010). Oral exposure is generally the main exposure pathway though dermal exposure may occur in workers (EC, 2010). General levels of consumer exposure have been estimated at 1.45 µg/kg bw/d from food and drink and 0.0091 µg/kg bw/d from other (indirect) sources (EC, 2010).

BPA is permitted for use in food contact plastics in the European Union, subject to a specific migration limit of 0.6 mg/kg food (Commission Directive 2002/72/EC). A TDI of 0.05 mg/kg bw/d has been established which is based on a no observable adverse effect level (NOAEL) of 5 mg/kg bw/d for adult bodyweight effects and pup body and organ weight effects in rats and liver effects in mice (Tyl *et al*., 2002; 2007); an uncertainty factor of 100 was applied to allow for inter-species differences (EFSA, 2010). Effects on fertility (reduced litter size) have been noted at 500 mg/kg bw/d but not 50 mg/kg bw/d in a rat multigeneration study (effects possibly associated with maternal toxicity) and in a murine continuous breeding study at 600 mg/kg bw/d or greater. An overall NOAEL of 50 mg/kg bw/d has been established for fertility endpoints. An occupational exposure limit for inhalation of 5mg/m^3 TWA has been adopted in several EU Member states and an EU Indicative OELV has been proposed at 10 mg/m^3 TWA (EC, 2010).

The worst-case predicted daily intake of BPA for adults, toddlers and infants afforded MOS of 78, 26 and 17 times respectively, when compared with the TDI. These were based on modelled levels in drinking water using measured data from a UK study of river water (Kasprzyk-Hordern *et al.*, 2009b) assuming conventional water treatment. Predicted intakes following advanced water treatment were zero in all cases.

An intake level MOS approaching 10 times, as seen for infants supplied with water derived from a conventional source, would warrant further consideration. However, the established oral TDI for BPA provides an uncertainty factor of 1000 in relation to potentially endocrine-relevant effects of the substance. In addition, it is recognised that BPA is the subject of ongoing regulatory discussion in Europe with, most recently, the French Competent Authority making proposals for European harmonised labelling for BPA[8]; such developments may be anticipated to place continued pressure for reducing the level of exposure experienced by European consumers.

Nonylphenol and the Nonylphenol ethoxylates

The term "nonylphenol" (NP) has historically been applied to a large number of isomeric substances that vary in structure with respect to the substitution position of the nonyl groups on the phenol, and the degree of branching of the nonyl group (EC, 2002). The majority of production of NP is used as a starting material for synthesis of nonylphenol ethoxylates (NPEs), with the remainder being used as monomer in polymer production. In turn, NPEs are manufactured by reacting NP with ethylene oxide to form polyethylene oxide chains of the desired length. NPEs are non-ionic surfactants widely used (e.g. ingredients in cleaners and detergents, household cleaning, personal-care products and for emulsion polymerisation and polymer stabilisation, textile processing, in agricultural chemicals, pulp and paper processing, metal and mineral processing, latex paints, wetting agents and emulsifiers, foaming agents, inks, adhesives, and in pharmaceuticals). NPEs are subject to microbial degradation in STWs and may also undergo environmental degradation into NP (EC, 2002). NP bioconcentrates to a significant extent in aquatic species despite relatively rapid excretion/metabolism (EC, 2002).

As a consequence of the very wide use pattern of these substances, possible routes of human exposure include: dermal contact and inhalation for workers involved in manufacture and use; dermal contact and inhalation for consumers of household pesticide products; and orally via various environmental sources for the general population (EC, 2002). The overall human exposure from environmental sources in the EU (excluding point source emissions) has been estimated as 5.31 µg/kg/day (EC, 2002). Canadian Authorities have estimated exposure to be mainly attributable to surface water (0.39 µg/kg/day), food packaging (17 µg/kg/day) and meat (17 µg/kg/day); with other sources only contributing a minimal additional load (IPCS, 2004).

[8] See http://albertoalemanno.eu/articles/bisphenol-a-toward-a-eu-wide-ban

A number of rigorous assessments have clearly established that the principal cause for concern with regard to nonylphenol and its ethoxylates (i.e. NP and NPEs) is the level of exposure to NP, since the NPEs are of much lower mammalian toxicity and not of direct concern with regard to endocrine or reproductive effects. Given this, it was deemed appropriate to concentrate the risk assessment on the predicted levels of NP, rather than consider the much less toxic NPEs. Furthermore, although possessing some oestrogenic effect (10^{-3} to 10^{-6} that of oestradiol) and showing both reproductive and development toxicity, risk assessments have established that the principle basis for regulation is the sub/chronic toxicity of NP and that control on this basis is adequately protective of all other hazardous effects.

Given this, the identified repeat dose lowest observable adverse effect level (LOAEL) of 15 mg/kg bw/d for NP that was identified by the EC (2002) is considered to be an acceptable basis against which exposure to these substances via drinking water might be assessed. If a highly precautionary study-specific uncertainty factor of 1,000 (10 for use of a LOAEL as the basis, 10 for interspecies extrapolation and 10 for inter-individual variation) were to be applied to the repeat dose LOAEL, this might suggest a limit for nonylphenol of 15 µg/kg bw/d. However, given the uncertainties with regard to the extent to which other forms of NP (e.g. NP1EC, NP2EC) might conceivably contribute to the overall hazard posed, it is proposed to introduce an additional uncertainty factor of 10 (i.e. 10,000 in all) giving a study-specific exposure limit for NP of 1.5 µg/kg bw/d. The worst-case predicted daily intakes of NP for adults, toddlers and infants afforded MOS of 178, 56 or 36 times respectively when compared to this study-specific exposure limit; these are based on modelled levels in drinking water based on measured data from a UK study of river water (Kasprzyk-Hordern *et al*., 2009b) and conventional water treatment. Predicted intakes following advanced water treatment were zero in all population subgroups.

The modelled predictions of intakes for NP are based on a PEC_{intake} of 0.3 µg/L which resulted in drinking water concentrations of only 2 and 270 ng/L for advanced and conventional treatments respectively. These values compare favourably with the UK Operational EQS for NP release of 1 µg/L and very favourably with the more stringent EQS values derived under the Water Framework Directive. Further reassurance regarding exposure to NP and its ethoxylates is provided by the restrictions on the use of NPEs in various applications now in place across the EU (IPCS, 2004; EA, 2010; EA, 2012) which demonstrate that future exposures of the European population would be confidently expected to show a continued reduction and hence any residual concern would be extremely low.

Ramipril

Ramipril is a prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor class of medications. It is metabolized to ramiprilat in the liver and, to a lesser extent, kidneys. Ramipril is a potent, competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system (RAAS). Ramipril may be used in the treatment of hypertension, congestive heart failure, nephropathy, and to reduce the rate of death, myocardial infarction and stroke in individuals at high risk of cardiovascular events (DrugBank.com).

Occupational exposure to ramipril may occur through inhalation of dust and dermal contact with this compound at workplaces where it is produced or dispensed. Direct exposure to ramipril among the general population is most probably limited to those administered the compound medically.

Using a precautionary approach, this substance was identified as of possible concern following expert advice from the clinician within the project team. However, no evidence suggestive of endocrine disrupting activity per se was identified for ramipril in subsequent literature review.

A study-specific exposure limit of 2.5 µg/kg bw/d was derived based on the minimum human therapeutic dose of 1.25 mg/d (0.25 mg/kg bw/d for a 5kg infant) to which was applied an uncertainty factor of 100 (based on expert clinical advice).

The worst-case predicited daily intake of ramipril in adults, toddlers and infants gave MOS of 250, 80 and 53 times respectively when compared to the study-specific exposure limit. These estimates were based on modelled levels in drinking water derived from UK prescription data assuming conventional water treatment. Predicted exposures in the case of advanced water treatment were zero for adults and approaching zero for toddlers and infants.

Given that no evidence of endocrine disrupting potential has been identified during detailed literature review, and considering the highly precautionary approach employed at each stage of assessement, no significant concerns are warranted for ramipril.

Tris (1,3-dichloro-2-propyl)phosphate

TCDP is an additive flame retardant (i.e. it is physically combined with the material being treated rather than chemically combined). Most TDCP is used in the production of flexible polyurethane (PUR) foam, which is mainly used in the automotive and furniture industries.

Extensive risk assessment by the EU suggests a daily human environmental intake of 1.52×10^{-5} mg/kg/day; the highest occupational daily intake was estimated at 6.99×10^{-4} mg/kg/day. Exposure pathways for humans are through oral, inhalation and dermal routes (EC, 2008).

In the absence of an established TDI, a study-specific exposure limit for TCDP of 0.05 mg/kg bw/d was defined based on a NOAEL of 5 mg/kg bw/d for reproductive and developmental toxicity in rats (Stauffer Chemical Company, 1981) and an uncertainty factor of 100 to allow for inter- and intra-species differences.

The worst-case predicted daily intake of tris (1,3-dichloro-2-propyl) phosphate for adults, toddlers and infants afforded MOS of 290, 97 and 65 respectively, when compared with the study-specific exposure limit. These figures were based on modelled levels in drinking water calculated using measured data from a study carried out on drinking water sources in Cyprus (Makris and Snyder, 2010) undergoing conventional water treatment. It should be noted that the MOS following advanced water treatment were slightly increased to a minimum of 127 times (for infants).

From the findings of this study, it appears that significant concern regarding this substance is not appropriate. Nonetheless, it would seem prudent to seek to gather additional measured data relevant to UK water sources to order to fully inform on intake.

Simazine

Simazine is a pre-emergence triazine herbicide. Although banned in the majority of Europe due to concerns regarding environmental persistence (Pan UK, 1993), the herbicide is used under derogation in the UK to control broad-leaved and grass weeds in artichokes, asparagus, berries, broad beans, citrus fruits, hops, maize, olives, orchards, ornamentals, sugar-cane, tea, tree nurseries, turf and vineyards, as well as in non-crop areas (BCPC, 1991). Triazines have been demonstrated to be relatively biopersistent, therefore, where it is used, consumers and farmers may be potentially exposed to simazine indirectly via drinking water and foods or directly by exposure during application (Kim *et al*., 2003).

A TDI of 0.5 µg/kg bw/d has been established for simazine based on a NOAEL of 0.52 mg/kg bw/d for carcinogenicity and long-term toxicity indicated by alterations in body weight and haematological parameters in rats. An uncertainty factor of 1000 was applied (10 for inter-species variation, 10 for intra-species variation, and 10 for possible carcinogenicity; WHO, 2003). Co-exposure of simazine with oestradiol in ovarectomised Sprague Dawley rats has been shown to result in a significant dose-related decrease in uterine weight, suggesting oestrogen receptor antagonism (Tennant *et al.*, 1994).

The worst-case predicted daily intakes of simazine for adults, toddlers and infants afforded MOS of 130, 40 and 27 when compared to the TDI. These figures were based on modelled levels in drinking water using measured UK-specific data from a study carried out on drinking water sources in Europe (Loos *et al.*, 2009), and conventional water treatment. It should be noted that MOS following advanced water treatment was a minimum of 260 times (for infants).

The predicted environmental concentration of simazine calculated in this study was 0.13 µg/L, which is well below the WHO drinking water guideline of 2 µg/L (WHO, 2003). Due to the current restrictions on the use of this herbicide in the UK, it seems unlikely that concentrations will rise above the conservative predictions provided by this study. In light of this, no significant concerns are raised.

3,4,5,6,-Tetrabromo-o-cresol

3,4,5,6-Tetrabromo-o-cresol is a brominated phenolic used as an antiseptic and fungicide. For example, it has been used for hand disinfection and for topical preparations for the treatment of fungal infections (Daughton & Ternes, 1999; Pharmacopoeia Site, undated). The main route of exposure for humans is following dermal application.

No established environmental standards or guide values have been identified during the literature review phase of this project for 3,4,5,6-tetrabromo-o-cresol. This substance was identified as of possible concern because of its use profile and a report of its presence in relevant water bodies (Kasprzyk-Hordern *et al.*, 2009b). Subsequent QSAR modelling by the Study Team indicated that it might show protein binding properties. However, no evidence suggestive of endocrine disrupting activity per se was subsequently identified for 3,4,5,6-Tetrabromo-o-cresol and little significant information on its hazard profile - other than its recognised irritant properties - is available. Although registration was anticipated by ECHA in 2010, this substance has yet to be registered in the EU (ECHA, undated); if this occurs, additional toxicity data may become available.

A study-specific exposure limit was defined for use in assessment of risk. This was based on application of a conservative uncertainty factor of 10,000 (100 for use of acute data only, and 10 for interspecies and 10 for inter-individual extrapolation) to the only available toxicity data (Rat oral LD_{50} = >500 mg/kg); this would suggest that a potential study-specific exposure limit of the order of 5 µg/kg bw/d.

The worst-case predicted daily intakes of 3,4,5,6-tetrabromo-o-cresol for adults, toddlers and infants respectively resulted in MOS of 150, 50 and 33 when compared to the study-specific value. The figures were based on modelled levels in drinking water calculated using measured UK-specific data (Kasprzyk-Hordern *et al.*, 2009b) and conventional water treatment. It should be noted that MOS based on advanced water treatment were a minimum of 625 times (for infants). In any event, the study-specific exposure limit incorporates a very high uncertainty factor of 10,000.

In the absence of further hazard information, it is not possible to undertake a more meaningful risk assessment at this time. However, it is recommended that the significance of any exposure to this substance should be re-evaluated in the future should additional toxicity and exposure data become available.

Atenolol

The cardioselective β_1-selective βadrenergic receptor blocker Atenolol is used for hypertension management and the long-term management of patients with ischaemic heart disease.

Occupational exposure to atenolol may occur through inhalation of dust and dermal contact with this compound at workplaces where atenolol is produced or formulated for use (e.g. hospital pharmacy). The general population may be exposed orally to atenolol through direct medical administration of this compound or potentially from environmental sources.

LOAELs for developmental and neurobehavioural effects in the rat have been established at 5 and 7.5 mg/kg/day atenolol respectively; atenolol is selectively embryotoxic in the rat, as developmental effects are induced at doses below those associated with maternal toxicity (Tabacova *et al*., 2003). There is some evidence, predominantly in vitro, that suggests that atenolol has some oestrogenic activity although the relationship to its developmental and reproductive toxicity is uncertain. The minimum therapeutic dose (0.5 mg/kg/d), used for neonates, is 10-fold lower than the LOAEL established for developmental effects. As a precautionary measure, a further uncertainty factor of 100 was applied to the minimum human therapeutic dose to derive a study-specific exposure limit for risk assessment purposes.

The worst-case predicted daily intakes of atenolol for infants afforded a MOS of 90 times the study-specific exposure limit based on reproductive and developmental endpoints. This relates to modelled levels in drinking water, calculated on the basis of UK prescription data and conventional water treatment. It should be noted that MOS following advanced water treatment were a minimum of 5000 times (for infants).

Given that the MOS for atenolol is towards the higher level considered to trigger detailed risk assessment in this study, and that a highly precautionary approach was employed at each stage of assessment, no significant concerns are merited.

Warfarin

Warfarin is the original and still most frequently prescribed oral anticoagulant. Anticoagulants are used in the treatment of patients with recent deep vein thrombosis to prevent extension and embolisation of the thrombus and in longer term treatments to reduce the risk of pulmonary embolism or recurrent thrombus formation. Warfarin and its sodium salt are also registered for use in controlling rodents (rats and mice) in and around homes, animal and agricultural premises, and commercial and industrial sites. However, only the pharmaceutical use is considered here.

Occupational exposure to warfarin may occur through inhalation of dust and dermal contact with this compound at workplaces where warfarin is produced or where used as a rodenticide. The general population may be exposed to warfarin via medical administration of this compound for the treatment of certain blood conditions, or indirectly through release into the environment during its use as a rodenticide.

This substance was identified as of possible concern by QSAR modelling by the Study Team which indicated that it may show protein binding properties. However, no evidence suggestive of endocrine disrupting activity per se has been identified in subsequent literature review.

A TDI of 0.0003 mg/kg/d (0.3µg/kg/d) was established by the US EPA based on increased prothrombin time in humans (LOAEL of 2 mg/day, equivalent to 0.029 mg/kg/day based on a 70 kg adult, 0.033 mg/kg/d for a 60 kg adult). An uncertainty factor of 100 was applied: 10 to account for the use of a LOAEL and 10 to protect for sensitive individuals in the population.

The worst-case predicted daily intakes of warfarin in infants gives a MOS of 14 compared with the TDI for haematopoietic endpoints when modelled levels in drinking water are based on usage figures from UK prescription data (making no allowance for toxicokinetic influences, as for all substances in this study) and assuming conventional water treatment. It should be noted that the MOS with advanced water treatment were a minimum of 100 times (for infants).

Given that no evidence of endocrine disrupting potential has been identified during detailed literature review, and considering the highly precautionary approach employed at each stage of assessment, no significant concerns are warranted for this substance.

4.1.3 Chemicals for which estimated worst-case intakes afford a margin of safety of ≤10 times the TDI or study-specific exposure limit, for one or more of the sub-populations considered

For six substances the predicted reasonable worst-case daily intakes from drinking water suggested a margin of safety of ≤10 fold when compared to established TDIs or study-specific exposure limits. These substances were subject to further consideration since it was considered possible that there might be erosion of the MOS when intakes from other potential sources - notably air and, more likely, foodstuffs - are also considered. In each case a detailed evaluation of the nature of the risks posed was undertaken, as presented below.

p-Benzylphenol

p-Benzylphenol is used in plastics as a germicide, antiseptic and a preservative, and it is possible that the chemical may be present in food packaging and consumer goods (EC 2002). The main pathway of human exposure is through the oral route, although dermal exposure may also occur in workers where plastics are manufactured (EC 2002). p-Benzylphenol is moderately soluble in water and has been shown to bioaccumulate; it may therefore be found in the wider environment (EC, 2002).

No current regulatory environmental or occupational exposure limit in the EU or US could be identified for p-benzylphenol during the literature review phase of this project. A study-specific exposure limit of 0.04 mg/kg bw/d was therefore defined for use in assessment of risk. This value was based on a NOAEL of 40 mg/kg bw/d for uterine toxicity in rats with applying a combine uncertainty factor of 100 to allow for both inter-and intra-species differences (Yamasaki *et al*., 2003).

The worst-case predicted daily intakes of p-benzylphenol for adults, toddlers and infants afforded MOS of 6.8, 1.4 and 1 times when compared to the study-specific exposure limit. These figures are based on modelled levels in drinking water calculated using measured data from a UK study of river water (Kasprzyk-Hordern *et al*., 2009b) assuming subsequent conventional water treatment. It should be noted for advanced water treatment MOS was a minimum of 400 times (for infants).

An intake level MOS approaching 10 may warrant further consideration. Although the study-specific exposure limit used in the risk assessment embodies an inherent uncertainty factor of 100 compared with the experimental no-observed-adverse-effect level (NOAEL) noted in rats, it may still be prudent to seek to gather additional exposure measurement data. Such data would allow better assessment of the concentrations of p-benzylphenol present in a representative set of UK drinking waters. In addition, other potentially important exposure sources of p-benzylphenol, particularly for toddlers and infants, should be assessed to better quantify the overall exposure.

Dibutylphthalate (DBP)

An estimated 76% of manufactures dibutylphthalate (DBP) is used as a polymer plasticiser, 14% in adhesives, 7% in printing inks and the remaining 3% for other applications (EFSA, 2005). DBP may therefore be present in a large number of consumer products (e.g. food wrap and food packaging).

Exposure of humans may occur via inhalation or dermal exposure but oral (dietary) exposure is generally considered the most significant pathway (ECB, 2003).

The total human intake of DBP via air, drinking water and food has been estimated at 0.359 µg/kg bw/d (ECB, 2003); this is similar to the 'worst-case' intake estimated in our study based on an adult drinking water at 0.533 µg/kg bw/d. The Health-Based Recommended Occupational Exposure Level (HBROEL): 5 mg/m^3 for respirable dust and 10 mg/m^3 for total inhalable dust. A TLV of 5 mg/m^3 was established by the ACGIH (ECB, 2003).

Effects on reproduction and development are considered the most sensitive endpoints on which to base the risk assessment of this substance (EFSA, 2005) and a TDI of 0.01 mg/kg bw/d has been established (EFSA, 2005). This value is based on a LOAEL of 20 mg/kg bw/d for developmental effects (germ cell development and mammary gland change) in rats with an uncertainty factor of 200 applied (Lee *et al*., 2004).

The worst-case predicted daily intake of DBP from drinking water only for adults, toddlers and infants give MOS of 19, 6 and 4 compared to the TDI. These figures are based on modelled levels in drinking water undergoing conventional water treatment. As no UK-based studies could be identified, data were calculated on the basis of worst-case measured data from a study of French river waters (Baugros *et al*., 2008). It should be noted that predicted intakes following advanced water treatment give a minimum MOS of 344-fold (in infants).

An intake level giving a MOS <10, as seen for infants and toddlers, may be of potential concern when taking into account exposure from all soureces. However, the TDI has an inherent uncertainty factor of 200 below the level at which no observed adverse effects occurred in rats. In addition, exposure data reflect a non-UK study. Given the apparent absence of UK-specific exposure data, it is suggested that it would be prudent to gather measurement data for DBP from both relevant UK-water bodies, and other sources of expsoure, to enable a total exposure pattern for DBP.

4-Nitrophenol

4-Nitrophenol is used in the manufacture of drugs (e.g. acetaminophen), fungicides, consumer dyes and methyl and ethyl parathion insecticides (ATSDR, 1992). 4-Nitrophenol is also present in light-duty gasoline and diesel exhaust fumes. General exposure may therefore occur via exposure to contaminated air, water and soil. Inhalation of contaminated air and oral exposures from contaminated drinking water are considered the most relevant pathways for human exposure; 4-nitrophenol has not been identified in foods.

No current regulatory environmental or occupational exposure limit appears to have been established in the EU or US during the literature review for this project.

A study-specific exposure limit of 0.1 µg/kg bw/d was derived for use in our assessment of risk. This value was based on a NOAEL of 0.01 mg/kg bw/d for testicular toxicity (decreased weight in seminal vesicles, ventral prostate, levator ani plus bulbcavernosus muscle and gland penis) in immature male rats in a Hershberger screening assay (Li *et al*., 2006) applying an uncertainty factor of 10 for inter- and 10 for intra-species differences.

The worst-case predicted daily intake of 4-nitrophenol in adults, toddlers and infants gave MOS of 28, 8 and 5 respectively, when compared with the study-specific exposure limit. This was based on modelled levels in drinking water calculated using UK-specific measurement data from a study of European river water (Loos, 2009) and assumed conventional water treatment. The MOS based on advanced water treatment was a minimum of 50 (for infants).

An intake level MOS below 10, as estimated here for toddlers and infants, is of potential concern. However, the study-specific exposure limit does incorporate an inherent uncertainty factor of 100

below the level at which no observed adverse effects were noted in rats, which suggests that concerns should not be over-emphasised.

Nonetheless, on the basis of the findings from this study, it would seem prudent to gather additional measurement data to better inform on levels of 4-nitrophenol in UK water bodies used as drinking water sources. In addition, consideration of other potential exposure sources of 4-Nitrophenol should be considered to better inform on the likely overall scale of exposure.

Digoxin

Digoxin is a purified cardiotonic glycoside, consisting of three sugars and the aglycone digoxigenin, extracted from the foxglove plant (*Digitalis lanata*). The positive inotropic and negative chronotropic activity of digoxin has led to its use to control ventricular rate in atrial fibrillation and in the management of congestive heart failure (HSDB, 2003).

Occupational exposure to digoxin may occur through inhalation of dust and dermal contact with this compound at workplaces where it is produced or formulated for use. The general population may be exposed orally to digoxin through direct medical administration of this compound or potentially indirectly via environmental sources.

This substance was identified as of possible concern by QSAR modelling for protein binding activity by the Study Team. However, no evidence suggestive of endocrine disrupting activity per se was subsequently identified in a detailed literature review.

A study-specific exposure limit of 0.06 µg/kg bw/d was derived for use in the assessment of risk based on the minimum human therapeutic dose of 6 µg/kg/bw for neonates, to which an uncertainty factor of 100 was applied (based on expert clinical advice).

The worst-case predicted daily intakes of digoxin for adults, toddlers and infants gave MOS of 15, 5 and 4 times respectively when compared to the study-specific exposure limit. The exposures were based on modelled levels in drinking water calculated using UK prescription data and assuming conventional water treatment. The intake based on advanced water treatment was zero for all age groups.

An intake level margin of safety below 10 times, as seen here with respect to toddlers and infants, is of potential concern. Nonetheless, since no evidence of endocrine disrupting potential has been found, and in the light of the highly conservative assumptions used throughout the modelling process, significant concern is not considered to be warranted in this case.

Fluticasone

Fluticasone is a synthetic glucocorticoid which may be applied dermally to relieve inflammatory and pruritic symptoms of dermatoses and psoriases, intranasally to manage symptoms of allergic and non-allergic rhinitis and for the treatment of asthma.

Occupational exposure to fluticasone may occur through inhalation and dermal contact with this compound at workplaces where fluticasone is produced. Direct exposure to fluticasone among the general population will most probably be limited to those administered medications that contain fluticasone.

This substance was initially identified as of possible concern by the Study Team on the basis of QSAR modelling for protein binding. Subsequent literature review showed fluticasone propionate to be a highly selective agonist of the human glucocorticoid receptor but to have negligible activity with androgen, oestrogen and mineralo-corticoid receptors. In preclinical studies, fluticasone propionate reportedly exhibited weak progesterone-like activity but the clinical importance of this finding is

unclear. Reproductive studies in rats provide evidence of decreased prostate weight after subcutaneous exposure to fluticasone propionate.

A study-specific exposure limit of 0.1 µg/kg bw/d was derived based on a LOAEL of 100 µg/kg bw/d for foetal toxicity in rats dosed via the subcutaneous route to which an uncertainty factor of 1000 was applied (100 for intra- and inter-species differences and 10 for use of a LOAEL end point).

The worst-case predicted daily intake of digoxin in adults, toddlers and infants gave MOS of 20, 6 and 4 respectively compared to the study-specific exposure limit. This was based on modelled levels in drinking water calculated using UK prescription data and assuming conventional water treatment. The MOS for advanced water treatment were adequate at 100, 33 or 20 for adults, toddlers and infants respectively.

An intake level MOS below 10 times, as seen here for toddlers and infants, is of some concern. However, while there is some evidence of endocrine disrupting potential from human and animal studies, the clinical significance of reported activities is undefined. Notwithstanding the limited size of the MOS calculated here, oral uptake of fluticasone is reported as <10% of that ingested (American Society of Health System Pharmacists, 2009) which – if corrected for - would result in adjusted MOS over 10-fold higher, which would not be considered of particular concern.

Salbutamol

Salbutamol (Ventolin HFA) is a β_2-adrenergic agonist used in the treatment and prevention of bronchospasm in patients with asthma and chronic obstructive airways disease. Salbutamol is also used in combination with other drugs as a growth promoter in livestock.

Occupational exposure to salbutamol may occur through inhalation and dermal contact with this compound at workplaces where it is produced, and potentially in animal husbandry circumstances. Direct exposure to salbutamol among the general population is most probably limited to those administered medications that contain the compound or through indirect environmental contamination.

The susbstance was identified for inclusion at the initial screening stage by the Study Team based on QSAR modelling that indicated possible oestrogen binding activity. Subsequent literature review showed that salbutamol administered to pregnant women is associated with significantly higher growth hormone levels in infants. This is likely to be secreted from the pituitary glan in response to blood glucose fluctuations in the baby through adrenergic stimulation induced by salbutamol (Desranges *et al*., 1987). A significant up-regulation oy CYP17 gene expression (13-fold) was also observed following exposure to salbutamol in the H295R cell bioassay, indicating potential effects on the steroidogenic pathway (Garcia *et al*., 2007).

A study-specific exposure limit of 0.2 µg/kg was derived based on the minimum human therapeutic dose of 100 µg/day (20 µg/kg bw/d for infants) to which was applied an uncertainty factor of 100 (based on expert clinical advice).

The worst-case predicited daily intake of salbutamol in adults, toddlers and infants gave MOS of 13, 4 and 3 times respectively when compared to the study-specific exposure limit. These estimates were based on modelled levels in drinking water derived from UK prescription data assuming conventional water treatment. Predicited exposures in the case of advanced water treatment were zero for all age groups considered.

An intake level MOS below 10, as seen for toddlers and infants, may warrant further investigation given the (albeit limited) evidence of endocrine disrupting potential. However, because of the highly conservative nature of the expeoure estimate, it is considered that there is no appreciable need for concern.

4.2 Metals and associated compounds

Most of the literature reviewed during the course of this study focused on the ED potential of organic substances in the pesticide, industria chemical and pharmaceutical sectors and, to a lesser extent, natural hormones of animal or human origin and plant metabolites. However, a number of other xenobiotics have been identified as of potential ED concern including several metals (Iavicoli *et al*., 2009).

Based on the prioritisation exercise, four metals and two metal-containing inorganic compounds were noted to posses potential ED properties in humans and to possibly occur in drinking waters. These are:

- Cadmium;
- Cadmium chloride;
- Chromium;
- Chromium trioxide;
- Cobalt; and
- Copper

The exposure/water treatment models utilised in this project are unsuited to metals, for which specialised modelling, beyond the scope of the current study, would be required. Therefore, the relevance of any ED potential was considered separately in the light of established regulatory drinking water standards for each of the parent metal elements/compounds listed above.

4.2.1 Cadmium

Cadmium (Cd) is widely distributed in the environment, existing in only one oxidation state, Cd^{2+} . The metal can be found as reservoirs at high concentrations in soil, arising from emissons from industry (Jarup *et al*., 1998; Bhattacharyya *et al*., 2000). In surface- and ground-waters, Cd may occur as the hydrated ion or in the form of ionic complexes with other inorganic or organic substances.

Occupational exposures to Cd occur during extraction and use in foundaries, metallurgical and the electroplating industries. Exposure of the general population occurs through indirect routes including ingestion of contaminated foods (meat, fish and fruit) or through dermal contact with Cd-containing consumer products (e.g. nickel/cadmium batteries; Jarup *et al* 1998; Zadorozhnaja *et al*., 2000).

A number of adverse health effects have been linked to Cd exposure including increased incidence of renal pathologies, osteoporosis, leukaemia, hypertension and lung cancer (Satoh *et al*., 2002). Cd is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC, 1993). The health effects have been extensively reviewed elsewhere (e.g. ASTDR, 2008a) and will not be described further here. However, it is the impact of Cd on the endocrine system in humans that forms the focus of the following discussion.

An association between inhalation exposure to Cd and adverse reproductive effects in humans is inadequate. A number of occupational cohort studies have assessed the extent to which Cd exposure influences male worker fertility. Gennart *et al*. (1992) found no significant influence of Cd on probability of live birth and Mason (1990) reports that, in males exposed to Cd levels sufficiently high to cause renal damage, no changes in serum level of testosterone, luteinizing hormone or follicle-stimulating hormone were evident. In non-occupationally exposed male smokers and non-smokers, no significant correlation has been found between Cd level in seminal fluid and semen quality or fertility (Saaranen *et al*., 1989; Xu *et al*., 1993). However, Cd levels in blood are reported to show a significant inverse relationship with sperm density ($r=-0.15$, $p<0.05$) in oligospermic (sperm density <20 million/mL) but not normospermic men (Xu *et al*. (1993). The authors observed a significant reduction in sperm count in men with blood cadmium of >1.5 µg/L and a weak negative correlation

between defective sperm and concentration of cadmium in semen (r=-0.21, p<0.05). The volume of semen was also inversely proportional to semen cadmium concentration (r=-0.29, p<0.05). In a study of female workers occupationally exposed to cadmium at up to 35 mg/m^3, no irregularity of menstrual cycle was reported but other indices of reproduction were not assessed (Tsvetkova 1970).

Oral exposure to Cd has been extensively monitored in the US NHANES biomonitoring study. Using data from this study, Schwartz *et al*. (2003) investigated possible associations between Cd exposure (as measured by urinary level) and prevalence of impaired fasting glucose and diabetes in a cohort of 8,722 participants (≥40 years old). A dose-related increase in impaired fasting glucose and diabetes was noted after adjusting for age, ethnicity, sex and BMI.

Effects specifically of oral Cd exposures on male fertility have been evaluated in humans through measurement of levels of sex steroid hormones but results are inconclusive. Significant associations were reported between blood Cd level and serum luteinizing hormone, follicle stimulating hormone, prolactin and testosterone among infertile men (sperm count <20 million/cm^3 or no spermatozoa in semen). However, the authors did not include control for smoking. In a further study of infertile men that did include adjustment for age, smoking status, alcohol consumption and co-incident levels of lead, copper, zinc or selenium, Jurasović *et al*. (2004) found significant associations between blood Cd level and increases in serum oestradiol, follicle stimulating hormone and testosterone levels. Conversely, a study of Chinese men living in areas with high Cd in rice did not report any significant correlation between urinary or blood Cd level and serum testosterone, follicle stimulating hormone, or luteinizing hormone levels after adjusting for BMI, age, smoking or alcohol consumption (Zeng *et al*., 2004a); these authors do report that the incidence of abnormally elevated serum testosterone levels (>95th percentile of controls) increased with exposure to Cd.

A limited number of studies have suggested adverse effects on other indices of male fertility. Akinloye *et al* (2006) describe a significant association between serum Cd level and abnormal sperm morphology and altered sperm counts, sperm motility, and sperm viability for a group of infertile men. However, no significant correlation between blood Cd level and sperm quality were observed in another cohort of infertile men with or without adjustment for smoking (Jurasović *et al*., 2004). Blood Cd level has also been found to be significantly higher in men with abnormal digital rectal examinations of the prostate and with prevalence of abnormal prostate specific antigen (Zeng *et al*., 2004b).

Exposure of pregnant women to Cd (as Cd^{2+}) has been associated with lower birth weight and increased numbers of spontaneous abortions or pre-term birth (Nishijo *et al*., 2002; Frery *et al*., 1993). The authors suggest Cd may influence the synthesis of hormones such as hCG that play a key role in maintaining and progressing pregnency but do not provide measurement data on this.

Inhalation studies in animals show that exposure of female rats to 1 mg Cd/m^3 (as cadmium oxide dust) for 20 weeks increases the oestrous cycle (Baranski and Sitarek 1987); a LOAEL of 1 mg Cd/m^3 was determined. An increase in oestrous cycle length was also observed in rats exposed to 0.88 mg Cd/m^3 (as cadmium oxide) for 13 weeks (NTP, 1995); a LOAEL of 0.88 mg Cd/m^3 was determined. In reproductive toxicity studies, male and female rats exposed to Cd at 1.06 mg/m^3 (as cadmium chloride) for 62 days and then mated with unexposed animals showed no reduction of reproductive success as measured by viable embryos and pre-implantation losses. However males had increased relative testes weights (Kutzman *et al*. 1986); a NOAEL of 1.06 mg/m^3 was determined for male. Similarly, no alterations in fertility in female rats exposed to 0.16 mg Cd/m^3 (as cadmium oxide for 5 months prior to mating with unexposed males and during the mating and gestation periods (Baranski, 1984).

No adverse effects were seen in parathyroid glands of female Wistar rats given 8 mg Cd/kg/day via drinking water for 90 days (Kawamura *et al*., 1978) or in the adrenal gland from male Sprague-Dawley rats given 8 mg/kg/day via drinking water for 24 weeks (Kotsonis and Klaassen, 1978). Pituitary, adrenals, thyroid, and thymus were also unaffected in Wistar rats given 3 mg/kg bw/day

cadmium via the diet for 3 months (Loeser and Lorke, 1977a). However, Wilson *et al*. (1941) reported pancreatic atrophy and pancreatitis in rats following exposure to Cd at 2.79 mg/kg/day via the diet for 100 days. In rabbits given 14.9 mg Cd/kg bw/day via drinking water for 200 days, the pancreas had moderate concentrations of Cd but no interstitial fibrosis or other pathologic alteration were noted (Stowe *et al*. 1972).

Acute oral exposure of male rats and mice to Cd at between 60 and 100 mg/kg bw caused testicular atrophy and necrosis (Andersen *et al*., 1988; Bomhard *et al*., 1987; Borzelleca *et al*., 1989) and decreased fertility (Kotsonis and Klaassen, 1978); acute exposures at doses between 25 and 50 mg/kg bw did not result in reproductive toxicity in male animals (Andersen *et al*., 1988; Bomhard *et al*., 1987; Dixon *et al*., 1976).

In a 28 day oral study in Sprague-Dawley rats given cadmium chloride ($CdCl_2$) in drinking water at between 5 and 100 ppm, Cd differentially affected secretory patterns of the pituitory hormones gonadotropin, prolactin, adrenocorticotropic hormone (ATCH), growth hormone (GH) and thyroid stimulating hormone (TSH) (Lafuente *et al*., 2003). In a 28 day oral study in Sprague-Dawley rats administerd cadmium chloride ($CdCl_2$) in drinking water at concentrations between 5 and 100 ppm, Cd was seen to differentially affect secretory patterns of the pituatory hormones gonadotropin, prolactin, adrenocorticotropic hormone (ATCH), growth hormone (GH) and thyroid stimulating hormone (TSH) (Lafuente *et al*., 2003).

Similarly, other sub-chronic studies via the oral route report no evidence of testicular histopathologic lesions or effects on male reproductive success. These included dosing regimens of: 0.25 mg Cd/kg/day via gavage for 10 weeks (Bomhard *et al*., 1987); 5 mg/kg/day via water for 30–90 days (Dixon *et al*., 1976); 2.5 mg/kg/day via food for 4 weeks (Groten *et al*., 1990); 8 mg/kg/day via water for 24 weeks (Kotsonis and Klaassen, 1978); 3 mg/kg/day via food for 12 weeks (Loeser and Lorke, 1977a; 1977b); 2.9 mg/kg/day via water for 14 weeks (Pleasants *et al*., 1992); and 4.64 mg/kg/day via water for 70–80 days (Zenick *et al*., 1982). In contrast, a number of other sub-chronic dosing regimen studies have reported reproductive toxicity in male rats. Administration of 8.58 mg Cd/kg/day in water for 10 weeks resulted in necrosis and atrophy of seminiferous tubule epithelium (Cha, 1987); rats exposed to 5.8 mg/kg/day via water for 14 weeks (Pleasants *et al*., 1992) or 11.6 mg/kg/day via water for 14 weeks (Pleasants *et al*., 1993) developed increased testes weight; rats exposed to 12.9 mg/kg/day in water for 120 days developed significantly increased relative testis weight, decreased sperm count and motility, decreased seminiferous tubular diameter, and seminiferous tubular damage (pyknotic nuclei, multinucleated giant cells, interstitial edema, and dilated blood vessels; Saxena *et al*., 1989). In sheep, Leoni *et al*. (2002) demonstrated that exposure to concentrations of only 2µM Cd induced significant modification of the acrosome membrane of sperm (essential to functional integrity) and decreased sperm viability.

In carcinogenicity studies, male rats fed up to 14 mg Cd/kg/day via food for 77 weeks showed an increase in incidence of prostatic hyperplasias. Testicular tumours (exclusively benign interstitial tumours) were also significantly higher at the high dose. In male Wistar rats given Cd in the drinking water at 25 to 200 ppm tumours were noted in the prostate (50 ppm), testes (200 ppm) and hematopoietic system (50 ppm; Waalkes and Rehm, 1992).

In female rats, Baranski and Sitarek (1987) report a significant increase in oestrous cycle duration following dosing at 40 mg/kg by gavage for 5 days/week, for 14 weeks. Female rats exposed to 2.61 mg/Cd kg/day via drinking water for 60 days prior to gestation or during gestation or 5.23 mg/kg/day via drinking water for 111 days (including 90 days prior gestation plus 21 days during gestation, did not show any increase in number of stillborn pups (Petering *et al*., 1979). Pond and Walker (1975) report no effect on number of pups born following exposure of female rats to Cd at doses of 19.7 mg/kg/day via food for 21–25 days, including Gd 1 through lactation day (Ld) 1. However, administration of Cd by subcutaneous injection to pregnant Sprague-Dawley rats on days 7 and 16 of gestation inhibited progesterone synthesis (Piasek and Laskey, 1994); decreased progesterone synthesis was also shown using *in vitro* human ovarian granulosa cells treated with $CdCl_2$ at

concentrations between 8 and 64 µM for 2 – 28 h, reaching significance at 16 µM (Paksy *et al*., 1997). A subsequent study by Piasek and Laskey (1999) provided evidence that Cd interferes directly with hormone production in steroid-production ovary cells. It has also been suggested that exposure to Cd at concentrations of 5 µM and above decreases progesterone synthesis in placenta (Jolibois *et al*., 1999a; 1999b; Kawai *et al*., 2002), however findings from a number of other *in vivo* and *in vitro* studies do not suppot this at present (Powlin *et al*., 1997; Massanyi *et al*., 2000). Exposure of ICR mice to $CdCl_2$ during gestation has been associated with poor gonadal development in male offspring, resulting in poor mating performance and a higher number of sterile matings (Kotsonis and Klassen, 1978).

Cd (in the form of Cd^{2+}) is able to exert an oestrogenic effect in both *in vivo* and *in vitro*. Treatment of female Sprague-Dawley rats with a single dose of $CdCl_2$ (5 µg/kg) resulted in an increase in uterine wet weight, promoted growth of mammary glands and induced hormone-regulated genes in ovariectomised rats. In addition, onset of puberty in female offspring was seen to occur early (Johnson *et al*., 2003). Cd was also seen to mimic oestrogen action in the human breast cancer MCF-7 cell line (Garcia-Morales *et al*., 1994).

A two-generation reproductive study in male and female rats orally exposed to 2.5 mg/Cd kg/day via drinking water for 180 days showed a decrease in litter size and increased interval between litters; three of five pairs failed to breed in the second generations (Schroeder and Mitchener 1971).

Summary

The exposure route most relevant to this study is oral intake, with exposures of the general population anticipated to include ingestion of Cd-contaminated drinking water.

A chronic-duration oral MRL of 0.1 µg/kg/day has been proposed for cadmium by ASTDR (2008a). Many of the available studies consider the relationship between urinary Cd (or cumulative Cd intake) and effects in the general population or populations living in known Cd-polluted areas. While a number of adverse health effects have been associated with chronic exposure, effects on the kidney and – to a lesser extent - liver are considered the most sensitive.

Of studies reporting potential endocrine disrupting properties, there have been suggested links to impaired fasting glucose function and diabetes as well as elevated serum testosterone level, abnormal sperm morphology and decreased sperm counts, motility and viability, increased prostate specific antigen, and in the offspring of exposed females, lower birth weight and increased pre-term births.

Findings from experimental studies on the effects of oral Cd exposure are inconclusive. Of the studies reporting positive associations with Cd indicative of tentative endocrine disrupting effects, changes reported include, in males, effects on the secretory pattern of pituitary hormones, necrosis and atrophy of seminiferous tubule epithelial, increased relative testis weights, decreased sperm count and motility, decreased seminiferous tubular diameter and seminiferous tubular damage in males. For females, changes include increased oestrous cycle duration, poor gonadal development in their male offspring, decreased litter size and increased interval between litters. Carcinogenic effects involving endocrine-related tissues include increased incidences of prostatic hyperplasia (not itself evidence of cancer per se) and tumours of the prostate and testis. However, several other studies have failed to confirm these findings.

It is thus not possible to identify, with confidence, a specific NOAEL for the endocrine or reproductive effects that may associate with chronic oral exposure to Cd for either humans or animals. A sub-chronic oral exposure study in female rats gave a NOEAL of 4 mg/kg/d based on oestrous cyclicity (Baranski and Sitarek, 1987; ASTDR, 2008a) however a lower NOAEL noted for males was 2.5 mg/kg/d in relation to testicular histopathology and male reproductive success (Groten *et al*., 1990; ASTDR, 2008a).

In the UK, Cd levels in drinking water are regulated to no more than 5µg/L, which would equate to an estimated intake of 0.17, 0.5 or 1.88 µg/kg bw/d for adults, toddlers and infants respectively. This would suggest, compared to the male NOAEL from the study by Groten *et al*. (1990), a MOS of at least 1000 times with respect to potential endocrine-related effects.

4.2.2 Chromium

Chromium (Cr) occurs naturally in the earth's crust and can be detected in all environmental media, however larger concentrations result following release from the metal industries, combustion of coal and oil, cement works and waste incineration. Cr is a transition element with common oxidation states of 0 (elemental) $^{+}2$, $^{+}3$ and $^{+}6$. The toxicity of Cr is dependent on its oxidation state with hexavalent Cr being more toxic than the trivalent form. Exposure in the general population occurs through indirect routes including ingestion of contaminated foods (meat, fish and fruit) or through dermal contact with Cr containing consumer products.

Chromium is an essential nutrient in humans as its plays a key role in glucose, fat and protein metabolism by enhancing the action of insulin (Anderson, 1989). However a number of adverse health effects have been linked to high levels of Cr exposure including liver and kidney toxicity, GI effects, and increased incidence of lung and sinonasal cancers; these have been reported extensively elsewhere and will not be described further here (WHO, 1987, 1996; IPCS, 1988; HSE, 1989; IARC, 1990, 1999; ATSDR, 2008b; USEPA, 2002, 2003. Cr(VI) has been classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC, 1990, 1999). However, it is the potential for Cr to act as an ED in humans that will be the focus of subsequent discussion.

A study of 50 chrome plating workers in India reported increased serum amylase activity (a marker for pancreatic function) when compared to a control group with no history of exposure to Cr(VI) through inhalation; serum amylase activity was found to be significantly correlated to urine Cr levels ($r=0.289$; $p<0.05$; Kalahasthi *et al*., 2007). In a cohort of workers in a chromium sulphate manufacturing plant, also in India, the effect of Cr on reproductive function was evaluated; a significant increase in number of morphologically abnormal sperm was reported in exposed workers with a significant positive correlation ($r=0.301$; $p=0.016$) between blood Cr levels and percentage of abnormal sperm (Kumar *et al*., 2005). In addition, sperm count and motility were shown to be significantly decreased (47 and 15% respectively) in a group of 21 workers employed at a chrome plating plant in Henan, China, compared to age-matched, unexposed controls (Li *et al*., 2001).

Complications during pregnancy and childbirth in a cohort of women workers at a dichromate manufacturing facility in Russia were reported in 20 of the 26 exposed women compared with 6 of 20 women in the control group (Shmitova, 1980). Bonde *et al.* (1992) further reported that wives of stainless steel workers were at an increased risk of spontaneous abortions. However this was not supported by results of a more robust study examining the occurrence of spontaneous abortion among 2,520 pregnancies of spouses of 1,715 married Danish metal workers exposed to hexavalent Cr from 1977 through 1987 (Hjollund *et al*., 1995). The study findings reported that the risk of spontaneous abortion was not increased for pregnant women whose spouses worked in the stainless steel welding industry when compared to controls (odds ratio 0.78, 95% confidence interval [CI] 0.55–1.1).

No studies assessing endocrine related effects in humans following oral exposure to Cr(VI) or Cr(III) compounds could be identified.

In animal studies, male Sprague-Dawley rats exposed for 3 months to 1.15 mg Cr(VI)/m^3 (as chromium trioxide; Kim *et al*., 2004) or in male and female CDF rats exposed to 30 mg Cr(III)/m^3 (as chromic oxide or basic chromium sulphate; Derelanko *et al*., 1999), did not show any histopathological changes to endocrine tissues; Kim *et al.*, determined a LOAEL of 1.15 mg/m^3 for male rats. This was supported by findings of a further study where no histopathological changes were reported in the adrenals of male rats exposed for 22 h/d for 18 months to 0.1 mg Cr(VI)/m^3 (as sodium dichromate) or to a mixture of Cr(VI) and Cr(III) (0.06 mg chromium(VI)/m^3 plus 0.04 mg

chromium(III)/m^3 as chromium(VI) trioxide and chromium(III) oxide; Glaser *et al*., 1986, 1988); a NOAEL for endocrine effects of 0.1 mg/m^3 was determined for male rats. No histopathological lesions were observed in the prostate, seminal vesicle, testes, or epididymis of male rats or in the uterus, mammary gland, or ovaries of female rats exposed to 15.5 mg Cr(IV)/m^3 as chromium dioxide for 2 years (Lee *et al*., 1989).

Following oral exposure of rats and mice for durations of between 3 months and 2 years to Cr(VI) (as sodium dichromate dihydrate) and Cr(III) (as chromium nicotinate and chromium picolinate) no histopathological changes to endocrine tissues (including the adrenal gland, parathyroid, and thyroid) were observed (NTP, 2007, 2008, 2010; Shara *et al*., 2005, 2007).

Numerous reproductive toxicity studies have been carried out in animals orally exposed to Cr(VI). Effects on male reproductive organs have been reported including decreased testes weight, histopathological changes of the epididymis, disrupted spermatogenesis, and decreased sperm count and motility in male bonnet monkeys (Macaca radiate) (Aruldhas *et al*.. 2004, 2005, 2006; Subramanian *et al*., 2006) exposed via drinking water to between 2.1 and 8.3 mg Cr(VI)/kg/day (as potassium dichromate) for 180 days; a LOAEL of 2.1 mg/kg/d was determined for reproductive effects in males. In male Wistar rats administered 5.2 and 10.4 mg Cr(VI)/kg/day (as chromic acid by gavage) for 6 days, decreased sperm count and histopathological changes to the testes were reported (Li *et al*., 2001). Male reproductive effects were also noted in mature male Charles Foster strain rats administered between 20 and 60 mg Cr(VI)/kg/day (as sodium dichromate(VI) by gavage) for 90 days (Chowdhury, 1995); a LOAEL of 20 mg/kg/d was determined. Significant reductions in testis weight, population of Leydig cells, seminiferous tubular diameter, testicular protein, DNA, and RNA were reported at 40 and 60 mg Cr(VI)/kg/day; a LOAEL of 40 mg/kg/d was determined.

In addition to effects on physical and functional parameters, significant alterations in sexual and aggressive behaviours have been seen following exposure of male Sprague-Dawley rats to 42 mg Cr(VI)/kg/day (as potassium dichromate in drinking water) for 12 weeks; a LOAEL of 42 mg/kg/d was determined (Bataineh *et al*., 1997).

Exposure of female Swiss albino mice to Cr(VI) (potassium dichromate in drinking water) for 20 days resulted in reduction in, the number of follicles at different stages of maturation at ≥60mg Cr(VI)/kg/day, number of ova per mice and histological alterations in the ovaries at ≥120 mg Cr(VI)/kg/day, and significant increase in oestrus cycle duration at 180 mg Cr(VI)/kg/day (Murthy *et al*., 1996); a NOAEL of 60 mg/kg/d was determined for effects on follicles and 120 mg/kg/d for effects on number of ova. The severity of the reproductive effects appeared to be dose-related. In contrast, female BALB/c mice and Sprague-Dawley rats fed up to 9.8 and 48 mg Cr(VI)/kg/day, respectively (as potassium dichromate(VI) in the diet) continuously for 9 weeks did not exhibit exposure related effects to the ovaries (NTP, 1996); a NOAEL of 9.8 was determined. Similarly, female F344/N rats and B6C3F1 mice administered sodium dichromate dihydrate (in drinking water) at doses up to 20.9 and 27.9 mg Cr(VI)/kg/day, respectively, for 3 months or at doses up to 7.0 and 8.6 mg Cr(VI)/kg/day, respectively, for 2 years did not produce histopathological changes to the ovaries (NTP, 2007, 2008); a NOAEL of 20.9 mg/kg/d was determined for the 2 year study.

Pregnant mice exposed to 50 mg Cr(VI) /kg bw/d) showed severe developmental effects in offspring including embryo death, decreased litter sizes and gross abnormalities (Trivedi *et al*., 1989); a LOAEL of 50 mg/kg/d was determined. Similar foeto- and embryo-toxicity was noted in female rats administered potassium dichromate in drinking water for 12 weeks at doses of 40 mg Cr(VI) /kg bw/d (Kanojia *et al*., 1996). Dietary levels of Cr(VI) of 60 and 20 mg/kg bw/d have been reported not to cause reproductive toxicity in rats and mice respectively (USEPA, 2011).

Exposure of female rats and mice to Cr through the diet or drinking water prior to mating has been shown to have a number of reproductive effects including decrease in the number of corpora lutea and placental weights and an increase in preimplantation loss and resorptions (Junaid *et al*., 1996). Additional studies have also shown a decrease in the number of implantations and viable foetuses

(Elbetieha and Al-Hamood, 1997). A multi-generation study with BALB/c mice administered a diet containing potassium dichromate(VI) did not report reproductive effects (NTP, 1997).

Reproductive studies assessing effects following exposure to Cr(III) have produced conflicting results. Exposure to Cr(III) as chromium oxide did not cause reproductive effects in male or female rats administered 1,806 mg Cr(III)/kg/day as chromium oxide 5 days/week for 60 days before gestation and throughout the gestational period; normal fertility, gestational length, and litter size were reported (Ivankovic and Preussmann, 1975). However, Bataineh and colleagues (1997) observed significant alterations in sexual and aggressive behaviours and significantly lower absolute weight of testes, seminal vesicles, and preputial glands in male Sprague-Dawley rats exposed to 40 mg Cr(III)/kg/day (as chromium chloride in drinking water) for 12 weeks. Male fertility indices were not seen to be adversely affected, however females mated to treated males showed an increase in total numbers of resorptions. In addition, impaired fertility (decreased number of implantations and viable foetuses) was also observed in females exposed to 5 mg Cr(III)/kg/day mated to unexposed males (Elbetieha and Al-Hamood, 1997). This study also found increased testes and ovarian weights and decreased preputial gland and uterine weights at 5 mg Cr(III)/kg/day.

Effects on the morphology of sperm following exposure of BALB/c mice to 42.4 mg Cr(III)/ kg/day as chromium sulphate have been reported (Zahid *et al*., 1990). However, in contrast to this, exposure of rats and mice to high doses of Cr(III) compounds (chromium nicotinate and chromium picolinate) in the diet for 3 months or 2 years did not produce histopathological changes to male or female reproductive organs (NTP, 2010; Rhodes *et al*., 2005; Shara *et al*., 2005, 2007). It should be noted that the 3-month and 2-year studies only evaluated low doses (up to 1.5 mg Cr(III)/kg/day for 3 months and up to 0.25 mg Cr(III)/kg/day for 2 years) (Shara *et al*., 2005, 2007).

The reproductive system may also be a target for Cr toxicity in the foetus. Studies have reported delayed vaginal opening and decreased relative weights of the uterus, ovaries, testis, seminal vesicle, and preputial glands in mouse offspring exposed to potassium dichromate or 1998); a LOAEL of 66 mg/kg/d was determined. Impaired fertility was also observed in the chromium(III) chloride-exposed female offspring when they were mated with unexposed males (Al-Hamood *et al*., 1998); no effect on fertility was observed in the male offspring.

Cr has been shown to stimulate cell proliferation in the human breast cancer cell line, MCF-7, indicating oestrogen receptor (ERalpha) activation (Martin *et al*., 2003).

Summary

The exposure route of most relevance to this study is the oral route, with exposure of the general population most likely to occur through ingestion of Cr in drinking water.

A chronic-duration oral MRL of 0.001 mg Cr(VI)/kg/day has been derived for chronic (≥1 year) exposure to hexavalent chromium compounds (ASTDR, 2008b). Nonneoplastic lesions of the duodenum observed in mice in an chronic drinking water (NTP, 2010) was selected as the critical effect for derivation of a chronic-duration MRL for Cr(VI) compounds.

As stated previously, no studies were identified assessing endocrine related effects in humans exposed to either Cr(VI) or Cr(III) compounds.

Although numerous animal studies have been reported, findings from sub-chronic and chronic oral Cr exposure studies have proved inconclusive to date. Of the studies reporting potential endocrine disrupting effects, exposure to Cr(VI) was linked to decreased testes weight, histopathological changes of the epididymis; disrupted spermatogenesis; decreased sperm count and motility in rats and monkeys. In addition, significant alterations in sexual and aggressive behaviours have also been seen reported in male rats. Exposure of female rats to Cr(VI) was linked to reductions in numbers of follicles and ova; decrease in placental weights; significant increase in length of oestrous cycle; increase in preimplantation loss and resorptions; decrease in the number of implantations and viable

foetuses. Of the studies reporting potential endocrine disrupting effects, exposure to Cr(III) was linked in males to significant alterations in sexual and aggressive behaviours; significantly lower absolute weight of testes, seminal vesicles, and preputial glands. In females, Cr(III) exposure was linked to a decrease in number of implantations and viable foetuses; decrease in uterine weight; increased ovarian weights. Foetal reproductive systems have also been suggested to be a target for Cr toxicity in females only.

The lowest NOAELs reported for either endocrine or reproductive effects following chronic oral exposure of rats to Cr(VI) was 6.6 and 7.0 mg/kg/d for male and females respectively for no observed histopathological changes to reproductive tissues (NTP, 2010; ASTDR, 2008b). For Cr(III) the lowest NOAEL reported for endocrine related effects following chronic oral exposure of female rats was 0.25 mg/kg/d for no observed histopathological changes to endocrine tissues (Shara *et al*., 2007; ASTDR, 2008b). For reproductive studies, a NOAEL of 313 mg/kg/d was identified for female rats and 781 mg/kg/d for male mice for no observed histopathological changes to reproductive tissues (NTP, 2010; ASTDR, 2008b).

In the UK, Cr levels in drinking water are regulated to 50 µg/L (as Cr), equating to estimated intakes of 1.67, 5.0 and 18.75 µg/kg bw/d for adults, toddlers and infants respectively. This would suggest, compared to the male NOAEL from the NTP study, a MOS of at least 300 times with respect to potential endocrine-related effects.

4.2.3 Cobalt

Cobalt (Co) occurs naturally in the environment and the metal is mainly used as a base for superalloys that are used in gas turbine aircraft engines. Common oxidation states of cobalt are +2 and +3. Co compounds are used as pigments in glass, ceramics and paints, as catalysts in the petrolium industry, as paint driers and as trace element additives in agriculture and medicines (ASTDR, 2004a). The main sources of naturally occurring Co in the environment is from the burning of fossil fuels, application of Co-containing fertilisers, mining and smelting of Co-containing ores and the processing of Co-containing alloys. Exposure of the general population to Co occurs through inhalation of ambient air and consumption of food and water containing Co; intake via food consumption is much greater than from other routes (ATSDR, 2004a)

Co is an essential element in humans being a component of vitamin B_{12} and a recommended dietary allowance of the vitamin has been determined as 2.4 µg/day which represents an intake of 0.1 µg of Co. Adverse effects from exposure to high levels of Co have been identified. Respiratory effects include decreased pulmonary function, asthma, interstitial lung disease and wheezing; other targets include effects on the thyroid and allergic dermatitis, polycythemia, cardiomyopathy. The carcinogenic potential of Co in humans is undefined at present and IARC has classified the metal as Group 2B, '*possibly carcinogenic to humans*'. These adverse health effects have been previously well documented (e.g. ASTDR, 2004a) and will not be discussed further here; the focus of subsequent discussion is the potential for Co to act as an endocrine disrupters in humans.

Following inhalation exposure to Co (in the form of cobalt glaze containing cobalt-zinc sulphate) at concentrations of 0.05 mg Co/m^3, female workers showed significantly elevated levels of serum thyroxine (T4) and free thyroxine but no change in serum T3 levels; a LOAEL of 0.05 mg/m^3 was determined (Prescott *et al*., 1992). However in a further study of workers exposed to cobalt oxides, salts and metals, T4 levels remained unchanged and T3 levels were significantly reduced; at LOAEL of 0.125 mg/m^3 was determined (Swennen *et al*., 1993).

Historically, oral exposure to Co in humans was evidenced by examination of beer-drinkers who had heavily consumed beer containing cobalt sulphate as a foam stabiliser, being exposed to an average of between 0.04 and 0.14 mg/Co kg/d for a period of years. The thyroids from these individuals showed abnormalities on histological examination with irregular follicle morphology and decreased follicular size (Roy *et al*., 1968).

In rats, exposure to cobalt sulphate ($CoSO_4$) in aerosols for 16 days resulted in testicular atrophy at doses of 19 mg Co/m^3. Decreases in sperm motility and testicular atrophy were seen following exposure of mice to aerosols of $CoSO_4$ for 13 weeks at concentrations of 1.14 mg Co/m^3; a LOAEL of 1.14 mg/kg/d was determined. In the same study, female mice showed significant increases in oestrous cycle length at levels of 1.14 mg Co/m^3 (Bucher *et al*., 1990; NTP, 1991).

In animal studies, female rats administered up to 26 mg/Co kg/d in drinking water for 45 days showed histopathological changes to the thyroid gland (Shrivastrava *et al*., 1996). Following longer term exposure (2 -3 months) of rats to doses of Co between 13.3- 58.9 mg Co/kg/d (as cobalt chloride in drinking water) testicular degeneration and atrophy were reported (Corrier *et al*., 1985; Domingo *et al*., 1984; Mollenhauer *et al*., 1985; Nation *et al*., 1983; Pedigo and Vernon, 1993). Similar effects were reported in male mice exposed to 43.4 mg/Co/kg/d (as Cobalt Chloride in drinking water) for 13 weeks (Anderson *et al*., 1992; 1993).

In vitro, primary cultures of mouse Leydig cells exposed to concentrations of Co between 0 and 2.5 mM for 3 hrs showed decreased production of basal and LH-stimulated androgen production in a dose-dependent manner (Moger, 1983). Co has been shown to stimulate cell proliferation in the human breast cancer cell line, MCF-7, indicating oestrogen receptor (ERalpha) activation (Martin *et al*., 2003).

Summary

The exposure route of most relevance to this study is the oral route, with exposure of the general population most likely to occur through ingestion of Co in drinking water.

A chronic-duration oral MRL is not available for Co. An MRL of 0.01 mg Co/kg/day has been derived for intermediate-duration oral exposure (<365 days) to Co based on a LOAEL of 1 mg cobalt/kg/day for polycythemia as reported in a study by Davis and Fields (1958).

Although a small number of human studies have been reported, findings have proved inconclusive to date. Of the studies reporting potential endocrine disrupting effects, exposure to Co was linked to significantly elevated levels of serum thyroxine (T4) and free thyroxine in females. In males, abnormalities in thyroid glands were reported from histopathological analysis.

Findings from animal studies have also proved inconclusive. Of the studies reporting potential endocrine disrupting effects, oral exposure to Co was linked to histopathological changes to the thyroid gland in female rats and testicular degeneration and atrophy in male rats and mice.

It was not possible to identify NOAELs for either endocrine or reproductive effects following chronic oral exposure to Co in either the human or animal studies described here. The lowest NOAEL reported for reproductive effects following sub-chronic oral exposure to Co is 5 mg/kg/d for testicular degeneration (Nation *et al*., 1983; ASTDR, 2004a).

In the UK, Co levels in drinking water are regulated to 100 µg/L, equating to estimated intakes of 3.3, 10.0 and 37.5 µg/kg bw/d for adults, toddlers and infants respectively. This provides a 'worst-case' margin of safety (for infants) of 133 times against the identified NOAEL for male reproductive effects following exposure to Co.

4.2.4 Copper

Copper (Cu) is a naturally occurring element in the environment as either the free metal or in compounds; in most compounds copper occurs in +1 Cu(I) or +2 Cu (II) valence states. Cu is mainly utilised as a metal or alloy but copper sulphate can also be used as a fungicide, algicide and nutritional supplement. The general population will be exposed to Cu through inhalation of copper dust particles, consumption of food and water contaminated with Cu and dermal contact with air, soil and water containing Cu (ATSDR, 2004b).

Cu is an essential element for humans being incorporated into a number of mettalloenzymes such as cytochrome c oxidase and superoxide dismutase. As an essential element, levels of Cu are tightly regulated and Cu homeostasis plays a key role in preventing exposure to toxic levels *in vivo*. Exposure to excessive levels of Cu are associated with a number of adverse health effects including, liver and kidney damage, GI distress, anaemia, immunotoxicity and developmental toxicity. The carcinogenicity of Cu *per se* has not been determined at present. These health effects have been previously well documented (e.g. ATSDR, 2004b) and will not be discussed further here; the focus of subsequent discussion is the potential for Cu to act as an endocrine disruptor in humans.

Following inhaltion of Cu dust at levels between 111 – 434 mg Cu/m^3 in a group of 100 workers, 7 cases of enlarged sella turcica, nonsecretive hypophyseal adenoma and arterial hypertension were reported, associated with disturbance of copper metabolism. In the same group of workers, 16% also reported sexual impotence (Suciu *et al*., 1981).

No animal studies assessing reproductive effects following expsoure to Cu through inhalation were identified during literature review.

No human studies assessing reproductive effects following oral expsoure to Cu were identified.

Oral exposure to 12 mg Cu/kg bw/d (given as copper sulphate in feed) in minks, reproductive performance was assessed by length of gestation, numbers of off spring and average offspring weight and found not to be adversely affected. A NOAEL of 12 mg/kg/d was determined (Aulerich *et al*., 1982). In an intermediate-duration study, male and female rats exposed to 66 and 68 mg Cu/kg/d respectively, did not show any histological alterations in sperm morphology or vaginal cytology; no adverse effects were reported in male and female mice exposed to 398 and 536 mg Cu/kg/d respectively, in the same study. A NOAEL of 66 and 68 mg/kg/d respectively was determined for male and female rats, and 398 and 536 mg/kg d respectively, for male and female mice (NTP, 1993).

No human or animal studies assessing endocrine toxicity following dermal exposure were identified during literature review. However, following i.p. exposure for 26 days to 0.95 or 1.4 mg Cu/kg/d, Wistar rats showed significant reduction in testes, seminal vesicle and ventral prostate weights and in levels of plasma testosterone. At the highest dose, decreases in testicular activity of the enzymes Δ5-3β-hydroxysteroid de-hydrogenases and 17β-hydroxysteroid dehydrogenase (Chattopadhyay *et al*., 1999). Although the authors did not derive a NOAEL from this study, a LOAEL of 0.95 mg Cu/kg/d could be derived for use in the risk assessment phase of this project.

Cu has been shown to stimulate cell proliferation in the human breast cancer cell line, MCF-7, indicating oestrogen receptor (ERalpha) activation (Martin *et al*., 2003).

Summary

The exposure route of most relevance to this study is the oral route, with exposure of the general population most likely to occur through ingestion of Cu in drinking water.

A chronic-duration oral MRL is not available for Cu. An intermediate-duration oral MRL of 0.01 mg Cu/kg/day was derived for copper based on a NOAEL of 0.042 mg Cu/kg/day for gastrointestinal effects in humans with an uncertainity factor of 3 applied (Araya *et al*. 2003; ASTDR, 2004b).

No human studies could be identified to inform on possible endocrine-related effects following chronic oral exposure to Cu.

Findings from animal studies to date have shown no evidence of adverse effects on either endocrine or reproductive endpoints following chronic oral exposure.

It was not possible to identify NOAELs for either endocrine or reproductive effects following chronic oral exposure to Cu in the animal studies described here. The lowest NOAEL reported for

reproductive effects following sub-chronic oral exposure of Mink to Co is 12 mg/kg/d (Aulerich *et al*., 1982; ASTDR, 2004b).

In the UK, Cu levels in drinking water are regulated to 2 mg/L, equating to estimated intakes of 66.7, 200 and 750 µg/kg bw/d for adults, toddlers and infants respectively. This provides a 'worst-case' margin of safety (for infants) of 16 times against the identified reproductive NOAEL.

4.3 Mixture interactions (Objective 4; Task 4)

This study has identified a large number of substances that may potentially occur in surface waters – and, hence, possibly in drinking water supplies – for which some level of concern regarding endocrine disrupting activity has been recorded. It is therefore appropriate to give consideration to the possibility of mixture effects.

4.3.1 The problem with mixtures

Although substances are normally tested for their toxicity and are subject to regulatory controls on an individual basis, it is a well recognised reality that environmental exposures invariably involve mixtures of chemicals. To conduct standard toxicological experiments with a mixture of substances – even a well-defined mixture – requires first the establishment of independent dose-response curves for each component and then experiments on combinations of two or more components of the mixture. This results in numerous combinations and permutations of treatments which makes testing using standard methods highly impractical or impossible.

In light of these difficulties, but in the face of a real desire to better understand the effects of mixtures of substances, a number of expert groups and organisations have published guidance or 'frameworks' for the assessment of chemical mixtures. Most recently, for example, three EC committees – the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) have issued a preliminary opinion for public consultation on 'Toxicity and Assessment of Chemical Mixtures' (EC. Health and Consumer Protection Directorate General, 2011).

This document summarises the principal possible modes of joint action between different chemicals, namely:

- **Similar action** (dose/concentration addition), where chemicals act by the same mechanism, and effects can be estimated from the sum of the doses/ concentrations, scaled for relative toxicity (potency);
- **Independent action**, where substances act independently through different mechanisms and there is no interaction between them; and
- **Interaction**:
  - ***Antagonism***, where combined effects are weaker than would be expected on the basis of dose/concentration addition; and
  - ***Synergism*** (potentiation), where combined effects are stronger than would be expected on the basis of dose/concentration addition.

The report also summarises the present state of knowledge, stating that under certain conditions chemicals may act jointly in such a way that the overall level of toxicity is affected, and that chemicals with common modes of action may produce combination effects that are larger than the effects of each component applied singly at the concentration at which it is present in the mixture under consideration. For chemicals with different modes of action (independently acting), the report concludes that there is no robust evidence that exposure to a mixture of such substances is a health concern if the individual chemicals are present at or below their no-effect levels, and that interactions (including antagonism and potentiation/synergy) usually occur at medium or high doses relative to the lowest effect levels; at low exposures they either do not occur or are toxicologically insignificant.

In view of the almost infinite number of possible combinations of chemicals to which humans and environmental species are exposed, some form of filter to allow a focus on mixtures of potential concern is necessary, and the report offers several such criteria including, critically, assessment of whether significant human or environmental exposure to the mixture or its components can occur. Knowledge of mode of action is essential for assessing the effects of chemical mixtures; if this

information is not available the report proposes that dose/concentration addition assumptions should be applied in preference to the independent action approach. The prediction of possible interaction (i.e. synergy/potentiation or antagonism) requires expert judgement and hence needs to be considered on a case-by-case basis.

Similarly, in its report 'Chemical mixtures: a framework for assessing risks to human health', (IGHRC, 2008) concluded that chemical mixtures are best considered as a series of discrete, precisely defined problems for which clear boundaries can be set, accepting that a key factor in assessing chemical mixtures is the availability (or absence) of reliable data for the whole mixture or its components including mechanisms of action and the range of concentrations over which the individual substances may be present; this is critical to allow expert judgement to be made about the potential for interactions between components to affect the overall toxicity of the mixture. Where there is no clear information on the potential for interactions to occur, the report recommends that it is most appropriate to use a default approach assuming no interactions as the starting point for the preliminary risk assessment. Although this may appear to be a less precautionary approach, the report - like the EC report - makes it clear that interactions seem not to occur at dose levels below thresholds of effect. In this situation, dose addition is recommended as the most precautionary no-interaction model to use.

Very recently a WHO/IPCS framework for assessing combined exposure to multiple chemicals has been published (Meek *et al.*, 2011). This framework is intended to aid the identification of priorities for risk management of a wide range of situations where co-exposures to multiple chemicals are expected. It is based on a hierarchical approach that involves integrated and iterative consideration of exposure and hazard at all phases, with each tier being more refined but also more labour intensive. Initial questions relevant to problem formulation for framework analysis relate to the nature of exposures, whether there is existing information on the hazard of the mixture itself, whether exposure is likely taking into account the context, whether co-exposure is likely within the relevant timeframe, and the rationale for considering compounds in a particular assessment group (mixture). The initial tier begins with simple but conservative assumptions for both exposure and hazard which are then refined and replaced with increasingly detailed data and models, but only if necessary. If there is no cause for concern based on assessment at the initial tier using conservative defaults, no further resources are invested. However, if the results of an initial conservative assessment indicate the potential for excessive risk, then the assessment is refined so as to incorporate more data and more accurate models. At any tier, the outcome of a 'margin of exposure' analysis can be risk management, no further action, generation of additional data, or further assessment (i.e. additional refinement at a higher tier of the framework). In this framework, dose additivity is the default assumption for estimating risk in all tiers, based on analysis of empirical results for effects of combined exposure to chemicals that induce critical effects by different modes of action. The paper reports that it has been shown that where dose addition may under-predict effects as a result of synergistic interactions, the magnitude of the under-prediction is less than an order of magnitude. In concordance with this conclusion, in a recent article on low-dose synergy in chemical mixtures (Boobis *et al.*, 2011) evaluation of 6 available studies that provided useful quantitative estimates of synergy found that the magnitude of synergy at low doses did not exceed the levels predicted by additive models by more than a factor of 4.

One of the case studies used in this paper to illustrate the application of the framework is a fictionalised example of an assessment for a range of substances that co-occur in drinking water, using the threshold of toxicological concern. Surface water is taken to represent a real-world example of a complex mixture of numerous substances at often very low levels, many of which do not have established standards or health-based guidance values, or indeed very much toxicological information at all. This case study sets out to demonstrate the potential utility of applying the threshold of toxicological concern (TTC) approach in a first-tier assessment to prioritize further evaluation of a chemical mixture. The TTS approach sets a *de minimis* value below which exposure is considered unlikely to be a concern, based upon structural characteristics and existing toxicity data for other substances. The case study assumes dose addition, where all components are considered to contribute

to the toxicity of the mixture (Boobis *et al* 2011). Whilst informative, this example is of somewhat limited value for the present exercise because it is a fictionalised example and does not detail actual chemical species or consider specifically the case for endocrine disrupters.

4.3.2 Assessing mixtures of endocrine disrupters

4.3.2.1 Mechanisms and effects

EDCs can affect biological processes by several different modes of action: they may alter the rate of synthesis, transport or clearance of endogenous hormones, or may act as agonists or antagonists of hormones. Modes of action of EDCs include direct interaction with hormone receptors through agonistic action (whereby a ligand binds to and activates the receptor) or antagonistic action (whereby a ligand blocks the receptor or diminishes its activation) and indirect interactions involving alteration in hormone concentrations, the impairment of hormone production, effects on hormone binding proteins, and interference with hormone metabolism, for example (Saalu & Osinubi, 2009).

Theoretically, EDCs can affect every possible cellular hormonal pathway, but most information is available about interference of EDCs with the hormone receptors of the nuclear receptor (NR) family. These are involved in virtually all vital functions – e.g. foetal development, reproduction and metabolism - and include the steroid hormone (oestrogen) receptors (ERs), androgen receptor (AR), progesterone receptor (PR), glucocorticoid receptor (GR), and thyroid receptor (TR). The other key mechanism of ED action is involvement with the aryl-hydrocarbon receptor (AhR), which is a key regulator of the cellular response to xenobiotic exposure. It is strongly activated by organic compounds, including a number of natural and endogenous substances. Most research on EDC to date has focused on the ERs (alpha and beta), AR and TR (Swedenborg *et al.*, 2009, Ruegg *et al.*, 2009).

Ishinawa *et al.*, (2010) has highlighted the likely importance of genetic polymorphisms in ED effects by demonstrating an abundant polymorphism in the AhR coding region in field mice that resulted in intraspecies variation in dioxin sensitivities. Vastermark *et al*., (2011) studying AR gene polymorphisms in relation to the risk, histological type and progression of testicular gene cell cancer, concluded that androgen action is important in the aetiology and pathogenesis of testicular malignancy, and that polymorphisms are involved in gene-environment interactions and may increase susceptibility to the effects of endocrine disrupters.

The disruption by EDCs on the programming of endocrine signalling pathways during development can result in adverse effects that sometimes are not apparent until much later in life. In additional to the well described impacts on reproductive health (and, to a lesser extent, foetal development), recent reports increasingly indicate that such effects can include obesity and diabetes (Newbold, 2010).

The first Scientific Statement of the Endocrine Society (Diamanti-Kandarakis *et al.*, 2009) acknowledged the evidence that EDCs have effects on male and female reproduction, breast development and cancer, prostate cancer, neuroendocrinology, thyroid metabolism and obesity and cardiovascular endocrinology. It is stated that the mechanisms of EDCs involve divergent pathways including, but not limited to, estrogenic, antiandrogenic, thyroid, peroxisome proliferator-activated receptor gamma, retinoid, and actions through other nuclear receptors, steroidogenic enzymes, neuroreceptor transmitters and systems, and many other pathways that are highly conserved in wildlife and humans. The importance of mixtures is acknowledged in this report, accepting that effects of different classes of EDCs may be additive or even synergistic (Diamanti-Kandarakis *et al*., 2009).

The observation that EDCs can modulate the endocrine system in a receptor-independent manner - as demonstrated, for example, by Henley and Korach (2006) in a series of experiments on diethylstilboestrol (a non-steroidal synthetic oestrogen), genistein (a phytoestrogen), di-butyl phthalate (a phthalate ester plasticiser/stabiliser) and methoxy-acetic acid (major metabolite of ethylene glycol monomethyl ether, a common industrial solvent) - has required investigators to reassess the criteria for classifying a compound as an EDC. The varied and often complex

mechanisms of action of EDCs, combined with their physical and chemical diversity, suggest there may be numerous additional chemical endocrine disrupting properties that have yet to be uncovered.

4.3.2.2 EDC mixture effects

Crews *et al.* (2003) explain that in nature EDCs are usually found in mixtures in which the constituent parts are in concentrations well below their NOAEL as determined in single compound studies in the laboratory. He contends that this makes the evaluation of low dose mixtures problematic, in particular because consideration of exposure at the individual level must take into account the fact that at every life stage the naturally occurring endocrine milieu of the organism or tissue, any burden inherited from the mother or built up over the individual's life, and the social environment in which the individual develops and interacts as an adult, will influence their response to acute exposure. Some light has been shed on this suggestion by a recent study by Bruner-Tran & Osten (2011) who investigated pregnancy outcome in mice with a history of developmental TCDD exposure and found that animals exposed *in utero* to TCDD had reduced fertility and increased incidence of premature birth in the F1 generation and in the three subsequent generations. These results suggest that heritable *epigenetic* alterations can be induced by ancestral exposure to a toxicant. Moreover, offspring of F3 mice descended from dually exposed F1 mice continued to exhibit some degree of infertility and premature birth, further suggesting an epigenetic consequence of exposure.

Koppe *et al*. (2006) considering exposure to multiple environmental agents, especially with regard to potential health effects in children, contend that cumulative low dose insults can in some cases be more toxic than a single high-dose exposure, giving as an example the endocrine disruptive effects of a combination of exposure to PCBs and dioxins which disrupt thyroid hormone status.

The contention that effects of mixtures can be predicted by using dose addition of the individual components has been evaluated for EDCs in a review by Kortenkamp (2007), who concluded that there is good evidence to show that the combined effects of EDCs belonging to the same category (e.g. estrogenic, antiandrogenic or thyroid-disrupting agents) can indeed be predicted using dose addition and that this is true for a variety of end points representing a wide range of organisational levels and biological complexity. He contends that this holds true even when each chemical is present at low doses that individually do not induce observable effects. He acknowledges that comparatively little is known about mixtures of chemicals in different classes of EDC but nonetheless argues that combination effects may result from cumulative exposure to EDCs if they are present in sufficiently large numbers at levels equivalent to fractions of their individual NOAELs. However, he accepts that whether mixture effects will indeed occur is difficult to predict without comprehensive information about the levels and identity of EDCs in the environment and in human tissues.

Kortenkamp *et al*., (2007) present an assessment of literature on low-level exposure to mixtures of chemicals and likewise contend that there is good evidence demonstrating significant mixture effects with combinations of chemicals well below their individual NOAELs, both with mixtures composed of similarly and dissimilarly acting agents. They conclude that mixtures of dissimilarly acting chemicals at levels below their respective NOAEL should not be regarded as 'safe' and controversially argue that NOAELs cannot therefore be equated with zero-level effects.

In a later review, Kortenkamp (2008) reiterates the view that, if present in sufficiently large numbers, exposure to mixtures of oestrogenic, thyroid disrupting and anti-androgenic substances that individually produce very small effects may result in combination effects, and concludes that a lack of knowledge about exposure scenarios presents serious obstacles for better human risk assessment. In particular he encourages the development of biomarkers for use in epidemiological studies that capture cumulative exposure to EDCs.

Hass *et al.* (2007) assessed specifically whether combined effects of three androgen receptor antagonists on sexual differentiation in male rats after *in utero* and postnatal exposure can be predicted based on the dose-response data of the individual chemicals. They found that, using the

sensitive endpoint of ano-genital distance[9], the joint effects of the three anti-androgens were essentially dose additive. For nipple-retention, the observed responses were slightly higher than expected on the basis of dose addition. The authors concluded that effects of a mixture of similarly acting anti-androgens can be predicted fairly accurately on the basis of the potency of the individual mixture components by using the dose addition concept, and caution that exposure to mixtures of anti-androgens may induce marked responses even when individually they exert only small effects.

Similarly Metzdorf *et al.* (2007), broadening the range of end points investigated by Hass *et al*., 2007 and reporting some additional results, investigated the ability of a mixture of three androgen receptor antagonists to induce disruption of male sexual differentiation after perinatal exposure and assessed whether the combined effects could be predicted from the dose-response curves of the individual compounds. The end points examined were changes in reproductive organ weights and androgen-regulated gene expression in the prostate. With all end points the joint effects of the three anti-androgens were dose additive. In some cases, effects were seen with the mixture but not with the individual components. The authors concluded that anti-androgens cause additive effects on end points of varying complexity, and that exposure to anti-androgens that individually produce only small effects may induce marked responses in mixtures.

Pursuing the thesis that similarly acting EDCs act in combination according to the principles of dose addition, and that collectively mixtures of EDC in the environment may pose a significant risk even when each component is present at a low and individually ineffective concentration, Brian *et al.* (2007) investigated the ecological significance of EDC mixtures by assessing impacts on reproductive performance in fish. End points analysed included fecundity, expression of male secondary sexual characteristics, somatic indices, and vitellogenin induction. The authors showed evidence of mixture effects on reproductive fitness and fecundity and concluded that chemicals have the capacity to act together to affect reproductive performance even when each component is present below the threshold of detectable effects.

A review article by Crofton (2008) investigates specifically the mechanisms and effects of mixtures of thyroid disrupting chemicals. Broadly defined, these are xenobiotics that alter the structure or function of the thyroid gland or in any way affect circulating or tissue levels of thyroid hormones. They are considered in the present report to fall under the broad heading of EDCs, including but not necessarily limited to mechanisms associated with the thyroid receptor. Crofton concludes that the limited data from mixture studies suggest that dose addition is reasonably accurate in predicting the effects on T4 (thyroxine) concentrations, but acknowledges that an improved understanding is needed of how divergent mechanisms alter thyroid hormones and of the consequent impacts on nervous system development.

In an article on human exposure to endocrine disrupters and effects on semen quality, Phillips and Tanphaichitr (2008) discuss exposure to chemical mixtures, namely pesticides and tobacco smoke. Although the studies reviewed demonstrated a relationship between pesticide exposure and reduced semen quality, a number of different types of mixture were assessed. While the authors make no attempt to analyse possible mixture effects, they do recommend that studies of chemical mixtures should hypothesise what, if any, interactions may be going on within the mixture and their relation to adverse health effects, asserting that it is generally difficult to draw conclusions from epidemiological studies examining pesticide exposures and semen quality when there are multiple components of these potentially toxic substances, each of which may act through similar or divergent mechanisms.

An article reviewing progress made 15 years after the famous "Wingspread" statement on endocrine disrupters (Hotchkiss *et al*., 2008) underlines the fact that mixtures in the environment present a major emerging issue for research and risk assessment and emphasises the importance of developing and

[9] Ano-genital distance (AGD) is a sexually dimorphic measure of genital development and used as a sensitive marker for endocrine disruption in animal studies. It has recently been proposed as a predictor of normal male reproductive potential in humans (Eisenberg *et al*., 2011).

validating methods to accurately predict effects of mixtures of EDCs. This paper cites further evidence for dose additivity of oestrogenic substances impacting the oestrogen receptor and states that chemicals present at NOAEL levels can contribute to a cumulative effect of the mixture. For example, *in utero* exposure to mixtures of chemicals that target the androgen signalling pathway at multiple sites elicit dose-additive effects on the male rat reproductive tract. They conclude from this that the current framework should not only consider including chemicals from different classes with the same mechanism of toxicity, but should also include chemicals that disrupt differentiation of the same foetal tissue at different sites in the androgen signalling pathway. They back this up by citing the work on mixtures of thyroid disrupting chemicals which also found that chemicals targeting endocrine signalling at multiple levels adhere to dose additivity (see Crofton *et al*., 2008). Like other authors, Hotchkiss *et al*. (2008) come to the conclusion that individual chemicals present below their NOAELs contribute to overall mixture effects, and that true risk associated with exposure to EDCs can only be determined when the cumulative effects of chemicals that target a common signalling pathway are taken into consideration.

In a series of experiments by Rider *et al.* (2009) to consider the cumulative risk of chemicals that act via a common mechanism of toxicity, rats were dosed during pregnancy with six different anti-androgens, either singly or in pairs. All binary combinations produced cumulative, dose-additive effects on the androgen-dependent tissues. In addition a mixture study was conducted combining seven antiandrogens that elicit antiandrogenic effects at two different sites in the androgen signalling pathway (i.e. AR antagonist or inhibition of androgen synthesis). This complex mixture also behaved in a dose-additive fashion. The authors concluded that overall their results indicated that compounds that act by disparate mechanisms of toxicity (but on the same biological system) display cumulative, dose-additive effects when present in combination. They propose that in assessing effects of mixtures the primary focus should be on the biological system (e.g. androgen signalling pathway) rather than the mechanism of toxicity *per se*, and that a cumulative risk assessment could potentially include all chemicals that target that system during the same critical development period.

A paper by Iavicoli *et al.* (2009) focuses specifically on the endocrine disrupting properties of metals, including combination effects. It reports the role of cadmium, mercury arsenic, lead, manganese and zinc as endocrine disrupters, detailing possible mechanisms of action and consequent health effects, both in experimental animals and in humans. Some of the reported adverse effects are common to different metals while others are specific. The authors conclude that it is not clear whether metals exert their influence on the endocrine system through the same or different mechanisms of action and stress that, at the present time, only a few of these mechanisms have been elucidated. Interestingly there appears to be some evidence for hormesis, suggesting that effects at very low concentrations are much greater than would be expected from a straight line dose-response relationship. There is a lack of information on the effects of combinations of different metals or of possible mixture effects with other classes of EDCs. Since most experimental studies have identified effects of metals on the endocrine system at levels in excess of those encountered in the environment, the authors advocate further work on potential adverse endocrine effects produced by low level exposures and their respective mechanisms of action. They also call for the systematic collection of information on the endocrine disruptive effects of other metals.

4.3.3 Summary of the current knowledge base

- Knowledge of the mode of action is essential for assessing the effects of chemical mixtures.
- If chemicals in a mixture act via the same mechanism (or influence a common physiological or biochemical pathway) then dose/concentration addition is appropriate for assessing the total effect of the mixture.
- If information on mode of action is not available then dose/concentration addition should be the default approach.

- The prediction of possible interaction (i.e. synergy/potentiation or antagonism) requires expert judgement and hence needs to be considered on a case-by-case basis. However, interactions usually occur at medium or high doses relative to the lowest effect levels and at low exposures they either do not occur or are toxicologically insignificant.
- Exposure to a mixture of substances with different modes of action (independently acting) is not likely to be a health concern if the individual chemicals are present at or below their zero-effect levels.
- For substances with similar modes of action, combination effects may occur even at levels well below individual NOAELs (provided a sufficiently large number of chemicals is present), whereas for mixtures of chemicals with diverse modes of action, acting independently, no adverse effects of mixtures are expected so long as the levels of each component remain below their NOAEL.
- The above rules appear to hold true for EDCs.
- In assessing effects of mixtures of EDCs, the primary focus should be on the biological system (e.g. androgen signalling pathway rather than the mechanism of toxicity *per se*). A cumulative risk assessment could potentially include all chemicals that target that system during the same critical development period. Thus, a true risk associated with exposure to EDCs can only be determined when the cumulative effects of chemicals that target a common signalling pathway are taken into consideration. Hence, when discussing mixtures of EDCs, the meaning of the 'same mechanism of action' may need to include action on common signalling pathways.

4.3.4 Assessment of the potential toxicity of the mixture of oestrogenic compounds identified as being of potential concern in drinking water

As recommended in the literature for EDCs, our assessment of the effects of mixtures of EDCs potentially present in drinking water is primarily focused on the biological system involved. For the sake of practicality – including data availability – we have looked in detail only at the oestrogenic compounds that were considered here. All the identified substances are present at very low concentrations, certainly below their respective NOAELs, so we assumed firstly that any synergistic effects are extremely unlikely to occur, and secondly that there will be no adverse effects from mixtures of dissimilar acting substances.

The potency of oestrogenic chemicals is commonly assessed by comparing it to the potency of the natural sex hormone 17β-oestradiol (E2). Hence, using potency estimates derived from the literature, we calculated an equivalent E2 concentration for each oestrogenic substance and summed these concentrations, assuming additivity, to give an E2 equivalent concentration for the mixture of oestrogenic substances that are possibly present in drinking water.

As an endogenous hormone, the toxic potential of E2 is dependent on a number of biological indices. Nonetheless, the World Health Organisation (WHO, 2000) has derived an ADI of 0.05 μg kg^{-1} bw/day for E2, based on a No Observed Effect Level (NOEL) of 0.3 mg E2/person, with an uncertainty factor of 100 to incorporate normal variation and sensitive populations.

The total E2 equivalent concentration (TEQ) of the mixture of oestrogenic compounds identified in this study, which assumed a worst case exposure scenario and additive toxicity, was ~0.005 μg kg^{-1} bw/day (see Table 4.2), some ten-fold lower than the WHO ADI for E2. It can be concluded that exposure to the mixture of oestrogenic compounds potentially present in drinking water – at the worst case exposure levels relating to exposure of infants to drinking water assuming only a conventional treatment process, as derived from our modelling - does not present a regulatory concern. This is in agreement with a weight-of-evidence assessment of the exposure and risk of oestrogens in US

drinking water (Caldwell *et al*., 2010) which demonstrated significant margins of safety and suggested that a young child's exposure to oestrogenic compounds in milk (soy-based formula, breast or infant formula) would far exceed its exposure to trace concentrations of oestrogens predicted to be in drinking water.

We were not able, within the scope of this study and with the available data, to make detailed biological/mechanistic assessments of each substance, or to undertake a detailed level of mathematical modelling of mixture effects for specific chemical combinations. Nor is there sufficient information available to look at mixture effects other than for oestrogenic substances.

Table 4.2 Summary table for mixture evaluation of oestrogenic compounds[10]

Chemical	Anticipated worst case intake (infant with conventional treatment) µg/kg bw/d	Potency cf. E2[11] (%)	Reference	Equivalent E2 concentration	
p-benzylphenol	3.750	0.0223	Yamasaki *et al.* (2003) *Toxicology Letters* **142:** 119 – 131.	3.750 x (0.0223/100) =	8.3625×10^{-4}
Bisphenol A	0.289	0.315	Olsen *et al.* (2005) *Comparative Biochemistry and Physiology, Part C* **141**: 267 – 274.	0.289 x (0.315/100) =	9.1035×10^{-4}
Nonylphenol	4-NP = 0.041 NP1EC = 0.019 NP2EC = 0.255 NP1EO = 0.031 NP2EO = 0.096 NP3EO = 10.670	0.02	Olsen *et al.* (2005) *Comparative Biochemistry and Physiology, Part C* **141**: 267 – 274.	0.041 x (0.02/100) = 0.019 x (0.02/100) = 0.255 x (0.02/100) = 0.031 x (0.02/100) = 0.096 x (0.02/100) = 10.670 x (0.02/100) =	8.2×10^{-6} 3.8×10^{-6} 5.1×10^{-5} 6.2×10^{-6} 1.92×10^{-5} 3.134×10^{-3}
Dibutylphthalate (DBP)	1.320	0.0000001	Harris *et al.* (1997) *Environmental Health Perspectives* **105**: 802 – 811.	1.320 x (0.0000001/100) =	1.32×10^{-9}
				Total E2 equivalent concentration:	**4.969×10^{-3}**

[10] Excluding compounds for which mechanistic information on mode of action is absent or inadequate
[11] E2 = 17β-oestradiol

5. Discussion

There is continued interest in the quality of drinking water supplies and the perceived risks posed by exposure to endocrine-active substances. It has been suggested that many chemicals in wide use (including industrial, consumer and natural chemicals, pesticides and pharmaceuticals) may possess endocrine disrupting properties and may enter the environment via a range of pathways leading to the presence of the chemicals in sewage discharges, and fresh- and ground-water bodies and, consequently, risk of take up into drinking water treatment facility intakes. Indeed, evidence from the published literature indicates that some EDCs do occur in some water abstraction sources, but the consequence of this in terms of their presence persisting through to treated drinking water and consequently leading to adverse effects on public health, remains unclear.

As a first step in addressing this issue, the study reported here has comprehensively reviewed the existing literature (drawing on both published papers and unpublished reports) to identify reports of the presence in drinking water of any substances that have been suggested as possessing endocrine disruptive properties of potential relevance to human health. Given the large number of substances (325) identified as occurring in relevant water bodies by the initial search, it was necessary to apply a prioritisation scheme to select for further study those suspected EDCs considered to be of most relevance to the UK situation. The use of such prioritisation schemes has been widely applied to the ranking of chemicals for various purposes (e.g. Wearne *et al*., 1996; Swanson and Socha, 1997; Boxall *et al*., 2003a; IEH, 2004; Capleton *et al*., 2006) and is recognised as an effective approach to inform the selection of candidate chemicals for risk assessment.

A customised prioritisation scheme was therefore developed specific to the needs of this study (Figure 2.1) based on a step-wise decision tree using pre-defined inclusion/exclusion criteria. The criteria adopted at this initial screen were highly precautionary in nature to ensure that all the identified chemicals for which there was any appreciable concern as to risk to the mammalian endocrine system were carried forward.

Subsequently, a combined model addressing the environmental fate and behaviour of the selected chemicals and the consequences of various methods of water treatment was used to derive estimates of both the potential concentrations that might be present at drinking water abstraction points and ultimately in treated drinking waters spanning the range of potential treatment processes employed within the UK. Due to resource constraints, consideration of the risk posed by such levels was focused on those predicted to occur at a "worst case" level of >100 ng/L.

From the estimates of drinking water concentrations, intakes (as mg/kg bw/d) that could arise from drinking the treated water (based on standard default assumptions) were established for various population subgroups of concern (adult, toddler and infant). For each chemical, the highest intake value generated (which generally was for infants consuming drinking water from a conventional treatment process) was compared against either authoritative health-base criteria values where previously established (generally in the form of tolerable daily intake (TDI) values or, if unavailable, study-specific exposure limits based on available toxicity information). In the case of endocrine-active pharmaceuticals, however, a different approach was taken involving the application of an additional margin of safety (advised by an expert clinician) to the established minimum therapeutic dose. For all substances the predicted worst-case intake was compared to the TDI or study-specific value to determine the margin of safety (MOS). The chemicals were then further prioritised into three categories according to their MOS ($\geq$ 100 times; 10-100 times and < 10 times).

It should be emphasised that the modelling approach adopted here represents a screening level model based on generally worst-case assumptions regarding the use patterns and chemical properties of the substances and their fate and behaviour within the environment and during treatment processes.

Hence, the resultant predicted intakes represent somewhat unrealistic worst case (extreme) values and will potentially be subject to a high degree of uncertainty (e.g. in relation to usage data, chemical behaviour in sewage treatment and the performance of different water treatment technologies). Thus, for any substance for which potential concern with regard to its predicted MOS is identified in this study, it is stressed that there needs to be more detailed study, including detailed investigation of the level and nature of inputs to the environment and use of more refined exposure models, before robust conclusions can be drawn as to the extent of any risk posed to human health.

As a result of comparing predicted worst case drinking water intakes against the known toxicity profiles for the 35 chemicals carried forward to this final stage, it was found that 21 had a very high MOS (>100) and were therefore not considered to be of public health concern, and 8 had a MOS of between 10-100 and hence were considered to be of doubtful significance given the highly conservative nature of the assumptions made during the derivation of the estimates. However, for 6 chemicals, p-benzylphenol, dibutyphthalate, 4-nitrophenol, digoxin, fluticasone and salbutamol, MOS values of $\leq$ 10 times were identified. For these chemicals it would seem prudent to gather additional measurement data to better inform on levels in UK water bodies used as drinking water sources. In addition, consideration of other potential exposure sources of each of these substances should be considered to better inform on the likely overall scale of exposure.

Concerns with regard to potential mammalian-relevant endocrine disrupting properties were also identified for a number of metals or inorganic metal compounds, including cadmium, chromium, cobalt and copper substances. Although appropriate for the majority of the substances considered, the models used in this study were unsuitable for application to metal substances. Therefore an initial precautionary risk assessment was carried out within the scope of this study to compare the established drinking water regulatory limit for each metallic substance with its known toxicity profile – in particular its potential endocrine activity. No definitive concerns were identified for the metals considered, but it is stressed that this assessment was limited by resource constraints.

Alongside the consideration of the potential risks posed by exposure to individual substances via consumption of drinking water, this study also considered the state of current scientific understanding with regard to the role of mixture activities concerning endocrine disruption effects. The current consensus is that if chemicals in a mixture act via the same mechanism (or influence a common physiological or biochemical pathway) then dose/concentration addition is appropriate for assessing the total effect of the mixture. If information on mode of action is not available then dose/concentration addition should in any case be the default approach. Synergistic (potentiation) or antagonistic interactions between substances usually occur at medium or high doses relative to the lowest effect levels and at low exposures it is considered that they either do not occur or are toxicologically insignificant. Exposure to a mixture of substances with different modes of action (i.e. independently acting) is not likely to be a health concern if the individual chemicals are present at or below their zero-effect levels. While for substances with similar modes of action combination effects may occur even at levels well below individual NOAELs (provided a sufficiently large number of chemicals is present), for mixtures of chemicals with diverse modes of action, acting independently, no adverse effects of mixtures are expected so long as the levels of each component remain below their NOAEL.

As recommended in the literature for EDCs, our assessment of the effects of mixtures of EDCs potentially present in drinking water was primarily focused on the biological system involved. For the sake of practicality – including particularly limitations in data availability – we have looked in detail only at compounds for which evidence of oestrogenicity was established. All the identified substances are present at very low concentrations, certainly below their respective NOAELs, so we assumed firstly that any synergistic effects are extremely unlikely to occur, and secondly that there will be no adverse effects from mixtures of dissimilar acting substances.

Using potency estimates derived from the literature, we calculated an equivalent E2 concentration for each oestrogenic substance and summed these concentrations, assuming additivity, to give an E2

equivalent concentration for the mixture of oestrogenic substances that are possibly present in drinking water. The calculated value of ~0.005 µg kg^{-1} bw/day (see Table 4.2) was some ten-fold lower than the WHO ADI for E2 of 0.05 µg kg^{-1} bw/day. It is thus concluded that exposure to the mixture of oestrogenic compounds potentially present in drinking water – at the worst case exposure levels relating to exposure of infants to drinking water assuming only a conventional treatment process, as derived from our modelling - does not present a regulatory concern. This is in agreement with a weight-of-evidence assessment of the exposure and risk of oestrogens in US drinking water (Caldwell *et al*., 2010) which demonstrated significant margins of safety and suggested that a young child's exposure to oestrogenic compounds in milk (soy-based formula, breast or infant formula) would far exceed its exposure to trace concentrations of oestrogens predicted to be in drinking water.

We were not able, within the scope of this study and with the available data, to make detailed biological/mechanistic assessments of each substance, or to undertake a detailed level of mathematical modelling of mixture effects for specific chemical combinations. Nor was there sufficient information available to look at mixture effects other than for oestrogenic substances. These aspects could be investigated further in a future study if considered to be of potential regulatory concern.

As discussed in this report, for a number of substances various regulatory measures have been introduced on a European basis that would be anticipated to lead to further reductions in usage and hence, over time, in exposure levels. However, as also noted above, the study identified six substances that – within a worst case modelling scenario – show relatively low MOS (<10) that could potentially raise concerns regarding the level of protection provided to the public. It is suggested that further study is needed on these substances to clarify if actual levels of exposure in water bodies and drinking waters in the UK are such as to raise concerns. If these preliminary estimates were to be substantiated then there would be an obvious need to consider potential policy options to address this matter.

For some substances considered here it is also apparent that there is currently a lack of publicly available information on their toxicological properties, thus limiting the robustness of any potential risk assessment. We suggest that in such instances the database should be periodically reviewed, since the available body of evidence is expected to improve over the coming years as a result of the assessment processes required under REACH and, possibly, as a result of operation of the Biocides Directive in the case of p-benzylphenol and 4-nitrophenol. Furthermore, the operation of the Water Framework Directive (WFD) would be anticipated to result in further information being generated on the levels of substances present in water and is likely to result in improvements in the general standards of water treatment, thus acting to reduce any potential exposure even further.

6. Conclusions and recommendations

Conclusions

- A comprehensive review of the available literature identified 325 substances for which concerns have been expressed with regard to their potential endocrine disrupting activity as being potentially present within water bodies relevant to drinking water abstraction;

- Of these, the evidence base for 35 potentially endocrine-active chemicals was considered to warrant detailed exposure modelling and hazard characterisation so as to inform a robust risk assessment;

- Comparison of predicted worst-case drinking water intake against the hazard profile showed that for 21 chemicals there was a very high margin of safety (>100) and, hence, no concerns for human health;

- A further 8 chemicals showed margins of safety of 10–100 and hence were unlikely to be of concern in the light of the highly conservative nature of the assumptions made;

- For only 6 chemicals (p-benzylphenol, dibutylphthalate, 4-nitrophenol, digoxin, fluticasone and salbutamol) were margins of safety ≤10 times – these are identified as warranting further, more detailed consideration;

- In the light of current scientific understanding of the way mixtures of chemicals interact, it was found that even drinking waters containing the predicted worst-case level of each of the oestrogenically-active chemicals identified here would not constitute a significant risk to human health when considered in terms of the equivalence to consumption of the natural hormone oestradiol.

Further recommendations

Whilst the principal conclusion of this study is that the identified EDCs pose only limited (if any) health risk to the UK population, this was a purely desk-based exercise and as such was subject to a number of inherent limitations:

- For the 6 chemicals (p-benzylphenol, dibutylphthalate, 4-nitrophenol, digoxin, fluticasone and salbutamol) considered by this preliminary investigation to warrant further, more detailed consideration, it is suggested that further research is carried out to characterise the extent and nature of exposure to these substances in the UK waters used for abstraction and drinking water supplies, so as to establish the actual situation with regard to exposures of the populations of England and Wales to these substances.

- Due to resource constraints it was only possible to undertake detailed risk characterisation for those chemicals with PECs ≥100 ng/L in this initial investigation and it may, therefore, be prudent to undertake further study of those chemicals estimated to have PECs of 10–100 ng/L; and

- The assessment of the risk posed by mixtures of EDCs in drinking water was somewhat simplistic in nature and it is suggested that a more detailed study, based on detailed exposure assessment and mathematical modelling of the potential mixture effects, may warrant consideration.

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Annex 1- Literature search strategies

A1.1

The following search strategy was adopted to identify literature relating to the presence of EDCs in water sources:

SET 1a – EDC

Endocrine disrupter* (used in Scopus)
Endocrine disruptor* (used in Scopus)
Endocrine disrupting (used in Scopus)
Endocrine disrupt*

SET 1b – Activity

Estrog* OR Oestrog*
Androg*
Adren*
Thyro*
Obesogen*
Progestagen* OR Progestogen OR gestagen*

SET 2a – Water

Freshwater OR fresh water
Groundwater OR ground water
Surface water
Drinking water
Potable water
Bottled water
Water suppl*
Water distribution

Sewage treatment work*
Sewage treatment plant*
Wastewater treatment plant*
Waste water treatment plant*
Wastewater treatment work*

SET 2c - Effluent

Final effluent
Secondary effluent
Tertiary effluent
Discharge

SET 3 - Exposure

Exposure

Search strategy:

((Set 1 a OR Set 1b) AND (Set 2a OR Set 2b OR Set 2c) AND Set 3)) for Article and Review using SCOPUS (includes Medline & Embase) and CSA Illumina (Aqualine, Biological Sciences,

Environment Abstracts, Environment Science and Pollution Management, Medline, Risk Abstracts, Toxline, Water Resources Abstracts).

A1.2

The following search strategy was adopted to identify literature relating to the identification, use, toxicological profile and EDC potency of chemicals identified as being of concern.

SET 1a – Chemical

Chemical name

SET 2 – Toxicity end-point

Health effect(s) (used in Scopus)
Adverse effect(s) (used in Scopus)
Health effect*
Adverse effect*
Toxic*
Teratogen*
Carcinogen*
Genotox*
Mutagen*

Search strategy:

((Set 1 a) AND (Set 2)) for title and keyword, unlimited review using SCOPUS (includes Medline & Embase) and CSA Illumina (Aqualine, Biological Sciences, Environment Abstracts, Environment Science and Pollution Management, Medline, Risk Abstracts, Toxline, Water Resources Abstracts).

A1.3

The following search strategy was adopted to identify literature relating to the current knowledge of mixture effects and mixture toxicity of EDCs.

SET 1a – EDC

Endocrine disrupter* (used in Scopus)
Endocrine disruptor* (used in Scopus)
Endocrine disrupting (used in Scopus)
Endocrine disrupt*

SET 1b - Activity

Estrog* OR Oestrog*
Androg*
Adren*
Thyro*
Obesogen*
Progestagen* OR Progestogen OR gestagen*

SET 2

Mixture*
Synerg*
Additiv*
Potentiation
Toxic* mechanism*
Multiple
Combin*
Water distribution

Search strategy:

((Set 1 a OR Set 1b) AND (Set 2)) for title and keyword, limited to review from year 2005 onwards using SCOPUS (includes Medline & Embase) and CSA Illumina (Aqualine, Biological Sciences, Environment Abstracts, Environment Science and Pollution Management, Medline, Risk Abstracts, Toxline, Water Resources Abstracts).

Annex 2 – Potential EDCs in European water sources

Table A2.1: Potential EDCs identified in European groundwater, surface water, freshwater, drinking/potable water or sewage treatment effluent following review of published and grey literature

No.	EDC Name	CAS number	Nature / Origin	Country of Measurement	Decision Code	Status
1	**6-Acetyl-1,1,2,4,4,7-hexa-methyltetralin**	-	Consumer chemical	UK	Code B	Exclude
2	**16α-Hydroxyestrone**		Natural hormone/conjugate	EU	Code 1	Include
3	**1H-Benzotriazole (Tolytriazole)**	95-14-7	Industrial/Consumer chemical	EU	Code B	Exclude
4	**2,4-Dichlorophenoxyacetic acid (2,4-D)**	94-75-7	Herbicide	EU	Codes 3, 4	Include
5	**2,4-Dinitrophenol**	51-28-5	Industrial Chemical / Pharmaceutical	EU	Code 8	Include
6	**2-Hydroxybiphenyl**	90-43-7	Industrial chemical degradation product	EU	Code 1	Include
7	**3-(4-Methylbenzylidine)camphor**	38102-62-4	Consumer chemical	UK	Code 9	Include
8	**3,4,5,6-Tetrabromo-o-cresol**	576-55-6	Consumer chemical	UK	Code 9	Include
9	**3-t-Butyl-4-hydroxyanisole**	121-00-6	Industrial chemical	EU	Code 8	Include
10	**4-Chloro-3-methylphenol**	59-50-7	Industrial chemical	EU	Code 3	Include
11	**4-Chloroxylenol**	88-04-0	Consumer chemical	UK	Code B	Exclude
12	**4'-Isobutylacetophenone**	38861-78-8	Synthetic by-product	EU	Duplicate	Exclude
13	**4-Nitrophenol (p-Nitrophenol, PNP)**	100-02-7	Industrial chemical	EU	Code EJ	Include
14	**4-Nonylphenodiethoxycarboxylate (NP2EC)**		Industrial Chemical	EU	Code 1	Include
15	**4-Nonylphenolmonoethyoxycarboxylate (NP1EC)**		Industrial Chemical	EU	Code 1	Include
16	**4-Nonylphenoltriethoxylate (NP3EO)**		Industrial Chemical	EU	Code 1	Include

17	**4-tert Octylphenol (OP)**	104-66-9	Industrial Chemical	EU & UK	Codes 1, 2	Include
18	**4-t-Octylphenol monoethoxylate (OP1EO)**	-	Industrial Chemical	EU	Code 1	Include
19	**4-t-Octylphenoltriethoxylate (OP3EO)**			EU	Code 1	Include
20	**5-Aminosalicylicacid**	89-57-6	Human/Veterinary drug degradation product	UK	Duplicate	Exclude
21	**5β-Androstane-3*α*, 17β-diol-11-one**	1158-94-7	-	UK	Code 9	Include
22	**Acebutolol**	37517-30-9	Pharmaceutical	EU	Code C	Exclude
23	**Acetaminophen (Paracetemol)**	103-90-2	Pharmaceutical	EU & UK	Code 9	Include
24	**Acetylsalicylic acid**	50-78-2	Human/Veterinary Drug	EU	Code 10	Include
25	**Acipimox**	51037-30-0	Human/Veterinary Drug	EU	Code 11	Include
26	**Alachlor**	15972-60-8	Pesticide	EU	Codes 2, A	Exclude
27	**Amitriptyline**	50-48-6	Human/Veterinary drug	UK	Code 9	Include
28	**Amoxicillin**	26787-78-0	Human/Veterinary Drug	EU	Code 8	Include
29	**Amphetamine**	300-62-9	Pharmaceutical / recreational drug use	EU & UK	Code B	Exclude
30	**Ampicillin**	69-53-4	Human/Veterinary Drug	EU	Code B	Exclude
31	**Androstanedione**	846-46-8	Natural hormone/conjugate	UK	Code 9	Include
32	**Androsterone**	53-41-8	Natural hormone/conjugate	UK	Code 7	Include
33	**Anthracene**	120-12-7	Industrial Chemical	EU	Code B	Exclude
34	**Arsenic**	7440-38-2	Metal/metal-containing compound	EU	Code 5	Include
35	**Aspirin**	50-78-2	Human/Veterinary drug	EU & UK	Code 9	Include
36	**Atenolol**	29122-68-7	Pharmaceutical	EU & UK	Code 7	Include
37	**Atorvastatin**	134523-00-5	Human/Veterinary Drug	EU	Code 9	Include
38	**Atraine**	93616-39-8	Herbicide	EU	Duplicate	Exclude
39	**Atrazine**	1912-24-9	Pesticide / Herbicide	EU	Code 2	Include
40	**Atrazine-desethyl**	6190-65-4	Herbicide degradation product	EU	Code 6	Include
41	**Azithromycin**	83905-01-5	Human/Veterinary Drug	EU	Code 9	Include
42	**Bendroflumethiazide**	73-48-3	Human/Veterinary drug	UK	Code B	Exclude

43	**Bentazone**	25057-89-0	Pesticide	EU	Code 4	Include
44	**Benzo(a)anthracene**	56-55-3	Coal mining waste products	EU	Code C	Exclude
45	**Benzo(a)pyrene**	50-32-8	Industrial Chemical	EU	Code 1	Include
46	**Benzo(b)fluoranthene**	205-99-2	Coal mining waste products	EU	Code 1	Include
47	**Benzo(g,h,i)perylene**	191-24-2	Coal mining waste products	EU	Code 1	Include
48	**Benzo(k)fluoranthene**	207-08-9	Coal mining waste products	EU	Code 1	Include
49	**Benzophenone**	119-61-9	Industrial Chemical	EU & UK	Code 3	Include
50	**Benzotriazole**	95-14-7	Industrial Chemical	EU	Code B	Exclude
51	**Benzoylecgonine**	519-09-5	Illicit Drug Metabolite	UK	Code C	Exclude
52	**Benzylparaben**	94-18-8	Consumer Chemical / Pharmaceutical	EU	Code 8	Include
53	**Betaxolol**	63659-18-7	Human/Veterinary drug	EU	Code B	Exclude
54	**Bezafibrate (BZF)**	41859-67-0	Pharmaceutical	EU & UK	Code B	Exclude
55	**Biochanin A**	491-80-5	Natural plant substance/degradation product	EU	Code 1	Include
56	**Bis(2-ethylhexyl)phthalate (DEHP)**	117-81-7	Industrial Chemical	EU & UK	Code 2	Include
57	**Bisoprolol**	66722-44-9	Human/Veterinary drug	EU	Code 7	Include
58	**Bisphenol A (BPA)**	80-05-7	Industrial Chemical	EU & UK	Code 1	Include
59	**Bisphenol F (BPF)**	2467.-02-9	Industrial Chemical	EU	Code 6	Include
60	**Bromoform**	75-25-2	Natural / industrial Chemical	EU	Code C	Exclude
61	**Butylated hydroxy aniline (anisole)**	25013-16-5	Food additive/contact material	EU	Code 6	Include
62	**Butylated hydroxytoluene (BHT)**	128-37-0	Industrial Chemical	EU	Code B	Exclude
63	**Butylbenzyl phthalate (BBP)**	85-68-7	Industrial Chemical	EU	Code 2	Include
64	**Butylparaben**	94-26-8	Consumer Chemical	EU & UK	Code 8	Include
65	**Butylphenol**	98-54-4	Industrial Chemical	EU	Code 2	Include
66	**Cadmium**	7440-43-9	Metal/metal-containing compound	EU	Code 5	Include
67	**Cadmium chloride**	10108-64-2	Metal/metal-containing compound	EU	Code 5	Include

68	**Caffeine**	58- 08-2	Natural / Consumer chemical	EU	Code C	Exclude
69	**Captopril**	62571-86-2	Human/Veterinary Drug	EU	Code 9	Include
70	**Carazolol**	57775-29-8	Human/Veterinary drug	EU	Code 7	Include
71	**Carbamazapine**	298-46-4	Pharmaceutical	EU & UK	Code B	Exclude
72	**Carisoprodol**	78-44-4	Human/Veterinary drug	EU	Code B	Exclude
73	**Cashmeran**	33704-61-9	Consumer chemical	EU	Code B	Exclude
74	**Cefuroxime**	55268-75-2	Human/Veterinary Drug	EU	Code 9	Include
75	**Celestolide**	13171-00-1	Consumer chemical	EU	Code B	Exclude
76	**Celiprolol**	56980-93-9	Pharmaceutical	EU	Code 9	Include
77	**Chloramphenicol**	56-75-7	Human/Veterinary drug	EU	Code C	Exclude
78	**Chlorazepate**	28425-34-5	Human/Veterinary Drug	EU	Code B	Exclude
79	**Chlordanes (total)**	N/A	Pesticide/pesticide degradation products	EU	Code 2	Include
80	**Chloridazon**	1698-60-8	Herbicide	EU	Code 4	Include
81	**Chlorocathecols**	-	Industrial chemical	EU	Code B	Exclude
82	**Chloroform**	67-66-3	Industrial Chemical	EU	Code B	Exclude
83	**Chloroguaiacols**	-	Industrial chemical	EU	Code B	Exclude
84	**Chlorophene**	120-32-1	Consumer chemical	EU & UK	Code 8	Include
85	**Chlorophenols**	-	Industrial chemical	EU	Code B	Exclude
86	**Chlorotetracycline**	57-62-5	Human/Veterinary Drug	EU	Code 8	Include
87	**Chromium**	7440-47-3	Metal/metal-containing compound	EU	Code 5	Include
88	**Chromium trioxide**	1333-82-0	Metal/metal-containing compound	EU	Code 5	Include
89	**Chrysene**	218-01-9	Coal mining waste product	EU	Code B	Exclude
90	**Cimetidine**	51481-61-9	Pharmaceutical	EU & UK	Code B	Exclude
91	**Ciprofloxacin**	85721-33-1	Pharmaceutical	EU	Code 9	Include
92	**Clarithromycin**	81103-11-9	Human/Veterinary Drug	EU	Code 8	Include
93	**Clenbuterol**	37148-27-9	Human/ Veterinary drug	EU	Code B	Exclude
94	**Clofibrate (Clofibric acid)**	54504-70-0	Human/Veterinary Drug	EU & UK	Code 8	Include

95	**Clotrimazole**	23593-75-1	Human/Veterinary drug	EU	Code B	Exclude
96	**Cobalt**	7440-48-4	Metal/metal-containing compound	EU	Code 5	Include
97	**Cocaine**	50-36-2	Illicit Drug	UK	Code C	Exclude
98	**Codeine**	76-57-3	Natural Chemical / Pharmaceutical	EU & UK	Code 8	Include
99	**Copper**	7440-50-8	Metal/metal-containing compound	EU	Code 5	Include
100	**Cortisol**	50-23-7	Pharmaceutical / Glucocorticoid	EU	Code 7	Include
101	**Cortisone**	53-06-5	Pharmaceutical / Glucocorticoid	EU	Code 7	Include
102	**Co-trimoxazole**	8064-90-2	Human/Veterinary Drug	EU	Code C	Exclude
103	**Coumestrol**	479-13-0	Natural plant substance//degradation product	EU	Code 6	Include
104	**Cyclophosphamide**	50-18-0	Human/ Veterinary drug	EU	Code 9	Include
105	**Daidzein**	486-66-8	Natural plant substance/degradation product	EU	Code 1	Include
106	**Dehydroabietic Acid**	1740-19-8	Industrial chemical	UK	Code B	Exclude
107	**Dehydrotestosterone**	846-48-0	Natural hormone/conjugate	UK	Code 6	Include
108	**Demeclocycline**	127-33-3	Human/Veterinary Drug	EU	Code 7	Include
109	**Desethylatrazine (DEA)**	6190-65-4	Pesticide degradation product	EU	Code 6	Include
110	**Desethylterbutylazine (DET)**	61-51-8	Pharmaceutical / recreational drug use	EU	Code 9	Include
111	**Dexamethasone**	50-02-2	Pharmaceutical / Glucocorticoid	EU	Code 7	Include
112	**Di-(2-ethylhexyl) phthalate**	117-81-7	Industrial chemical/Consumer chemical	EU	Code 3	Include
113	**Diatrizoic acid**	117-96-4	Human/Veterinary Drug	EU	Code 9	Include
114	**Diazepan**	439-14-5	Human/Veterinary Drug	EU	Code B	Exclude
115	**Diazinon**	333-41-5	Pesticide	EU	Code A	Exclude

116	**Dibenzo(a,h)anthracene**	53-70-3	Coal mining waste products	EU	Code C	Exclude
117	**Dibromochloromethane**	124-48-1	Industrial Chemical / Natural Chemical	EU	Code C	Exclude
118	**Dibutyl phthalate (DBP)**	84-74-2	Industrial Chemical	EU	Code 2	Include
119	**Dichlorobromomethane**	75-27-4	Industrial Chemical	EU	Code C	Exclude
120	**Diclofenac (DCF)**	15307-86-5	Pharmaceutical	EU & UK	Code B	Exclude
121	**Diethyl phthalate (DEP)**	84-66-2	Industrial Chemical	EU & UK	Code 3	Include
122	**Diethylstilbestrol (DES)**	56-53-1	Pharmaceutical	EU	Code 1	Include
123	**Diethyltoluamide (DEET)**	134-62-3	Consumer Chemical / Pharmaceutical	EU	Code 9	Include
124	**Digoxigenin**	1672-46-4	Human/Veterinary drug degradation product	EU	Code 9	Include
125	**Digoxin**	20830-75-5	Human/Veterinary drug	EU	Code 9	Include
126	**Diisobutyl phthalate (DIBP)**	84-69-5	Industrial Chemical	EU	Code B	Exclude
127	**Diisodecylphthalate**	26761-40-0	Industrial chemical	EU	Code 3	Include
128	**Di-isononylphthalate**	28553-12-0	Industrial chemical	EU	Code 3	Include
129	**Dilantin**	57-41-0	Human/Veterinary Drug	EU	Code 9	Include
130	**Diltiazem**	42399-41-7	Human/Veterinary drug	EU & UK	Code 9	Include
131	**Dimethoate**	60-51-5	Pesticide	EU	Code 4	Include
132	**Dimethyl phthalate**	131-11-3	Industrial Chemical / Pharmaceutical	EU	Code B	Exclude
133	**Dimethylaminophenazone**	58-15-1	Human/Veterinary Drug	EU	Code 9	Include
134	**Diuron**	330-54-1	Pesticide	EU	Code 4	Include
135	**Dodecyl benzene sulfonic acid (LAS)**	27176-87-0	Industrial Chemical / Pesticide	EU	Code 8	Exclude
136	**Domperidone**	57808-66-9	Human/Veterinary Drug	EU	Code 9	Include
137	**Doxazosin**	74191-85-8	Human/Veterinary drug	EU	Code 9	Include
138	**Doxycline**	564-25-0	Human/Veterinary Drug	EU	Code 8	Include
139	**Enalapril**	75847-73-3	Pharmaceutical	EU	Code C	Exclude
140	**Epi-androsterone**	481-29-8	Natural hormone/conjugate	UK	Code 6	Include

141	**Erythromycin**	114-07-8	Natural Chemical / Pharmaceutical	EU	Code 9	Include
142	**Erythromycin-H2O (Erythromycin dihydrate)**	59319-72-1	Natural Chemical / Pharmaceutical	EU & UK	Code 9	Include
143	**Estradiol-17-glucuronide**	-	Natural hormone/conjugate	EU	Code 6	Include
144	**Estrone-3-sulfate**	481-97-0	Natural hormone/conjugate	EU	Code 6	Include
145	**Ethylparaben**	120-47-8	Consumer Chemical	EU & UK	Code 9	Include
146	**Ethynyloestradiol (EE2)**	57-63-6	Pharmaceutical Synthetic hormone	EU & UK	Code 2	Include
147	**Etofenamate**	30544-47-9	Human/Veterinary Drug	EU	Code B	Exclude
148	**Etofibrate**	31637-97-5	Human/Veterinary Drug	EU	Code 9	Include
149	**Famotidine**	76824-35-6	Pharmaceutical	EU	Code B	Exclude
150	**Fenobibrate**	49562-28-9	Human/Veterinary Drug	EU	Code 9	Include
151	**Fenofibric acid**	42017-89-0	Human/Veterinary drug metabolite	EU	Code 9	Include
152	**Fenoprofen (FNP)**	31879-05-7	Pharmaceutical	EU & UK	Code B	Exclude
153	**Fenoterol**	13392-18-2	Human/ Veterinary drug	EU	Code 8	Include
154	**Fentiazac**	18046-21-4	Human/Veterinary Drug	EU	Code B	Exclude
155	**Ferric chloride**	7705-08-0	Metal/metal-containing compound	EU	Code 5	Include
156	**Flumethasone**	2135-17-3	Pharmaceutical	EU	Code 8	Include
157	**Flunixin**	38677-85-9	Veterinary Product	EU	Code 9	Include
158	**Fluorene**	86-73-7	Coal mining waste products	EU	Code B	Exclude
159	**Fluoroanthene**	206-44-0	Coal mining waste products	EU	Code B	Exclude
160	**Fluoxetine**	54910-89-3	Pharmaceutical	EU	Code B	Exclude
161	**Flurbiprofen**	5104-49-4	Human/Veterinary Drug	EU	Code B	Exclude
162	**Fluticasone**	80474-14-2	Human/Veterinary Drug	EU	Code 9	Include
163	**Formononetin**	485-72-3	Natural plant substance/ degradation product	EU	Code 1	Include
164	**Furosemide**	54-31-9	Human/Veterinary Drug	EU	Code B	Exclude

165	**Gabapentin**	60142-96-3	Pharmaceutical	EU & UK	Code B	Exclude
166	**Gemfibrozil (GFB)**	25812-30-0	Pharmaceutical	EU & UK	Code B	Exclude
167	**Genistein**	446-72-0	Natural plant substance/ conjugate/ degradation product	EU	Code 1	Include
168	**Gentisic acid**	490-79-9	Human/Veterinary drug metabolite	EU	Code B	Exclude
169	**Glycitein**	40957-83-3	Natural plant substance/ conjugate/ degradation product	EU	Code 6	Include
170	**Hexachlorobenzene**	118-74-1	Pesticide	EU	Code 2	Include
171	**Hexachlorohexane**		Pesticide/pesticide degradation products	EU	Code A	Exclude
172	**Hexahydrohexamethylcyclopentabenzopyran (Galoxilide)**	1222-05-5	Consumer chemical (Polycyclic musk)	EU & UK	Code B	Exclude
173	**Hydrochlorothiazide**	58-93-5	Pharmaceutical	EU	Code C	Exclude
174	**Hydrocinnamic acid**	501-52-0	Cosmetic	EU	Code B	Exclude
175	**Hydroxazine**	68-88-2	Human/Veterinary Drug	EU	Code B	Exclude
176	**Hydroxyacetophenone**	99-93-4	Pesticide / Pharmaceutical	EU	Code 9	Include
177	**Hydroxylphenyl propanone**	-	-	EU	Code 9	Include
178	**Hydroxyprogesterone**	-	Natural Hormone	EU	Code 6	Include
179	**Ibuprofen (IBP)**	15687-27-1	Pharmaceutical	EU & UK	Code B	Exclude
180	**Ifosfamide**	3778-73-2	Human/ Veterinary drug	EU	Code B	Exclude
181	**Indapamide**	26807-65-8	Human/Veterinary Drug	EU	Code 9	Include
182	**Indenol(1,2,3-cd)pyrene**	193-39-5	Coal mining waste products	EU	Code C	Exclude
183	**Indomethacin (IDM)**	53-86-1	Pharmaceutical	EU & UK	Code 9	Include
184	**Iohexol**	66108-95-0	Human/Veterinary Drug	EU	Code 9	Include
185	**Iomeprol**	78649-41-9	Human/Veterinary Drug	EU	Code 9	Include
186	**Iopamidol**	60166-93-0	Human/Veterinary Drug	EU	Code 9	Include
187	**Iopromide**	73334-07-3	Pharmaceutical / Contrast medium	EU	Code 9	Include
188	**Iothalamic acid**	2276-90-6	Human/Veterinary Drug	EU	Code 9	Include

189	**Irgarol**	28159-98-0	Algaecide	EU	Code A	Exclude
190	**Isoproturon**	34123-59-6	Pesticide	EU	Code A	Exclude
191	**Kaempferol**	520-18-3	Natural plant substance/degradation product	EU	Code 8	Include
192	**Ketoprofen (KTP)**	22071-15-4	Pharmaceutical	EU & UK	Code B	Exclude
193	**Ketorolac**	74103-06-3	Pharmaceutical	EU	Code B	Exclude
194	**Lansoprazole**	103577-45-3	Pharmaceutical	EU	Code B	Exclude
195	**Lead**	7439-92-1	Metal/metal-containing compound	EU	Code 5	Include
196	**Lead acetate**	301-04-2	Metal/metal-containing compound	EU	Code 5	Include
197	**Levonorgestrel**	797-63-7	Pharmaceutical	EU	Codes 3, 6	Include
198	**Lincomycin**	154-21-2	Pharmaceutical	EU	Code B	Exclude
199	**Loratadine**	79794-75-5	Pharmaceutical	EU	Code B	Exclude
200	**Manganese chloride**	7773-01-5	Metal/metal-containing compound	EU	Code 5	Include
201	**MCPA**	94-74-6	Herbicide	EU	Code B	Exclude
202	**Meclocycline**	73816-42-9	Human/Veterinary Drug	EU	Code 9	Include
203	**Meclofenamic acid**	644-62-2	Human/Veterinary Drug	EU	Code B	Exclude
204	**Mecoprop**	93-65-2	Pesticide	EU	Code A	Exclude
205	**Mefenamic acid**	61-68-7	Human/Veterinary Drug	EU	Code B	Exclude
206	**Mercury**	7439-97-6	Metal/metal-containing compound	EU	Code 5	Include
207	**Mestranol**	72-33-3	Synthetic Hormone	EU	Code 1	Include
208	**Methylbenotriazole**	29385-43-1	Industrial Chemical	EU	Code B	Exclude
209	**Methyl ester**	n/a	Landfill leachate	EU. Monitored in Germany only	Code C	Exclude
210	**Methylparaben**	99-76-3	Consumer Chemical	EU & UK	Code 9	Include
211	**Metolachlor**	51218-45-2	Pesticide	EU	Code A	Exclude
212	**Metoprolol**	51384-51-1	Pharmaceutical	EU & UK	Code 7	Include

213	**Metrinidazole**	443-48-1	Pharmaceutical	EU	Duplicate	Exclude
214	**Metronadiazole**	81103-11-9	Human/Veterinary Drug	EU	Duplicate	Exclude
215	**Metronidazole**	443-48-1	Human/Veterinary drug	EU	Duplicate	Exclude
216	**Musk ketone**	81-14-1	Industrial/Consumer chemical	EU	Code 11	Include
217	**n,n-Diethyl-meta-toluamide**	94271-03-1	Pesticide/pesticide degradation products	EU	Code B	Exclude
218	**Nadolol**	42200-33-9	Human/Veterinary Drug	EU	Code 7	Include
219	**Naproxen**	22204-53-1	Pharmaceutical	EU & UK	Code B	Exclude
220	**Napthalene**	91-20-3	Coal mining waste products	EU	Code B	Exclude
221	**Napthalene sulfonate**	(napthalene 91-20-3	Pesticide	EU	Code B	Exclude
222	**Napthalene-1,5-disulfonate**	(napthalene 91-20-3	Pesticide	EU	Code B	Exclude
223	**Naringenin**	480-41-1	Natural plant substance/degradation product	EU	Code 1	Include
224	**Nickel**	8049-31-8	Metal/metal-containing compound	EU	Code 5	Include
225	**Nickel chloride**	7718-54-9	Metal/metal-containing compound	EU	Code 5	Include
226	**Nimesulide**	51803-78-2	Human/Veterinary Drug	EU	Code B	Exclude
227	**Nitrophenols**	25154-55-6	Industrial Chemicals	EU	Code B	Exclude
228	**Nitrosopiperidine**	100-75-4	Industrial Chemical	EU	Code B	Exclude
229	**Nitrosopyrrolidine**	35884-45-8	Industrial Chemical	EU	Code B	Exclude
230	**N-Nitrosodimethylamine (NDMA)**	62-75-9	Industrial Chemical	EU	Code B	Exclude
231	**N-Nitrosomethylethylamine**	10595-95-6	Industrial Chemical	EU	Code B	Exclude
232	**Nonylphenoldiethyoxylate (NP2EO)**	9016-45-9	Industrial Chemical	EU & UK	Code 1	Include
233	**Nonylphenolmonoethoxylate (NP1EO)**	27986-36-3	Industrial Chemical	EU	Code 1	Include
234	**Nonylphenoxyacetic acid**	3115-49-9		EU	Code 1	Include
235	**Nonylphrnol (NP)**	25154-52-3	Industrial Chemical	EU & UK	Code 1	Include
236	**Norethindrone**	68-22-4	Human/Veterinary drug	EU	Code 6	Include

237	**Norfloxacin**	70458-96-7	Pharmaceutical	EU	Code 9	Include
238	**Nortriptyline**	894-71-3	Human/Veterinary drug	EU	Code 9	Include
239	**Oestradiol (E2)**	50-28-2	Natural Hormone	EU & UK	Code 1	Include
240	**Oestriol (E3)**	50-27-1	Natural Hormone	EU & UK	Code 1	Include
241	**Oestrone (E1)**	53-16-7	Natural Hormone	EU & UK	Code 1	Include
242	**Ofloxacin**	82419-36-1	Pharmaceutical	EU	Code 9	Include
243	**o-Hydroxyhippuric acid**	487-54-7	Human/Veterinary drug metabolite	EU	Code 9	Include
244	**Oxazepam**		Human/Veterinary Drug	EU	Code 9	Include
245	**Oxytetracycline**	6153-64-6	Pharmaceutical	EU	Code 8	Include
246	**p,p-DDE**		Pesticide/pesticide degradation products	EU	Code 1	Include
247	**p,p-DDT**		Pesticide/pesticide degradation products	EU	Code 1	Include
248	**Palmitic acid**	57-10-3	Natural Chemical	EU	Code B	Exclude
249	**Papaverine**	61-25-6	Human/Veterinary drug	EU	Code B	Exclude
250	**Paroxetine**	61869-08-7	Human/Veterinary drug	EU	Code B	Exclude
251	**p-Benzylphenol**	101-53-1	Consumer chemical	UK	Code 3	Include
252	**Pentachlorophenol**	87-86-5	Pesticide/Fungicide	EU	Code A	Exclude
253	**Pentylphenol (PP)**	1322-06-1	Industrial chemical	EU	Code B	Exclude
254	**Perfluorodecanoic acid (PFDA)**	335-76-2	Industrial chemical	EU	Code B	Exclude
255	**Perfluorohaxanoate**	68259-11-0	Industrial chemical	EU	Code B	Exclude
256	**perfluorohepanoate**	375-85-9	Industrial chemical	EU	Code B	Exclude
257	**Perfluorohexane sulfonate**	355-46-4	Industrial Chemical	EU	Code B	Exclude
258	**Perfluorooctanoic acid**	335-67-1	Industrial Chemical	EU	Code B	Exclude
259	**Perfluorooctanoic sulfonic acid (PFOS)**	1763-23-1	Industrial Chemical	EU	Code B	Exclude
260	**Perflurononanoate**	375-95-1	Industrial Chemical	EU	Code B	Exclude
261	**Pergfluoroundecanoate**	2058-94-8	Industrial Chemical	EU	Code B	Exclude
262	**Phenanthrene**	99257-48-4	Coal mining waste products	EU	Code B	Exclude
263	**Phenazone**	60-80-0	Human/Veterinary Drug	EU	Code 9	Include

264	**Phenolic acid**	29656-58-4	Natural Chemical	EU	Code B	Exclude
265	**Phenylphenol**	90-43-7	Consumer Chemical	EU	Code 3, 8	Include
266	**Phloretin**	60-82-2	Natural plant substance/degradation product	EU	Code 6, 8	Include
267	**p-hydroxybiphenyl**	92-69-3	industrial chemical	UK	Code 1	Include
268	**Pindolol**	13523-86-9	Human/Veterinary drug	EU	Code B	Exclude
269	**Piroxicam**	36322-90-4	Human/Veterinary Drug	EU	Code 9	Include
270	**Polychlorinated biphenyl congeners**	na	Industrial chemical	EU	Code 1, 2	Include
271	**Potassium bromate**	7758-01-2.	Disinfection by-product	EU	Code B	Exclude
272	**Pravastatin**	81093-37-0	Human/Veterinary Drug	EU & UK	Code 8	Include
273	**Prednisolone**	50-24-8	Pharmaceutical / Glucocorticoid	EU	Code 7	Include
274	**Prednisone**	53-03-2	Pharmaceutical / Synthetic Glucocorticoid	EU	Code 7	Include
275	**Primidon**	718-71-8	Human/Veterinary Drug	EU	Code B	Exclude
276	**Progesterone**	57-83-0	Natural Chemical / Pharmaceutical	EU	Code 1	Include
277	**Propanil**	709-98-8	Herbicide	EU	Code 3	Exclude
278	**Propazine**	139-40-2	Pesticide	EU	Code 3	Exclude
279	**Propoxur**	114-26-1	Pesticide	EU & UK	Code 4	Include
280	**Propranolol**	525-66-6	Pharmaceutical	EU & UK	Code 7	Include
281	**Propylparaben**	94-13-3	Consumer Chemical	EU & UK	Code 8	Include
282	**Propyphenazone**	479-92-5	Human/Veterinary drug	EU	Code 9	Include
283	**Pyrene**	1718-52-1	Coal mining waste products	EU	Code B	Exclude
284	**Quercetin**	74893-81-5	Natural plant substance/degradation product	EU	Code 9	Include
285	**Ramipril**	87333-19-5	Human/Veterinary Drug	EU	Code 9	Include
286	**Ranitidine**	66357-35-5	Pharmaceutical	EU & UK	Code B	Exclude
287	**Retene**	483-65-8	Natural plant substance	EU	Code B	Exclude

288	**Roxithromycin**	80214-83-1	Human/Veterinary drug	EU	Code 9	Include
289	**Salbutamol**	35763-26-9	Pharmaceutical	EU & UK	Code 8	Include
290	**Salicylic Acid (SA)**	22204-53-1	Pharmaceutical	EU & UK	Duplicate	Exclude
291	**Sertraline**	79617-96-2	Human/Veterinary drug	EU	Code 9	Include
292	**Simazine**	122-34-9	Pesticide	EU	Code 4	Include
293	**Simvastatin**	79902-63-9	Human/Veterinary drug	EU & UK	Code 9	Include
294	**Sodium arsenate**	7778-43-0	Metal/metal-containing compound	EU	Code 5	Include
295	**Sotalol**	3930-20-9	Pharmaceutical	EU	Code 9	Include
296	**Spiramycin**	8025-81-8	Pharmaceutical	EU	Code B	Exclude
297	**Stearic Acid**	57-11-4	Natural Chemical	EU	Code B	Exclude
298	**Sulfadimethoxine**	122-11-2	Human/Veterinary Drug	EU	Code 8	Include
299	**Sulfamethoxazole**	723-46-6	Pharmaceutical / antibacterial agent	EU & UK	Code 8	Include
300	**Sulfapyridine**	144-83-2	Pharmaceutical	EU & UK	Code 8	Include
301	**Sulfasalazine**	599-79-1	Human/Veterinary drug	UK	Code 8	Include
302	**Tamoxifen**	10540-29-1	Human/Veterinary drug	EU	Code 7	Include
303	**Tebuconazole**	107534-96-3	Pesticide	EU & UK	Code 4	Include
304	**Terbutalin**	23031-25-6	Human/ Veterinary drug	EU	Code 8	Include
305	**Terbutryn**	886-50-0	Herbicide	EU	Code 4	Include
306	**Terbutylazine**	5915-41-3	Herbicide	EU	Code A	Include
307	**Tetracyclin**	60-54-8	Human/Veterinary Drug	EU	Code 8	Include
308	**Timolol**	26839-75-8	Human/Veterinary drug	EU	Code C	Exclude
309	**Tolfenamic acid**	13710-19-5	Human/Veterinary Drug	EU	Code 9	Include
310	**Tonalid**	21145-77-7	Cosmetic	EU	Code B	Exclude
311	**Tramadol**	27203-92-5	Pharmaceutical	EU & UK	Code B	Exclude
312	**Tri(2-butoxyethyl)phosphate**	78-51-3	Industrial Chemical	EU	Code C	Exclude
313	**Tri-(2-chloroisopropyl)phosphate**	13674-84-5	Industrial Chemical	EU	Code C	Exclude
314	**Triamcinolone**	124-94-7	Pharmaceutical	EU	Code 7	Include

315	**Triamcinolone acetonide**	76-25-5	Pharmaceutical / Glucocorticoid	EU	Code 6	Include
316	**Tributylphosphate**	126-73-8	Industrial Chemical	EU	Code 9	Include
317	**Tributyltin**	688-73-3	Pesticide	EU	Codes 1, 2	Include
318	**Triclosan**	3380-34-5	Pharmaceutical / antibacterial / fungicide	EU & UK	Code 8	Include
319	**Trimethoprim**	738-70-5	Pharmaceutical / antibacterial	EU & UK	Code B	Exclude
320	**Tris(1,3-dichloro-2-propyl) phosphate**	13674-87-8	Industrial chemical	EU	Code 9	Include
321	**Tris(2-chloroethyl) phosphate**	115-96-8	Industrial chemical	EU	Code 9	Include
322	**Valsartan**	137862-53-4	Human/Veterinary drug	EU & UK	Code B	Exclude
323	**Vanadium**	740-62-2	Metal/metal-containing compound	EU	Code 5	Include
324	**Warfarin**	81-81-2	Human/Veterinary drug	EU	Code 9	Include
325	**β-Sitosterol**	83-46-5	Natural plant substance/Human/Veterinary drug	EU & UK	Code 1	Include

Inclusion codes - Codes 1-3: Listed by IUPAC, EU or EUROPA as 'of concern'; Code 4: Currently included as a UK registered pesticide; Code 5: Metal or metal-containing compound – not subject to modelling; Code 6: A Conjugated hormone/EDC or a phytoestrogen; Code 7: Clinical ranking of extent of potential EDC activity (High, medium, low - **for pharmaceuticals only**); Code 8: OECD QSAR toolbox results for oestrogen binding activity; and Code 9: OECD QSAR toolbox results and weight of evidence (WoE) for protein binding potential.

Exclusion codes - Code A: Not listed by IUPAC, EU or EUROPA as 'of concern' and not a UK registered pesticide; Code B: As Code A plus not a metal or metal containing-compound, conjugated hormone/EDC or phytoestrogen, not a pharmaceutical ranked as likely to have a 'high' or 'moderate' clinical endocrine activity, no evidence of oestrogen or protein binding using OECD QSAR toolbox; and Code C: As Code A and B but some evidence of protein binding using OECD QSAR toolbox, plus no concern following assessment of WoE for endocrine toxicity.

Annex 3 – Effect of water treatment

Table A3.1 Extent of Substance removal by coagulation/filtration and chlorination (Conventional Treatment)

Compound	Removal by coagulation/filtration (%)	Removal by Cl2 (%)	Removal by both (%)
16α-Hydroxyestrone	0	64	64
2,4-Dichlorophenoxyacetic acid (2,4-D)	25	28	46
2,4-Dinitrophenol	0	46	46
2-Hydroxybiphenyl	0	74	74
3-(4-Methylbenzylidine)camphor	0	46	46
3,4,5,6-Tetrabromo-o-cresol	25	64	73
3-t-Butyl-4-hydroxyanisole	0	64	64
4-Chloro-3-methylphenol	0	55	55
4-Nitrophenol (p-Nitrophenol, PNP)	25	55	66
4-Nonylphenodiethoxycarboxylate (NP2EC)			
4-Nonylphenolmonoethyoxycarboxylate (NP1EC)			
4-Nonylphenoltriethoxylate (NP3EO)			
4-tert-Octylphenol (OP)	0	64	64
4-t-Octylphenol monoethoxylate (OP1EO)	0	55	55
4-t-Octylphenoltriethoxylate (OP3EO)			
5β-Androstane-3α, 17β-diol-11-one	0	46	46
Acetaminophen (Paracetemol)	0	74	74
Acetylsalicylic acid	0	46	46
Acipimox	25	41	56
Amitriptyline	0	64	64
Amoxicillin	25	83	87
Androstanedione	0	18	18
Androsterone	0	37	37
Atenolol	0	74	74
Atorvastatin	25	83	87
Atrazine	0	51	51
Atrazine-desethyl	0	51	51
Azithromycin	0	83	83
Bentazone	25	51	63
Benzo(a)pyrene	0	83	83
Benzo(b)fluoranthene	0	74	74

Compound	Removal by coagulation/filtration (%)	Removal by Cl2 (%)	Removal by both (%)
Benzo(g,h,i)perylene	0	83	83
Benzo(k)fluoranthene	0	74	74
Benzophenone	0	46	46
Benzylparaben	25	74	81
Biochanin A	25	74	81
Bis(2-ethylhexyl)phthalate (DEHP)	0	46	46
Bisoprolol	0	64	64
Bisphenol A (BPA)	0	83	83
Bisphenol F (BPF)	0	83	83
Butylated hydroxy aniline (anisole)	0	64	64
butylbenzyl phthalate (BBP)	0	64	64
Butylparaben	0	64	64
Butylphenol	0	64	64
Captopril	25	51	63
Carazolol	0	78	78
Cefuroxime	25	78	84
Celiprolol	0	74	74
Chlordanes (total)	0	0	0
Chloridazon	0	51	51
Chlorophene	0	64	64
Chlorotetracycline	0	83	83
Ciprofloxacin	0	37	37
Clarithromycin	0	83	83
Clofibrate	0	46	46
Codeine	0	51	51
Cortisol	0	46	46
Cortisone	0	28	28
Coumestrol	25	83	87
Cyclophosphamide	0	37	37
Daidzein	25	83	87
Dehydrotestosterone	0	37	37
Demeclocycline	0	83	83
Desethylterbutylazine (DET)	0	51	51
Dexamethasone	0	37	37
Diatrizoic acid	0	37	37
Dibutyl phthalate (DBP)	0	46	46
Diethyl phthalate (DEP)	0	46	46
Diethylstilbestrol (DES)	0	83	83
Diethyltoluamide (DEET)	0	46	46
Digoxigenin	0	64	64
Digoxin	0	83	83
Diisodecylphthalate	0	46	46

Compound	Removal by coagulation/filtration (%)	Removal by Cl2 (%)	Removal by both (%)
Di-isononylphthalate	0	46	46
Dilantin	0	51	51
Diltiazem	0	74	74
Dimethoate	0	55	55
Dimethylaminophenazone	0	60	60
Diuron	0	46	46
Domperidone	0	60	60
Doxazosin	0	74	74
Doxycycline	25	83	87
Epi-androsterone	0	37	37
Erythromycin	0	83	83
Erythromycin-H2O (Erythromycin dihydrate)	0	83	83
Estradiol-17-glucuronide			
Estrone-3-sulfate	0	46	46
Ethylparaben	0	64	64
Ethynyloestradiol (EE2)	0	74	74
Etofibrate	0	41	41
Fenofibrate	0	37	3
Fenofibric acid	25	37	53
Fenoterol	0	83	83
Flumethasone	0	28	28
Flunixin	25	32	49
Fluticasone	0	28	28
Formononetin	25	64	73
Genistein	25	83	87
Glycitein	25	83	87
Hexachlorobenzene	0	0	0
Hydroxyacetophenone	0	46	46
Hydroxylphenyl propanone	0	28	28
Hydroxyprogesterone	0	28	28
Indapamide	0	79	79
Indomethacin (IDM)	25	51	63
Iohexol	0	83	83
Iomeprol	0	83	83
Iopamidol	0	83	83
Iopromide	0	83	83
Iothalamic acid	25	37	53
Kaempferol	25	83	87
Levonorgestrel	0	37	37
Meclocycline	25	83	87
Mestranol	0	55	55

Compound	Removal by coagulation/filtration (%)	Removal by Cl2 (%)	Removal by both (%)
Methylparaben	25	64	73
Metoprolol	0	74	74
Musk ketone	0	18	18
Nadolol	0	83	83
Naringenin	25	83	87
Nonylphenoldiethyoxylate (NP2EO)			
Nonylphenolmonoethoxylate (NP1EO)	0	60	60
Nonylphenoxyacetic acid	25	46	60
Nonylphenol (NP)	0	64	64
Norethindrone	0	37	37
Norfloxacin	0	37	37
Nortriptyline	0	64	64
Oestradiol (E2)	0	74	74
Oestriol (E3)	0	83	83
Oestrone (E1)	0	55	55
Ofloxacin	25	37	53
o-Hydroxyhippuric acid	25	74	81
Oxazepam	0	60	60
Oxytetracycline	0	83	83
p,p-DDE	0	18	18
p,p-DDT	0	9	9
p-Benzylphenol	0	74	74
Phenazone	0	51	51
Phloretin	25	83	87
p-Hydroxybiphenyl	0	74	74
Piroxicam	25	83	87
Polychlorinated biphenyl congeners			
Pravastatin	25	74	81
Prednisolone	0	46	46
Prednisone	0	28	28
Progesterone	0	18	18
Propoxur	0	51	51
Propranolol	0	74	74
Propylparaben	0	64	64
Propyphenazone	0	51	51
Quercetin	25	83	87
Ramipril	25	60	70
Roxithromycin	0	83	83
Salbutamol	0	83	83
Sertraline	0	46	46
Simazine	0	51	51

Compound	Removal by coagulation/filtration (%)	Removal by Cl2 (%)	Removal by both (%)
Simvastatin	0	46	46
Sotalol	0	83	83
Sulfadimethoxine	25	79	84
Sulfamethoxazole	25	78	84
Sulfapyridine	0	78	78
Sulfasalazine	0	83	83
Tamoxifen	0	74	74
Tebuconazole	0	10	10
Terbutalin	0	83	83
Terbutryn	0	69	69
Terbutylazine	0	51	51
Tetracyclin	25	83	87
Tolfenamic acid	25	55	66
Triamcinolone	0	46	46
Triamcinolone acetonide	0	28	28
Tributylphosphate	0	37	37
Tributyltin	0	37	37
Triclosan	25	46	60
Tris(1,3-dichloro-2-propyl) phosphate	0	0	0
Tris(2-chloroethyl) phosphate	0	9	9
Warfarin	25	51	63
β-Sitosterol	0	46	46

Table A3.2 Extent of Substance removal by coagulation, ozone, activated carbon and chlorination (Advanced Treatment)

Compound	Removal by coagulation (%)	Removal by ozone (%)	Removal by activated carbon (%)	Removal by chlorine (%)	Removal by all (%)
16α-Hydroxyestrone	0	80	50	64	96
2,4-Dichlorophenoxyacetic acid (2,4-D)	25	40	0	28	68
2,4-Dinitrophenol	0	60	50	46	89
2-Hydroxybiphenyl	0	90	50	74	99
3-(4-Methylbenzylidine)camphor	0	60	90	46	98
3,4,5,6-Tetrabromo-o-cresol	25	80	0	64	95
3-t-Butyl-4-hydroxyanisole	0	80	50	64	96
4-Chloro-3-methylphenol	0	70	50	55	93
4-Nitrophenol (p-Nitrophenol, PNP)	25	70	25	55	92
4-Nonylphenodiethoxycarboxylate (NP2EC)					
4-Nonylphenolmonoethyoxycarboxylate (NP1EC)					
4-Nonylphenoltriethoxylate (NP3EO)					
4-tert-Octylphenol (OP)	0	80	90	64	99
4-t-Octylphenol monoethoxylate (OP1EO)	0	70	90	55	99
4-t-Octylphenoltriethoxylate (OP3EO)					
5β-androstane-3α, 17β-diol-11-one	0	60	50	46	89
Acetaminophen (Paracetemol)	0	90	50	74	99
Acetylsalicylic acid	0	60	0	46	78
Acipimox	25	55	0	41	80
Amitriptyline	0	80	0	64	93
Amoxicillin	25	100	25	83	100
Androstanedione	0	30	50	18	71
Androsterone	0	50	50	37	84
Atenolol	0	90	50	74	99
Atorvastatin	25	100	25	83	100
Atrazine	0	65	50	51	91
Atrazine-desethyl	0	65	50	51	91
Azithromycin	0	100	50	83	100
Bentazone	25	65	25	51	90
Benzo(a)pyrene	0	100	90	83	100
Benzo(b)fluoranthene	0	90	90	74	100
Benzo(g,h,i)perylene	0	100	90	83	100

Compound	Removal by coagulation (%)	Removal by ozone (%)	Removal by activated carbon (%)	Removal by chlorine (%)	Removal by all (%)
Benzo(k)fluoranthene	0	90	90	74	100
Benzophenone	0	60	50	46	89
Benzylparaben	25	90	50	74	99
Biochanin A	25	90	50	74	99
Bis(2-ethylhexyl)phthalate (DEHP)	0	60	50	46	89
Bisoprolol	0	80	50	64	96
Bisphenol A (BPA)	0	100	50	83	100
Bisphenol F (BPF)	0	100	50	83	100
Butylated hydroxy aniline (anisole)	0	80	50	64	96
Butylbenzyl phthalate (BBP)	0	80	90	64	99
Butylparaben	0	80	50	64	96
Butylphenol	0	80	50	64	96
Captopril	25	65	25	51	90
Carazolol	0	95	50	78	99
Cefuroxime	25	95	25	78	99
Celiprolol	0	90	50	74	9
Chlordanes (total)	0	0	90	0	90
Chloridazon	0	65	50	51	91
Chlorophene	0	80	90	64	99
Chlorotetracycline	0	100	50	83	100
Ciprofloxacin	0	50	50	37	84
Clarithromycin	0	100	50	83	100
Clofibrate	0	60	50	46	89
Codeine	0	75	50	51	94
Cortisol	0	60	50	46	89
Cortisone	0	40	50	28	78
Coumestrol	25	100	25	83	100
Cyclophosphamide	0	50	50	37	84
Daidzein	25	100	25	83	100
Dehydrotestosterone	0	50	50	37	84
Demeclocycline	0	100	25	83	100
Desethylterbutylazine (DET)	0	65	50	51	91
Dexamethasone	0	50	50	37	84
Diatrizoic acid	0	50	0	37	69
Dibutyl phthalate (DBP)	0	60	90	46	98
Diethyl phthalate (DEP)	0	60	50	46	89
Diethylstilbestrol (DES)	0	100	90	83	100
Diethyltoluamide (DEET)	0	60	50	46	89
Digoxigenin	0	80	50	64	96

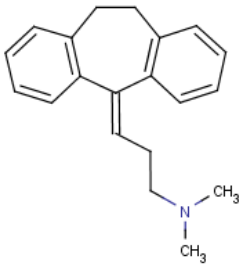
Compound	Removal by coagulation (%)	Removal by ozone (%)	Removal by activated carbon (%)	Removal by chlorine (%)	Removal by all (%)
Digoxin	0	100	25	83	100
Diisodecylphthalate	0	60	90	46	98
Di-isononylphthalate	0	60	90	46	98
Dilantin	0	75	25	51	91
Diltiazem	0	90	50	74	99
Dimethoate	0	70	50	55	93
Dimethylaminophenazone	0	75	25	60	93
Diuron	0	60	50	46	89
Domperidone	0	75	50	60	95
Doxazosin	0	90	50	74	99
Doxycycline	25	100	0	83	100
Epi-androsterone	0	50	50	37	84
Erythromycin	0	100	50	83	100
Erythromycin-H2O (Erythromycin dihydrate)	0	100	50	83	100
Estradiol-17-glucuronide					
Estrone-3-sulfate	0	60	50	46	89
Ethylparaben	0	80	50	64	96
Ethynyloestradiol (EE2)	0	90	50	74	99
Etofibrate	0	55	50	41	87
Fenofibrate	0	50	90	37	97
Fenofibric acid	25	50	25	37	82
Fenoterol	0	100	50	83	100
Flumethasone	0	40	50	28	78
Flunixin	25	45	50	32	86
Fluticasone	0	40	50	28	78
Formononetin	25	80	25	64	96
Genistein	25	100	25	83	100
Glycitein	25	100	25	83	100
Hexachlorobenzene	0	0	90	0	90
Hydroxyacetophenone	0	60	50	46	89
Hydroxylphenyl propanone	0	40	25	28	68
Hydroxyprogesterone	0	40	50	28	78
Indapamide	0	95	50	79	99
Indomethacin (IDM)	25	65	25	51	90
Iohexol	0	100	0	83	100
Iomeprol	0	100	0	83	100
Iopamidol	0	100	0	83	100
Iopromide	0	100	0	83	100
Iothalamic acid	25	50	0	37	76
Kaempferol	25	100	25	83	100

Compound	Removal by coagulation (%)	Removal by ozone (%)	Removal by activated carbon (%)	Removal by chlorine (%)	Removal by all (%)
Levonorgestrel	0	50	50	37	84
Meclocycline	25	100	0	83	100
Mestranol	0	70	90	55	99
Methylparaben	25	80	25	64	96
Metoprolol	0	90	25	74	98
Musk ketone	0	30	50	18	71
Nadolol	0	100	50	83	100
Naringenin	25	100	25	83	100
Nonylphenoldiethyoxylate (NP2EO)					
Nonylphenolmonoethoxylate (NP1EO)	0	75	90	60	99
nonylphenoxyacetic acid	25	60	25	46	88
Nonylphenol (NP)	0	80	90	64	99
Norethindrone	0	50	50	37	84
Norfloxacin	0	50	25	37	76
Nortriptyline	0	80	50	64	96
Oestradiol (E2)	0	90	50	74	99
Oestriol (E3)	0	100	50	83	100
Oestrone (E1)	0	70	50	55	93
Ofloxacin	25	50	0	37	76
o-Hydroxyhippuric acid	25	90	25	74	99
Oxazepam	0	75	50	60	95
Oxytetracycline	0	100	25	83	100
p,p-DDE	0	30	90	18	94
p,p-DDT	0	20	90	9	93
p-Benzylphenol	0	90	90	74	100
Phenazone	0	65	50	51	91
Phloretin	25	100	50	83	100
p-hydroxybiphenyl	0	90	90	74	100
Piroxicam	25	100	0	83	100
Polychlorinated biphenyl congeners					
Pravastatin	25	90	0	74	98
Prednisolone	0	60	50	46	89
Prednisone	0	40	50	28	78
Progesterone	0	30	50	18	71
Propoxur	0	65	50	51	91
Propranolol	0	95	50	74	99
Propylparaben	0	80	50	64	96
Propyphenazone	0	65	50	51	91

Compound	Removal by coagulation (%)	Removal by ozone (%)	Removal by activated carbon (%)	Removal by chlorine (%)	Removal by all (%)
Quercetin	25	100	25	83	100
Ramipril	25	75	50	60	96
Roxithromycin	0	100	50	83	100
Salbutamol	0	100	50	83	100
Sertraline	0	60	50	46	89
Simazine	0	65	50	51	91
Simvastatin	0	60	90	46	98
Sotalol	0	100	50	83	100
Sulfadimethoxine	25	95	25	79	99
Sulfamethoxazole	25	95	25	78	99
Sulfapyridine	0	95	50	78	99
Sulfasalazine	0	100	50	83	100
Tamoxifen	0	90	50	74	99
Tebuconazole	0	65	50	10	84
Terbutalin	0	100	50	83	100
Terbutryn	0	85	50	69	98
Terbutylazine	0	65	50	51	91
Tetracyclin	25	100	0	83	100
Tolfenamic acid	25	70	50	55	95
Triamcinolone	0	60	50	46	89
Triamcinolone acetonide	0	40	50	28	78
Tributylphosphate	0	50	50	37	84
Tributyltin	0	50	0	37	69
Triclosan	25	60	50	46	92
Tris(1,3-dichloro-2-propyl) phosphate	0	0	50	0	50
Tris(2-chloroethyl) phosphate	0	20	50	9	64
Warfarin	25	65	25	51	90
β-Sitosterol	0	60	90	46	98

Annex 4 – Hazard profiles

Hazard Assessment

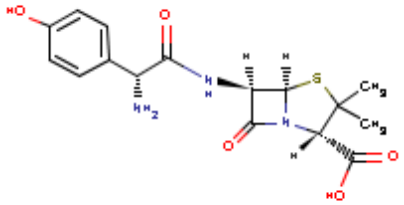
Substance name:	**Amitryptiline** 
CAS No.:	50-48-6
Synonym(s):	10,11-Dihydro-5-(γ-dimethylaminopropylidene)-5H-dibenzo(a,d)cycloheptene; 10,11-Dihydro-N.N-dimethyl-5H-dibenzo(a,d)heptalene-δ(sup5), γ-propulamine; 3-(10,11-Dihydro-5H-dibenzo(a,d)cyclohepten-5-yliden)-N,N0dimethylpropylamin; 3-(10,11-Dihydro-5H-dibenzo(a,d)cyclohepten-5-ylidene)-N,N-dimethyl-1-propanamine; 5-(γ-Dimethylaminopropylidene)-10,11-dihydro-5H-dibenzo(a,d)cycloheptene; Adepress; Adepril; Amitriptilina; Amitriptylin; Amitryiptylinum; Amytriptylin; Damilen; Damitryptyline; Lantron; Proheptadiene; Seroten; Triptanol; Triptilin; Tryptanol; Tryptizol; 1-Propanamine, 3-(10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5-ylidene)-N,N-dimethyl-.
Use and potential human exposure routes:	Cyclic antidepressants such as amitriptyline are used in the treatment of enuresis, obsessive-compulsive disorder, attention deficit hyperactivity disorder, school phobia and separation anxiety in paediatric populations, whilst in adults it is used in the treatment of depression, neuralgic pain, chronic pain and migraine prophylaxis. Literature reporting amitriptyline levels in UK water sources is sparse. Kasprzyk-Horden et al (2009) reported concentrations of amitriptyline in the River Ely, in Wales, of up to 17 ng/L. Occupational exposure to amitriptyline may occur through inhalation of dust and dermal contact with this compound at workplaces where amitriptyline is produced. The general population may be exposed via the oral route to amitriptyline through medical administration of this compound.
Established/Study-specific exposure limit:	**Therapeutic dose by age range:** < 1 month : not used 2 to 12 years : 200 µg/ kg bw 12 to 18 years : 10 mg (10 – 20mg) > 18 years : 10 mg (10 – 300 mg) Pregnancy Category C **Study-specific Exposure Limit** = 2 µg/kg bw/d.
Basis for exposure limit:	Assuming an uncertainty factor of 100 from minimum human therapeutic dose.
Evidence for human relevant endocrine	Adverse effects observed in humans taking amitriptyline include testicular swelling and gynecomastia in males; breast enlargement and galactorrhea in

Amitryptiline

disrupting potential:	females; increased or decreased libido; impotence; and, elevation and/or lowering of blood sugar levels (RxList, 2011).
Other significant toxic effects:	Acute oral toxicity in mice is 350 mg/kg. Amitriptyline has been shown to cross the placenta and although a causal relationship has not been established, there have been reports of increased incidence of adverse developmental abnormalities in infants whose mothers had taken amitriptyline during pregnancy. Reproductive toxicity studies using pregnant mice and hamsters administered oral doses of between 28 – 100 mg/kg bw/d (9 – 22 times maximum human therapeutic dose), have shown amitriptyline to be teratogenic, producing multiple malformations. However, no teratogenic effects were observed in mice, rats or rabbits given oral doses between 2 and 40 mg/kg bw/d amitriptyline (up to 12 times the maximum recommended human dose of 3 mg/kg/day). An oral dose of 25 mg/kg bw/d in rats resulted in delays in ossification of foetal vertebral bodies without other signs of embyrotoxicity. Similarly in rabbits, an oral dose of 60 mg/kg bw/d (20 times the maximum recommended human dose) was reported to cause incomplete ossification of cranial bones. Bautista-Ferrufino *et al*. (2011) demonstrated an increase in lipid peroxidation, resultant of oxidative stress in liver, lung, kidney, heart skeleteal muscle and serum of mice exposed to amitriptyline. Coenzyme Q concentrations, a component of the respiratory chain and potent antioxidant, were significantly increased in the brain, heart and skeletal muscle, while decreased in liver and lung following exposure. Amitriptyline (24 hour exposure to 50 µM or 100 µM) enhanced the production of oxidised products during lipid peroxidation, inverting the reduced/oxidised ratio to 25% reduction and 75% oxidation in human fibroblast cells *in vitro.* A decrease in the number of cultured cells was also reported in all dose groups. The study suggests that amitriptyline induces mitochondrial dysfunction and oxidative stress in human fibroblast cells (Moreno-Fernàndez *et al.*, 2008).
Primary mode of endocrine activity	None Identified
Study estimate of anticipated exposure via drinking water	Limited intake via drinking water is predicted to occur in all age groups following conventional treatment processes and in infants and toddlers following advanced treatment processes. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.015; Toddler 0.010; Adult 0.003. Intakes (ug/kg bw/d) based on advanced treatment: Infant 0.001; Toddler 0.001.
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	No evidence to either prove or disprove endocrine disrupting activity per se has been identified for this substance. Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes and conservative assumptions is 0.015 µg/kg bw/d for infants, this would suggest a margin of safety of around 130 times against the study-specific exposure limit. These figures were based on modelled levels in drinking water calculated with usage figures obtained from UK prescription data, undergoing conventional water treatment; as such they are conservative in nature. It

	should be noted that margins of safety following advanced water treatment were significantly increased to a minimum of 2000 times (for infants). As there is currently no evidence to support ED activity of amitripyline, the pharmaceutical is not considered of concern under the specific remit of this project, and will not be assessed further.
References:	Bautista-Ferrufino, M. R., Cordero, M. D., Sànchez-Alcàzar, J. A., Illandes, M., Fernàndez-Rodriguez, A., Navas, P., de Miguel, M. (2011) Amitriptyline induces coenzyme Q deficiency and oxidative damage in mouse lung and liver. Toxicology Letters 204: 32 – 37. Kasprzyk-Hordern, B., Dinsdale, M., Guwy, A.J. (2009) The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. *Water Research* **43** : 363–380. Moreno-Fernàndez, A. M., Cordero, M. D., de Miguel, M., Delgado-Rufino, M. D., Sànchez-Alcàzar, J. A., Navas, P. (2008) Cytotoxic effects of amitriptyline in human fibroblasts. Toxicology 243: 51 – 58. RxList (2011) The Internet Drug Index: http://www.rxlist.com/elavil-drug.htm [Accessed 19.09.2011]

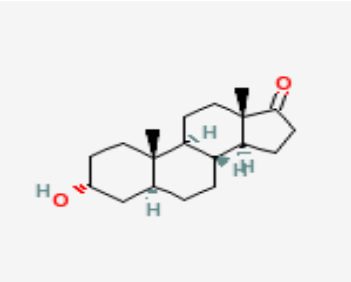
Hazard Assessment

Substance name:	**Amoxicillin** 
CAS No.:	CAS No. 26787-78-0
Synonym(s):	(-)-6-(2-Amino-2-(P-hydroxyphenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-(3.2.0)heptane-2-carboxylic acid; 6-(D-(-)-alpha-Amino-p-hydroxyphenylacetamido)penicillanic acid; 6-(D-(-)-p-Hydroxy-alpha-aminobenzyl)penicillin; 6-(p-Hydroxy-alpha-aminophenylacetamido)penicillanic acid; AMPC; Amoclen; Amolin; Amopenixin; Amoxi; Amoxi-Mast; Amoxicillin; Amoxiden; Amoxil; Amoxivet; Amoxycillin; Ampy-Penyl; Anemolin; Aspenil; BLP 1410; Bristamox; Cemoxin; Clamoxyl; D-(-)-alpha-Amino-p-hydroxybenzylpenicillin; D-2-Amino-2-(4-hydroxyphenyl)acetamidopenicillanic acid; D-Amoxicillin; Delacillin; DisperMox; EINECS 248-003-8; Efpenix; Flemoxin; HSDB 3204; Hiconcil; Histocillin; Ibiamox; Imacillin; Metafarma capsules; Metifarma capsules; Moxacin; Moxal; NSC 277174; Piramox; Ro 10-8756; Robamox; Sawamox PM; Sumox; Unicillin; Vetramox; alpha-Amino-p-hydroxybenzylpenicillin; p-Hydroxyampicillin
Use and potential human exposure routes:	Amoxicillin, an analogue of ampicillin, has a broad spectrum of bactericidal activity against many Gram-positive and Gram-negative micro-organisms. Occupational exposure to amoxicillin may occur through inhalation of dust and dermal contact with this compound at workplaces where amoxicillin is produced or dispensed. The general population may be exposed via the oral route to amoxicillin through medical administration of this compound.
Established/Study-specific exposure limit:	An ADI of 0.2mg/kg bw has been established by the Australian Government (Department of Health and Ageing. Australian Government, 2005).
Basis for exposure limit:	The ADI was based on a NOEL of 200 mg/kg bw, with an uncertainty factor of 1000 applied; specific study details used in establishing this ADI are not available.
Evidence for human relevant endocrine disrupting potential:	Using a precautionary approach, this substance was identified as of possible concern following expert advice from the clinician within the project team. However, no evidence suggestive of endocrine disrupting activity per se was identified for amoxicillin in subsequent literature review. In a multi-generation reproductive toxicity study in rats, no impairment of fertility or other adverse reproductive effects were observed at doses up to 500 mg/kg. This dose is approximately three times the therapeutic dose used

	in humans (RxList, 2008). Reproductive toxicity studies have been performed in mice and rats at doses up to ten times the human therapeutic dose and have not revealed any evidence of impaired fertility or adverse effects on the foetus as a result of treatment with amoxicillin. There have been no well-controlled studies in humans (RxList, 2008).
Other significant toxic effects:	The most common health effects are the side-effects of amoxicillin experienced following its therapeutic use; the most serious of these effects is the hypersensitivity reaction which can take the form of anaphylactic shock, serum sickness-like reactions, erythematous maculopapular rashes, erthema multi-forme, Stevens–Johnson syndrome, exfoliative dermatitis, toxic epidermal necrolysis, acute generalized exanthematous pustulosis, hypersensitivity vasculitis and urticaria. (RxList, 2008). Anaemia, including haemolytic anaemia, thrombocytopenia, thrombocytopenic purpura, eosinophilia, leukopenia, and granulocytosis has been reported during therapy with penicillins. The effects are reversible on discontinuation of therapy and are believed to be part of the hypersensitivity reaction (RxList, 2008). Gastrointestinal effects are common and include nausea, vomiting, diarrhoea, and haemorrhagic/pseudomembranous colitis. Pseudomembranous colitis may range in severity from mild to life-threatening (HSDB, 2003; RxList, 2008). Following treatment with amoxicillin a moderate rise in aspartate aminotransferase (AST) and/or alanine transaminase (ALT) has been noted in patients, but the significance of this change is not known. Hepatic dysfunction, including cholestatic jaundice, hepatic cholestasis and acute cytolytic hepatitis, has been reported (RxList , 2008). Interstitial nephritis resulting in oliguric renal failure has been reported in a small number of patients after overdose with amoxicillin. Renal impairment appears to be reversible with cessation of drug administration[4]. Glomerulonephritis caused by drug allergy is usually observed as part of a serum sickness. Deposition of antigen–antibody complexes occurs non-specifically along the glomeruli (Haddad, 1990). Effects on the central nervous system, such as reversible hyperactivity, agitation, anxiety, insomnia, confusion, convulsions, behavioural changes, and/or dizziness have been reported, but are rare (RxList, 2008). A prospective study of 51 paediatric patients at a poison-control centre suggested that overdoses of less than 250 mg/kg of amoxicillin are not associated with significant clinical symptoms (RxList, 2008). Amoxicillin administered to pregnant women had no reported adverse effects on the foetus. According to the USCDC recommendations, the use of amoxicillin in pregnancy for those with chlamydia infections is allowed (McEvoy, 1995). A population-based study conducted by Jepsen *et al*. (2003) also reported no effect on birth weight, preterm delivery, congenital malformation or spontaneous abortion following amoxicillin use in pregnant women. A controlled study (nursing mothers invited prior to administration of amoxicillin/clavulanic acid or cefuroxime) was undertaken to examine the effect of amoxicillin/clavulanic acid or cefuroxime on infants, via nursing

	mothers receiving treatment. Information is not available for the route of administration or the dose. Out of 156 infants, 16 infants had 1 adverse effect and 3 infants had more than 2 adverse effects. No severe adverse events were observed. Symptoms of the adverse effects included; constipation, rashes, diarrhoea, irritability and elevated liver enzymes (in one case only). Following cessation infant's symptoms disappeared (Benyamini *et al.*, 2005).
Primary mode of endocrine activity	None identified.
Study estimate of anticipated exposure via drinking water	Limited intake via drinking water is predicted to occur in all age groups following conventional treatment processes. Predicted intakes following advanced treatment processes are predicted to be zero in all age groups. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.039; Toddler 0.026; Adult 0.0086.
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	No evidence to either prove or disprove endocrine disrupting activity per se has been identified for this substance. Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes and conservative assumptions is 0.039 µg/kg bw/d for infants, this would suggest a margin of safety in excess of 10,000 times against the ADI. These figures were based on modelled levels in drinking water calculated with usage figures obtained from UK prescription data, undergoing conventional water treatment; as such they are conservative in nature. However given that the predicted margin of safety was so large, that predicted intakes following advanced water treatment were reduced to zero and that there is currently no evidence to support ED activity of amoxicillin, the pharmaceutical is not considered of concern for further evaluation here.
References:	Benyamini, L., Merlob, P., Stahl, B., *et al.* (2005) The Safety of amoxicillin/clavulanic Acid and Cefuroxime during Lactation. *Therapeutic Drug Monitoring*, 27(4), 499-502. Campbell, A.C.; McElnay, J.C.; Passmore, C.M. (1991) The excretion of ampicillin in breast milk and its effect on the suckling infant. *British Journal of Clinical Pharmacology*, 31:230p. [Abstract] ChemID Plus *Amoxicillin*. Available at: http://chem.sis.nlm.nih.gov/chemidplus [Accessed Dec 2011]. Department of Health and Ageing. Australian Government (2005) *ADI List: Acceptable Daily Intakes for Agricultural and Veterinary Chemicals.* Available at: http://www.health.gov.au/internet/main/publishing.nsf/Content/E8F4D2F95D616584CA2573D700770C2A/$File/ADI%20Report%20-%20Dec%202008.pdf. EMEA (2008) *Penicillins: Summary Report.* Available at: http://www.emea.europa.eu/pdfs/vet/mrls/penicillins.pdf. EMEA (2006a) *Amoxicillin (Extension to Eggs).* EMEA/CVMP/330177/2006. Available at: http://www.emea.europa.eu/pdfs/vet/mrls/mrlopinions/33017706en.pdf. EMEA (2006b) *Press Release Committee for Medicinal Products for Veterinary use Meeting of 12 to 14 September 2006.* EMEA/CVMP/323417/2006. Available at: http://www.emea.europa.eu/pdfs/vet/press/pr/32341706.pdf.

	Haddad, L.M. (1990) *Clinical Management of Poisoning and Drug Overdose*. 2nd ed. Philadelphia, PA, W.B. Saunders Co. , cited in HSDB (2003). HSDB (2003) *Amoxicillin*. Available at: http://toxnet.nlm.nih.gov [Accessed Dec, 2011]. Ito S, Blajchman A, Stephenson M et al. Prospective follow-up of adverse reactions in breast-fed infants exposed to maternal medication. Am J Obstet Gynecol. 1993;168:1393-9 [Abstract] IPCS (1991) *Benzylpencillin (Toxicological Evaluation of Certain Veterinary Drug Residues in Food).* WHO Food Additives Series 27. Available at: http://www.inchem.org/documents/jecfa/jecmono/v27je01.htm. Kafetzis, D.A., Siafas, C.A., Georgakopoulos, P.A., *et a*l (1981) Passage of Cephalosporins and Amoxicillin into the Breast Milk. *Acta Paediatrica Scandinavica*, 70(3), 285-288. [Abstract]. McEvoy, G.K. (ed.)(1995) *American Hospital Formulary Service*.American Society of Hospital Pharmacists, Inc, Bethesda MD. p. 920, cited in HSDB (2003) pp. RxList (2008) *AMOXIL® (Amoxicillin) Drug Informatio*n. Available at: http://www.rxlist.com/amoxicillin-drug.htm[accessed Dec2011]. US Pharmacopeia (2007) *Amoxicillin and Clavulanate: Veterinary Systemi*c. Available at: http://www.usp.org/pdf/EN/veterinary/amoxicillinAndClavulanate.pdf [Accessed Dec 2011].

Substance name:	**Androsterone** 
CAS No.:	53-41-8
Synonym(s):	3a-hydroxy-5a-androstan-17-one; 3alpha-hydroxy-5alpha-androstan-17-one; 3-alpha-hydroxyetioallocholan-17-one; 5a-androstan-3a-ol-17-one; 5alpha-androstan-3alpha-ol-17-one; 5alpha-androstan-3alpha-ol-17-one-16,16-d2; androstan-3a-ol-17-one; androsterine; androsterolone; androsterone cis-androsterone; 3-alpha-hydroxy-17-androstanone; 3alpha-Hydroxy-17-androstanone; 3-alpha-hydroxy-5-alpha-androstan-17-on; 3-Epihydroxyetioallocholan-17-one; 3-hydroxy-,(3-alpha,5-alpha)-androstan-17-on; 3-hydroxy-,(3alpha,5alpha)-androstan-17-on; 5alpha-Androstan-17-one, 3alpha-hydroxy; 5alpha-Androstane-3alpha-ol-17-one; 5alpha-Androsterone
Use and potential human exposure routes:	Androsterone is an hormonally-inactive, naturally-occurring metabolite of testosterone and other steroid hormones, that generally occurs in and is excreted by humans (males) in conjugated form. However it may be converted to the highly androgenic dihydrotestosterone (Wishart et al., 2007). Medical applications of the substance have been investigated (e.g. Carson et al, 1966). It is also available commercially and may be used by bodybuilders, despite warnings from regulatory agencies of the dangers associated with products containing this and similar substances (USFDA, 2009). It is also suggested as a supposed human pheromone attractant (e.g. see BodyBuilding Factory.com, undated; Pheromones, 2010) and as a deer repellent (PAN, 2011; DeYoe & Schaap, 1987)
Established/Study-specific exposure limit:	No established exposure limits have been identified for Androsterone. A highly precautionary study-specific exposure limit of 25 µg/kg/d is therefore proposed, based on the study by Greene et al (1939, see below)
Basis for exposure limit	Greene et al (1939) report adverse developmental changes in rats at a dosage of 250 mg/kg/d in rats but failed to define a NOAEL. Given this, it is proposed to apply an uncertainty Factor of 10,000 (100 for lack of a NOAEL or any information on dose response, and 10 each for intra- and inter-species variability)
Evidence for human relevant endocrine disrupting potential:	Androsterone was considered by IUPAC (Lintelmann et al, 2003) but not in the EU prioritisation exercise, and there is little evidence of the direct endocrine disrupting potential of this substance. Given that the substance may be converted to dihydrotestosterone, it can be anticipated that the toxicity profile will reflect that of this and other similar androgens. However, the only identified in vivo study on the reproductive and developmental toxicity of androsterone is the finding of masculinization of external genitalia and Wolffian duct persistence in female foetuses of pregnant rats given 250-800 mg androsterone during the later gestational

	period (Greene et al, 1939). However, no further details are available to establish the robustness of the study or to allow determination of a NOAEL. It is however of note that testosterone does not influence levels of sperm abnormalities in mice (IARC, 1974, 1979 and 1987), so effects on male fertility appear unlikely
Endocrine disrupting activities of concern:	Androgenicity (following metabolic activation)
Other significant toxic effects:	Androsterone is not classified in Annex I of Directive 67/548/EEC. Androsterone is an inactive metabolite of the principle natural male hormone, testosterone. It is also formed as a metabolite of adrenal androgens (e.g. dehydroepiandrosterone, dihydrotestosterone and androstenedione). Humans and other primates have a high level of circulating conjugated glucuronide and in the human prostate. If deconjugated, androsterone may be converted back to an active form, dihydrotestosterone (Wishart et al., 2007) Hence, androsterone's toxic profile would be anticipated to reflect that of this active form. Dihydrotestosterone has low acute toxicity (oral LD_{50} - rat = 7060 mg/kg; mouse = 3450 mg/kg; DrugBank, 2006). Also, dihydrotestosterone, as an androgenic (anabolic) steroid, is considered *probably carcinogenic to humans (Group 2A)* by IARC. For this group of steroids, associations with increased risk of various benign and malignant liver tumours and prostate cancer has been noted in humans while, in animals, a range of tumour types (including cervical-uterine, prostatic and other genital tract and mammary tumours) has been reported following treatment with various androgens. However testosterone is not considered mutagenic per se (IARC, 1974, 1979 and 1987). A range of other adverse side effects have been associated with very high exposure to anabolic steroids. These include, in males, shrinkage of testes, decreased sperm count, baldness, and gynecomastia while, in females, effects may include masculinisation (reduction in body fat and breast size, swelling of clitoris, deepening of voice, development of facial and body hair). Other, non-sex specific effects reported include increased risk of cardiovascular events, impaired immune-response, liver failure and psychiatric/psychological disturbance (MedicineNet.com, undated)
Study estimate of anticipated exposure via drinking water	Intakes (µg/kg/d) from drinking water from advanced treatment process: Infant 0.13; Toddler 0.009; Adult 0.003. Intakes (µg/kg/d) based on conventional treatment: Infant 0.081; Toddler 0.054; Adult 0.018
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	A highly precautionary, study-specific exposure limit of 25 µg/kg/d has been suggested for this substance based on a reproductive-relevant endpoint and an uncertainty factor of 10,000. Given that the highest intake via drinking water predicted is 0.018 µg/kg/d in the case of infants exposed to a conventional treatment system, this would give a Margin of Safety of in excess of a 100-fold. Hence, the 'worst case' predicted exposure is considered of no concern.
References	BodyBuilding Factory.com (undated) Available at Internet site http://store.bodybuildingfactory.com/sports-one-3-alpha-60c.html. Carson P et al. (1966) Effects of Clofibrate with Androsterone (Atromid) and without Androsterone (Atromid-S) on Blood Platelets and Lipids in Ischemic

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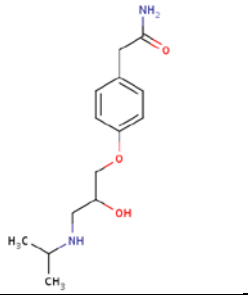
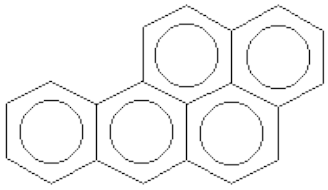
Hazard Assessment

Substance name:	**Atenolol** 
CAS No.:	29122-68-7
Synonym(s):	Aircrit; Alinor; Altol; Anselol; Antipressan; Atenil; Betablock; Betasyn; Blocotenol; Cardaxen; Corotenol; Cuxanorm; Duraatenolol; Duratenol; Evitocor; Farnormin; Hypoten; Internolol; Lotenal; Myocord; Normalol; Ormidol
Use and potential human exposure routes:	The cardioselective β_1-selective βrenergic receptor blocker, Atenolol, is used for hypertension management and the long-term management of patients with angina pectoris. Literature reporting atenolol levels in water sources is fairly abundant. In the UK, Kasprzyk-Horden et al (2007) reported concentrations of atenolol in the River Taff, in Wales, of between 3 – 60 ng/L, and of atenolol in the River Ely, also in Wales, of up to 510 ng/L (Kasprzyk-Horden et al, 2009). Occupational exposure to atenolol may occur through inhalation of dust and dermal contact with this compound at workplaces where atenolol is produced. The general population may be exposed via the oral route to atenolol through medical administration of this compound.
Established/Study-specific exposure limit:	**Therapeutic doses by age range:** <1 month: 0.5 mg/ kg (0.5 – 2 mg/ kg bw/d range) 1 month to 12 years: 0.5 mg/ kg (0.5 – 2 mg/ kg/ bw/d range) 12 to 18 years: 25 mg/day (20 – 50 mg/d range) >18 years: 25 mg/day (25 – 100 mg/d range) **Study-specific Exposure Limit** = 5 µg/ kg bw/d Assuming an uncertainty factor of 100 from the minimum human therapeutic dose, or an uncertainty factor of 1000 from the developmental LOAEL of 5 mg/ kg bw/d.
Basis for exposure limit:	LOAEL for developmental and neurobehavioural effects in the rat of 5 and 7.5 mg/ kg bw/d atenolol, respectively. Exposure to atenolol was shown to be selectively embryotoxic in rat, as developmental effects are induced at doses below those causing maternal toxicity (>2000 mg/kg bw/d). An uncertainty factor of 1000 was applied (Tabacova *et al*., 2003).
Evidence for human relevant endocrine disrupting potential:	Patients taking β-blockers have reported sexual dysfunction. A number of endocrine relevant *in vivo* endpoints have been reported in laboratory models (Khan *et al*., 2004). Increased embryo-foetal death (resorptions) was observed in rabbits administered 25 mg/ kg bw/d by i.p. injection (LOAEL). Placental weight changes and marked decreases in umbilical cord length were also observed following i.p. doses of 1 mg/ kg bw/d atenolol. Atenolol (9 and 18 mg/kg bw) was shown to affect both total and free testosterone levels in rat s, inducing a significant decrease in progressive

	motility of sperm, a significant increase in sperm heard and tail abnormalities and histopathological alterations in testis, epiditymis and seminal vesicles (Rosen *et al.*, 1988; el Sayed *et al*., 1998). Atenolol was shown to significantly ($p<0.05$) reduce rat Leydig cell testosterone release *in vitro* in a dose dependent manner. Mechanistic studies suggest that atenolol inhibits testosterone release by decreasing production of cAMP, rather than steroidogenic enzymes (Khan *et al*., 2004)
Other significant toxic effects:	Acute oral toxicity is low (Mice LD_{50} = 2000 – 3000 mg/ kg). Symptoms of atenolol overdose include a slow heart beat, shortness of breath, fainting, dizziness, weakness, confusion, nausea and vomiting.
Primary mode of endocrine activity	**Oestrogenic** Both the YES-test and E-screen assay have identified atenolol to be a weak oestrogen agonist (EC50 value of 40.74 mg/L and 5.43 mg/ L, respectively). The E-screen assay monitors cell proliferation of human oestrogen-receptor positive MCF-7 breast cancer cells, and believed to be the most useful evaluation of human oestrogenic activity in vitro, while the YES-test uses recombinant yeasts to testing for ERα binding (Isidori et al., 2009).
Study estimate of anticipated exposure via drinking water	Limited intake via drinking water is predicted to occur in all age groups following conventional treatment processes and in infants following advanced treatment processes. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.056; Toddler 0.037; Adult 0.012. Intakes (ug/kg bw/d) based on advanced treatment: Infant 0.001.
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	There is evidence to suggest that atenolol has oestrogenic activity, highlighting its potential capacity as an endocrine disrupter, however, daily intakes estimated in this project are well below both the s-TDI and therapeutic doses in infants, toddlers and adults. Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes and conservative assumptions is 0.056 µg/kg bw/d for infants, this would suggest a margin of safety of around 90 times against both the s-TDI and TD for reproductive and developmental endpoints for the substance.
References:	Isidori, M., Bellotta, M., Cangiano, M., Parrella, A. (2009) Estrogenic activity of pharmaceuticals in the aquatic environment. *Environment International* **35:** 826 – 829. Kasprzyk-Hordern, B., Dinsdale, M., Guwy, A.J. (2007) Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra performance liquid chromatography–positive electrospray ionisation tandem mass spectrometry *Journal of Chromatography A* **1161** :132 – 145. Kasprzyk-Hordern, B., Dinsdale, M., Guwy, A.J. (2009) The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. *Water Research* **43** : 363–380. Khan, U. A., Aslam, M., Saeed, S. A. (2004) Effect of beta adrenergic antagonist on the production of testosterone by rats Leydig cells. *JAMC* **16** (4): 26 – 28. el-Sayed, M. G., el-Sayed, M. T., Elazab, A. el S., Hafeiz, M. H., el-Komy, A. A., Hassan, E. (1998) Effects of some beta-adrenergic blockers on male

	fertility parameters in rats. Dtsch Tierarztl Wochenschr 105 (1): 10 – 12. Tabacova, S., Kimmel., C. A., Wall, K., Hansen, D. (2003) Atenolol developmental toxicity: animal-to-human comparisons. *Birth Defects Research (Part A)* **67**: 181 – 192.

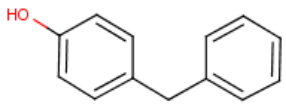
Hazard Assessment

Substance name:	**Benzo(a)pyrene (BaP)** 
CAS No.:	50-32-8
Synonym(s):	1,2-Benzpyrene; 3,4-Benzopyrene; 3,4-Benzpyrene; 3,4-Benz[a]pyrene; 6,7-Benzopyrene; Benzo[α]pyrene; Benzo[d,e,f]chrysene; B(a)P; BP; 3,4-Benzopirene; 3,4-Benzpyren; 3,4-BP; Rcra waste number U022; Benzopyrene
Use and potential human exposure routes:	BaP is not used in significant amounts by industry. The largest source of emissions of BaP and other polycyclic aromatic hydrocarbons (PAHs) is the incomplete combustion of organic substances in industrial processing and by combustion engines, as well as several other human activities. However, BaP in the environment is subject to photo- and bio-degradation by microorganisms and metabolism in higher biota (WHO, 1998). The main non-occupational exposures are via ambient air, smoke from open fireplaces, cooking, ETS and contaminated food and potentially drinking-water. Levels of PAHs in uncontaminated groundwater supplies and drinking-water are generally very low (0.1-1 ng/l) (WHO, 1998)
Established/Study-specific exposure limit:	UK Drinking Water Standard = 0.01 µg/L (HPA, 2008). WHO-Europe Ambient Air Quality Guideline = 1.2, 0.12 and 0.012 ng/m^3 for lifetime cancer risks of 1:10000, 1: 100000 and 1:1000000 respectively (HPA, 2008). US EPA maximum contaminant level (MCL) in drinking water = 200 ng/l (USEPA, 2011)
Basis for exposure limit	Carcinogenic activity of BaP (HPA, 2008).
Evidence for human relevant endocrine disrupting potential:	Limited evidence for anti-oestrogenic activity reported for BaP (Chaloupks et al, 1992) and basis for consideration as an endocrine disrupter discussed by Keith (1998). Most evidence indicates that BaP is a high-affinity ligand for AhR which may lead to cross-talk with steroid receptors, an effect which may be considered as adaptive, toxic and/or a perturbation of endogenous pathways (IARC, 2010). BaP is also an established animal reprotoxin (reduced fertility and gonadal effects) and also embryotoxic. Following in utero exposure, offspring may suffer adverse postnatal consequences such as increased tumours, immunological suppression and reduced fertility
Endocrine disrupting activities of concern:	AhR ligand (IARC, 2010). Possible anti-oestrogenic activity (Lintelmann et al, 2003).
Other significant toxic effects:	BaP is readily absorbed via inhalation, the GI-tract and skin. Once absorbed it is widely systemically available, preferentially occurring in lipid-rich compartments. PAHs can cross the placenta and have been detected in fetal tissues. Metabolic transformation of BaP is complex involving CYP-dependent mono-oxygenase reactions leading to epoxide formation and

	subsequent conjugation to form excretory products (e.g. glucuronides); toxic effects are believed to be mediated by electrophilic intermediates (WHO, 1998). Acute toxicity is moderate to low (LD_{50} in mice >1600 mg/kg). However, repeated dermal exposure may cause hyperkeratosis and may induce contact hypersensitivity in guinea-pigs and mice. BaP may also be phototoxic (WHO, 1998; HPA, 2008). BaP is also an immunotoxin affecting a range of immune organs and the blood. It also results in increased liver weight in non-responsive strains of mice at high doses (IARC, 2010; WHO, 1998). BaP is mutagenic in a range of in vitro test assays and a recognised genotoxin. It is carcinogenic in a range of species when administered by various routes and has been classified as a human carcinogen (Group 1; IARC 2010). BaP was classified under the EU Existing Substances Directive as: Carc Cat 2; Muta Cat 2; Repro Cat 2, R43, N, R45, R 46, R60, R61, R43, R50/53 (HPA, 2008)
Study estimate of anticipated exposure via drinking water	No intake is predicted from drinking water from the advanced treatment process. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.053; Toddler 0.035; Adult 0.012
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	The highest predicted drinking water level of BaP (i.e. 0.053 µg/L), based on drinking water from a conventional treatment system, is noted to be somewhat above the established regulatory limit (0.01 µg/L) for this pollutant in the UK. However, whilst this would raise concern, available monitoring data provides clear evidence that the regulatory limit is in actuality being adhered to across the UK (e.g., in 2010 only 4 exceedences were noted in England and Wales and each was attributable to site-specific transitory failure associated with recent repair/replacement works; DWI, 2011). This evidence indicates that the predicted levels derived in this study reflect inherently precautionary assumptions that are built into the environmental models employed. Overall, therefore, it is considered that risks from exposure to BaP via drinking water are not a concern in the UK. Furthermore, although BaP is a AhR ligand and may possess some anti-oestrogenic activity, the principal regulatory concern (on which the established limit was based) is its carcinogenicity. Together with its mutagenic and reproductive/developmental properties, this has meant that it is regulated as a 'CMR' and, hence, highly protective limits on exposure were established. This should provide further reassurance that any potential endocrine-related concerns are adequately addressed
References	ATSDR (1995) Toxicological Profile for Polycyclic Aromatic Hydrocarbons. Agency for Toxic SUBSTANCES AND Disease Registry, US Department of Health and Human Services. Available at Internet site http://www.atsdr.cdc.gov/ToxProfiles/tp69.pdf. Chaloupka K et al. (1992) Carcinogenesis, 13, 2233-2239, as cited in Lintelmann et al., 2003. DWI (2011) Drinking Water 2010. A report by the Chief Inspector of Drinking Water. Drinking Water Inspectorate, UK. Available at Internet site http://dwi.defra.gov.uk/about/annual-report/2010/index.htm.

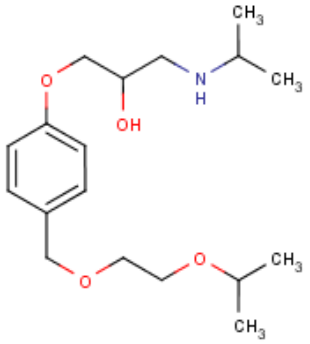
	HPA (2008) Polycyclic aromatic hydrocarbons (Benzo[a]pyrene). Health Protection Agency, UK. Available at Internet site http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1227169968068. IARC (2010) IARC Monographs on the Evaluation of Carconogenic Risks to Humans Volume 92. Some non-heterocyclic polyclic aromatic hydrocarbons and some related exposures. World Health Organisation, International Agency for Research on Cancer, Lyon, France. Available at Internet site http://monographs.iarc.fr/ENG/Monographs/vol92/mono92.pdf. Keith L.H. (1998) Environmental endocrine disruptors. Pure & Appl. Chem., 12, 2319-2326. Lintelmann J. et al. (2003) Endocrine Disruptors in the Environment (IUPAC Technical Report). Pure Appl. Chem, 75, 631-681. USEPA (2011). Basic Information about bezo(a) pyrene in drinking water. United States Environmental Protection Agency. Available at Internet siet http://water.epa.gov/drink/contaminants/basicinformation/benzo-a-pyrene.cfm. WHO (1998) Environmental Health Criteria 202. Selected Non-hetrocyclic Polycyclic Aromatic Hydrocarbons. United Nations Environment Programme, International Labour Oranisation and, World Health Organisation International Programme on Chemical Safety. Available at Internet site http://www.inchem.org/documents/ehc/ehc/ehc202.htm.

Hazard Assessment

Substance name:	**p-Benzylphenol** 
CAS No.:	101-53-1
Synonym(s):	4-(Phenylmethyl)phenol; 4-Benzylphenol; 4-Hydroxyphenylmethane; 4-Hydroxyditane; Fesiasept; Parabencifenilcarbamato; alpha-Phenyl-p-cresol
Use and potential human exposure routes:	4-Benzylphenol is used in plastics as a germicide, antiseptic and a preservative. Due to these uses, it may occur in food packaging and consumer goods (EC 2002). 4-benzylphenol is moderately soluble and bioaccumulative and may therefore occur in the environment (EC, 2002). Oral exposure is generally the main human exposure pathway though dermal exposure may occur in workers (EC 2002)
Established/Study-specific exposure limit:	Study-specific exposure limit = 0.004 mg/kg bw/d. No current regulatory environmental or occupational exposure limit were identified for the EU or US, nor any guidance from the WHO regarding exposure.
Basis for exposure limit:	An endocrine-relevant endpoint, uterine toxicity, was used to derive a study-specific exposure limit. This was based on a NOAEL of 40 mg/kg bw/d for increased uterine weight in rats (Yamasaki *et al*., 2003a). An uncertainty factor of 1000 was applied to allow for inter-species differences and the weak toxicity endpoint defined.
Evidence for human relevant endocrine disrupting potential:	In a uterotrophic assay using Crj:CD (SD) rats given 200 or 800 mg/kg bw/d of 4-benzylphenol, a significant increase in uterine blotted weight was found. In addition, at 200 mg/kg bw/d, in combination with ethynyloestradiol (EE2), uterine weight was decreased suggesting that 4-benzylphenol had oestrogen agonist properties but may also act antagonistically to EE2. Decreased body weight gain was also noted at 600 mg/kg bw/d (Yamasaki *et al*., 2003a; Yamasaki *et al.*, 2003b). In a Hershberger assay, male Crj:CD (SD) rats given 50 or 200 mg/kg bw/d showed increased bodyweight-relative weight of the gland penis. In combined exposure at 50 mg/kg bw/d with testosterone propionate (0.2 mg/kg bw/d) bodyweight-relative seminal vesicle weights were increased though no significant change occurred with 4-benzylphenol alone (Yamasaki *et al.*, 2003a). The results suggest that 4-benzylphenol affects or interacts with the androgenic effects of testosterone, but does not hold any androgenicity itself; however 4-benzylphenol does have the potential to interact with the oestrogen receptor. 4-Benzylphenol is an oestrogen receptor-α (ER-α) agonist using a reporter gene assay; Agonist PC_{10} = 1198024 (Yamasaki *et al.*, 2003b).
Endocrine disrupting activities of concern:	**Oestrogen**
Other significant toxic effects:	Little other data on the toxicity profile of 4-benzylphenol was identified during the literature review.

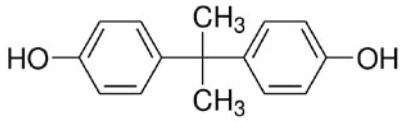
	Acute oral toxicity is, however, low (LD_{50}mouse = 20,000 mg/kg bw; Sigma Aldrich, 2011).
Study estimate of anticipated exposure via drinking water	Potential intake via drinking water is predicted with both conventional and advanced treatment processes in all age groups considered. Intakes (µg/kg bw/d) based on conventional treatment: Infant 3.750; Toddler 2.500; Adult 0.833. Intakes (ug/kg bw/d) based on advanced treatment: Infant 0.010; Toddler 0.007; Adult 0.002
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	The study-specific exposure limit, based on data from a uterotrophic assay, is 0.004 mg/kg bw/d. Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes using highly conservative assumptions is 3.75 µg/kg bw/d, this would suggest no margin of safety for 4-benzylphenol. However, it should be noted that the large inherent uncertainty factor of 1000 that was built into the study-specific exposure limit may have resulted in such a low overall margin of safety.
References*:*	EC (2002) European Commission DG ENV. Endocrine Disrupters: Study on Gathering Information on 435 Substances with Insufficient Data. Final Report EU DG ENVIRONMENT B4-3040/2001/325850/MAR/C2. Sigma Aldrich (2011) Material Safety Data Sheet (MSDS) 4-benzylphenol: http://www.sigmaaldrich.com/catalog/DisplayMSDSContent.do [Accessed 25.08.2011 12.56pm]. Yamasaki, K., Takeyoshi, M., Sawaki, M., Imatanaka, N., Shinoda, K., Takatsuki, M. (2003a) Immature rat uterotrophic assay of 18 chemicals and Hershberger assay of 30 chemicals. *Toxicology* **183**: 93 – 115. Yamasaki, K., Takeyoshi, M., Yakabe, Y., Sawaki, M., Takatsuki, M. (2003b) Comparison of the reporter gene assay for ER-alpha antagonists with the immature rat uterorophic assay of 10 chemicals. *Toxicology Letters* **142**: 119 – 131.

Hazard Assessment

Substance name:	Bisoprolol 
CAS No.:	66722-44-9
Synonym(s):	(+-)-1-((alpha-(2-isopropoxyethoxy)-p-tolyl)oxy)-3-(isopropylamino)-2-propanol; (RS)-1-(4-(2-Isopropoxyethoxymethyl)phenoxy)-3-(isopropylamino)-2-propanol; Bisocor; Bisoprolol; Bisoprolol; Bisoprololum
Use and potential human exposure routes:	Bisoprolol is a cardioselective β-blocker that competitively inhibits β1 adrenergic receptors, used in the management of hypertension and angina pectoris. Meta-analyses of randomised trials indicated a reduction in mortality in the Chronic Heart Failure (CHF) patients receiving beta-blockers, leading to the standard use of β-blockers in CHF treatment (Merck, 2001). Literature reporting bisoprolol levels in UK water sources is sparse. In Germany, Ternes (1998) reported bisoprolol in river water at concentrations up to 2.9 µg/L. Occupational exposure to bisoprolol may occur through inhalation of dust and dermal contact with this compound at workplaces where bisoprolol is produced. The general population may be exposed via the oral route to bisoprolol through medical administration of this compound.
Established/Study-specific exposure limit:	**Therapeutic doses by age range:** <1month : not used 1 month to 12 years : not used 12 to 18 years : not used > 18 years : 1.25 mg/day (1.25 – 10 mg/day) **Study-specific Exposure Limit**= 2.5 µg/kg/day.
Basis for exposure limit:	Assuming an uncertainty factor of 100 from the minimum human therapeutic dose (1.25 mg/ day) for a 5 kg infant.
Evidence for human relevant endocrine disrupting potential:	Using a precautionary approach, this substance was identified as of possible concern following expert advice from the clinician within the project team. However, no evidence suggestive of endocrine disrupting activity per se was identified for bisoprolol in subsequent literature review.
Other significant toxic effects:	Bisoprolol has an oral LD_{50} of 1116 mg/kg for the rat and 734 mg/kg for the mouse. Following intravenous administration LD_{50} values of 127 (mouse), 53 (rat) and 24 (dog) mg/ kg were found, which were considerably lower than via the oral route. No toxic effects were detected in rats following daily oral administration of 15, 50 and 150 mg/kg bisoprolol for 6 months. In an extended study, rats

	tolerated daily treatment with 25 mg/kg for 12 months with no adverse effects, however daily administration of 75 mg/kg led to a slight reduction in body weight gain. In a 12-month study in beagles, daily doses of 3, 10 and 30 mg/ kg were tolerated (Merck, 2001). Bisoprolol had no effect on fertility or general reproductive performance in rat and rabbit studies at daily doses of 1.0, 2.5 and 6.25 mg/ kg and 15.0 and 40.0 mg/kg, respectively. However, at doses above these, bisoprolol was seen to be embryolethal via a non-teratogenic mechanism (Merck, 2001). Bisoprolol is not considered to be carcinogenic.
Primary mode of endocrine activity	None Identified
Study estimate of anticipated exposure via drinking water	Limited intake via drinking water is predicted to occur in all age groups following conventional treatment processes and in infants and toddlers following advanced treatment processes. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.027; Toddler 0.018; Adult 0.006. Intakes (ug/kg bw/d) based on advanced treatment: Infant 0.001; Toddler 0.001.
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	No evidence to either prove or disprove endocrine disrupting activity per se has been identified for this substance. Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes and conservative assumptions is 0.25 µg/kg bw/d for infants, this would suggest a margin of safety of around 9 times against the study-specific exposure limit. These figures were based on modelled levels in drinking water calculated with usage figures obtained from UK prescription data, undergoing conventional water treatment; as such they are conservative in nature. It should be noted that margins of safety following advanced water treatment were significantly increased to a minimum of 250 times (for infants). As there is currently no evidence to support ED activity of bisoprolol, the pharmaceutical is not considered of concern under the specific remit of this project, and will not be assessed further.
References*:*	Merck (2001) Merck Cardio-vascular. Bisoprolol: Cardioselective Beta-blocker. February 2001. Available at http://www.bisoprolol-slides.info/homesite/teaser.pdf Ternes, T. (1998) Occurrence of drugs in German sewage treatment plants and rivers. *Wat. Res.* **32**: 3245 – 3260.

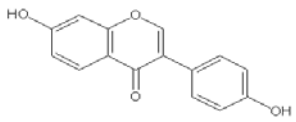
Hazard Assessment

Substance name:	**Bisphenol A (BPA)** 
CAS No.:	80-05-7
Synonym(s):	2,2-Bis(4-hydroxyphenyl)propane; Bis(4-hydroxyphenyl)dimethyl methane; 4,4'-Dihydroxydiphenyl propane; 4,4;-Dihydroxy-2,2-diphenyl propane; Diphenylolpropane; 4,4'-Isopropylidenediphenol
Use and potential human exposure routes:	Used mainly as a monomer in manufacture of polycarbonate and epoxy resins for food contact materials and consumer products such as tableware, reusable drinking bottles and infant feeding bottles (EU, 2010). Oral exposure is generally the main exposure pathway though dermal exposure may occur in workers (EU, 2010). General levels of consumer exposure estimated at 1.45 µg/kg bw/d from food and drink and 0.0091 µg/kg bw/d from other (indirect) sources (EU, 2010). BPA is permitted for use in food contact plastics in the European Union, subject to a specific migration limit of 0.6 mg/kg food (Commission Directive 2002/72/EC) and a TDI has been established (EFSA, 2010)
Established/Study-specific exposure limit:	Oral TDI = 0.05 mg/kg bw/d (EFSA, 2010). Occupational exposure limit for inhalation: 5mg/m^3 TWA in several EU Member states; EU Indicative OELV proposed at 10 mg/m^3 TWA (EU, 2010)
Basis for exposure limit:	NOAEL of 5 mg/kg bw/d from a rat oral 3-generation study (based on adult bodyweight effects and pup body and organ weight effects; Tyl et al.,2002) and a mouse 2 generation study (based on liver effects; Tyl et al., 2007). An uncertainty factor of 100 was applied (EFSA, 2010). A minority opinion by one member suggested that the TDI should be regarded as temporary because of concerns regarding developmental neurotoxicity findings in some non-regulatory compliant studies (EFSA, 2010)
Evidence for human relevant endocrine disrupting potential:	No robust epidemiological studies on the reproductive or developmental effects of BPA have been identified (EU, 2010). Unconjugated BPA is endocrine-active in a number of *in vitro* and *in vivo* screening assays; potency is 3-5 orders of magnitude less than oestradiol (EU, 2010). Effects on fertility (reduced litter size) noted at 500 mg/kg bw/d, but not 50 mg/kg bw/d, in a rat multigeneration study (effects possibly associated with maternal toxicity) and in a murine continuous breeding study at 600 mg/kg bw/d or greater. Other multigenerational studies of various designs in rats and mice at <600 mg/kg bw/d BPA have shown no similar effects. Overall NOAEL of 50 mg/kg bw/d established for fertility endpoints. No evidence of developmental effects noted in standard rat and mouse studies at <600 or <1000 mg/kg bw/d respectively (EU, 2010). However, some non-standard (investigational) neurodevelopment studies report subtle behavioural changes in female offspring (e.g. Ryan et al 2010) and in memory and

	learning functions (Stump et al., 2009). Overall a NOAEL of 50 mg/kg bw/d has been established for developmental toxicity (EU, 2010). Two non-standard studies (Jenkins et al, 2009; Betacourt et al 2010b) report a possible potentiation of mammary carcinogenesis in offspring of rats lactationally exposed to BPA.
Endocrine disrupting activities of concern:	Oestrogenic activity
Other significant toxic effects:	Studies in human volunteers and animals suggest that ingested BPA is generally rapidly and extensively absorbed and is subject to extensive first pass metabolism (conjugation via several routes) followed by urinary excretion, with a t½ of about 5 hours in humans. Acute oral toxicity is low (rodent LD50 >2000 mg/kg bw). BPA is an eye (but probably not) skin irritant and has low sensitizing potential. Significant repeat-dose oral toxicity is limited. Effects include reduced bodyweight at high doses and hepatic changes (particularly multinucleated giant hepatocytes in mice; NOEL not identified) considered of uncertain human significance, and renal weight change (EU, 2010)
Study estimate of anticipated exposure via drinking water	No intake via drinking water from advanced treatment process is predicted. Intakes (µg/kg/d) based on conventional treatment: Infant 0.289; Toddler 0.192; Adult 0.064
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	The lowest identified developmental NOAEL is 50 mg/kg bw/d, compared with the overall NOAEL of 5 mg/kg/day when all toxicity endpoints are considered (EFSA, 2010; EU, 2010). Thus, there is an uncertainty factor of a 1000 in relation to potentially endocrine-relevant effects of the substance when compared with the established oral TDI of 0.05 mg/kg bw/d (i.e. 5 µg/kg bw/d; EFSA, 2010). Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes and conservative assumptions is 0.289 µg/kg bw/d for infants, this would suggest a margin of safety of 17-times against the established overall TDI (and of > 170-times when compared with the experimental NOAEL for reproductive and developmental endpoints for the substance. Even when the estimated total intake of 1.45 µg/kg bw/d from both food and drink (EU, 2010) is compared with the TDI, this still gives a margin of safety of 3-times compared with the TDI or 34-times when compared to the known developmental NOAEL
References	Betacourt A.M. et al (2010) Environ. Health Perspect, published on line July 30th, as cited in EFSA (2010). Jenkins S. et al (2009) Environ. Health Perspect, 117, 910-915, as cited in EFSA (2010). EU (2010) European Union Risk Assessment Report 4,4'-Isopropylidenediphenol (Bisphenol-A) CAS Number 80-05-7. EINECS Number: 201-245-8.Risk Assessment. February 2010. Published by Office of Official Publications, European Commission, Luxemburg. Available at Internet site http://esis.jrc.ec.europa.eu/doc/existing-chemicals/risk_assessment/REPORT/bisphenolareport325.pdf EFSA (2010) Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on Bisphenol A: evaluation of a study investigating its neurodevelopmental toxicity, review of

	recent scientific literature on its toxicity and advice on the Danish risk assessment of Bisphenol A. *EFSA Journal* **8** (9): 1829. Available at Internet site http://www.efsa.europa.eu/fr/scdocs/doc/1829.pdf. Ryan B.C. et al (2010) Toxicological Sciences, 114, 133-148, as cited in EFSA (2010). Stump (2009) Study Report submitted by Polycarbonate/BPA Global Group Americal Chemistry Council, as cited in EFSA (2010). Tyl R.W. et al (2002) Toxicological Sciences, 68, 121-146, as cited in EFSA (2010). Tyl, R.W. et al. (2007) RTI International Centre for Life Sciences and Toxicology, Vol. 1-8, as cited in EFSA (2010)

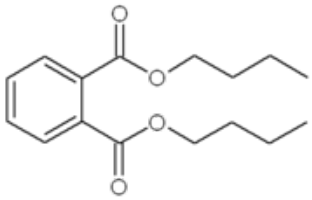
Hazard Assessment

Substance name:	**Daidzein** 
CAS No.:	486-66-8
Synonym(s):	4',7-Dihydroxyisoflavone; 7-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one; 4H-1-benzopyran-4-one, 7-hydroxy-3-(4-hydroxyphenyl)-; 7-Hydroxy-3-(4-hydroxy-phenyl)-chromen-4-one
Use and potential human exposure routes:	Daidzein is the aglycone (aglucon) form of daidzin and occurs naturally in plant material as the glycoside and as the glycosides 6"-O-malonylgenistin and 6"-O-acetyldaidzin. It is present mainly in legumes (such as soybeans and chickpeas) and hence also occurs at high levels in soya-based infant formula. Indeed, infants fed soy-based formula may have total plasma isoflavone levels about 13,000-22,000 times that of endogenous oestrogen levels (Rufer et al., 2008). Some adults may also take isoflavone suppliments because of its putative health benefits (BCERC COTC, 2007). Estimates of average dietary intakes are variable and depend on many factors, including dietary practice (e.g. veganism) and the phenotype of the individual and their intestinal flora. One estimate of normal dietary intake of daidzein (primarily as glycoside) is <0.3 mg/kg bw/d (Cayman Europe, undated) while FSA (2003) estimated the typical intake of the UK population to be <1 mg/person/d. Some workers (e.g. Ribeiro et al, 2009) have reported the presence of daidzein in European water bodies
Established/Study-specific exposure limit:	As a naturally-occurring constituent of some plant foods, no environmental limits or acceptable daily intakes have been considered to be appropriate
Basis for exposure limit	Not applicable
Evidence for human relevant endocrine disrupting potential:	Daidzein was recognised as a phytoestrogen by IUPAC (Lintelmann, 2003) but as a naturally-occurring substance was specifically excluded from consideration under the EU prioritisation exercise (BKH, 2000). Numerous studies have demonstrated in vivo that isoflavones including daidzein, are capable of binding to oestrogen receptors and can elicit weak oestrogenic activity. The oestrogenicity of daidzein has been estimated at approximately 10^{-3-} to 10^{-5}-times that of diethylstilbestrol (DES) in mice and, in a sheep uterine oestrogen receptor binding study, a binding affinity of 0.1% that of oestrogen was established (Zhange et al, 1999). In a comprehensive assessment, FSA (2003) indicated that the binding of daidzein to the ERα receptor was about 500 times less that of oestradiol but binding to ERβ was only 100 times less. When cell-based transcriptional assays were considered, relative potency was 2.4×10^{-6} to 1.4×10^{-4} that of oestradiol. However, under certain conditions, it may act as an anti-oestrogen (Miyaki, 2004).

	Although available, direct studies on the effects of phytoestrogens on human development and fertility are limited, often conflicting and not considered particularly robust. Furthermore, species differences between rodents, non-human primates and humans make inter-species extrapolation from experimental studies extremely difficult. Binding to sex-hormone binding globulin has also been reported but with a 1000-5000–fold lower affinity than that shown by oestradiol; this was not considered to be of significance to humans. Similarly, although in vitro inhibition of aromatase has been reported, levels required are not such as would be anticipated to occur in vivo. A wide range of other endocrine-related effects have been suggested, based largely on in vitro investigations, but are considered of unclear significance (FSA, 2003). Based on experimental studies, daidzein and some metabolites have been suggested to posses goitrogenic properties, at least under conditions of iodine insufficiency (possibly by inhibition of thyroid peroxidise) and there are a few historic reports of goiter in infants fed non-iodine supplemented soy-based formula (Doerge & Sheehan, 2002). Overall, it was concluded that - despite claims of both adverse and beneficial effects (ranging from impacts on the cardiovascular system, bone maintenance and cancer incidence) - the available data did not confirm any definitive effects of significance to human health (FSA, 2003)
Endocrine disrupting activities of concern:	Weak oestrogen and antioestrogen. Possible goitrogen (under specific conditions)
Other significant toxic effects:	Daidzein, as aglycone or glycone, is readily absorbed through the gastrointestinal tract, although bioavailability is higher when in the form of daidzin (4.5-times, with maximum systemic levels (C_{max}) being 6-times greater). The glucoside is not found in plasma, rather hydrolysis of the sugar moiety occurs by microbial or intestinal β-glucosidases before transport to the liver, with possible entero-hepatic circulation, before excretion of metabolites mainly via the urine (Rufer et al., 2008). Diadzein may be present in human milk with the maximum level of total isoflavone report of 32 ng/L for a vegan mother FSA(2003). As a constituent of plants considered appropriate for human nutrition, no hazard profile has been developed for daidzein other than in relation to its hormonal activity
Study estimate of anticipated exposure via drinking water	No intake is predicted from drinking water from advanced treatment process. Intakes (µg/kg/d) based on conventional treatment: Infant 0.096; Toddler 0.064; Adult 0.021
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	Because of its status as a natural plant constitutent, it has not been possible to either identify an authoritative exposure standard or to derive a meaningful study-specific value for this substance. Nonethless, the predictive drinking water intakes (given above) may be compared with estimates of the typical dietary intake by the UK population. Adopting the dietary intake estimate of 1 mg/person/d, would give a value of 0.017 mg/kg bw/d for an adult. Comparing this to the above estimate for adult drinking intake would indicate that dietary sources contributed much greater amounts. Hence, the estimated intakes from drinking water are considered not to be of particular concern

References	BCERC COTC (2007) Fact Sheet – Phytoestrogen Daidzein. Breast Cancer & the Environment Research Centers. Available at Internet site http://www.bcerc.org/COTCpubs/BCERC.FactSheet_Phytoestrogen_Daidzein.pdf. BKH (2000) Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption. Bkh consulting engineers. Available at Internet site http://ec.europa.eu/environment/endocrine/strategy/substances_en.htm. Cayman Europe (undated) 10005166 Daidzein. Cayman Europe. Available at Internet site http://www.caymaneurope.com/app/template/Product.vm/catalog/10005166/a/z. Doerge D.R. & Sheehan D.M. (2002) Goitrogenic and Estrogenic Activity of Say Isoflavones. Environ. Health Perspect., 110, 349–353. Available at Internet site http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1241182/pdf/ehp110s-000349.pdf). FSA (2003) COT Report - Phytoestrogens and Health. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, Food Standards Agency, UK. Available at Internet site http://www.food.gov.uk/multimedia/pdfs/phytoreport0503. Lintelmann J. et al. (2003) Endocrine Disruptors in the Environment (IUPAC Technical Report). Pure Appl. Chem, 75, 631-681. Miyaki K (2004) Novel approach for evaluation of estrogenic and anti-estrogenic activities of genistein and daidzein using B16 melanoma cells and dendricity assay. Pigment Cell Res, 17, 407-12. Ribeiro C. et al (2009) Spatial distribution and quantification of endocrine-disrupting chemicals in Sado River estuary, Portugal. Environmental Monitoring and Assessment, 159, 415-427. Rufer CE. et al (2008) Pharmacokineitcs of teh soybean isoflavone daidzein in its aglycone and glucoside formL: a rnadomised, double-blind, crossover study. American Hournal of Clinical Nutrition, 87, 1314-1423. Availabel at Internet site http://www.ajcn.org/content/87/5/1314.full. Zhange Y et al (1999) Daidzein and Genistein Glucuronides In Vitro Are Weakly Estrogenic and Activate Human Natural Killer Cells at Nutritionally Relevant Concentrations. Journal of Nutrition, 129, 399-405. Available at Internet site http://jn.nutrition.org/content/129/2/399.full

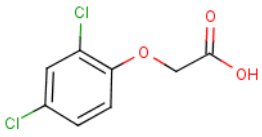
Hazard Assessment

Substance name:	**Di-Butylphthalate (DBP)** 
CAS No.:	84-74-2
Synonym(s):	Di-n-butylphthalate; 1,2-Benzenedicarboxylic acid; Dibutyl ester (9CI); Phthalic acid; Dibutyl ester (6CI, 8CI); Bis-n-butyl phthalate; Butyl phthalate; DBP; DBP (ester); Di(n-butyl) 1,2-benzenedicarboxylate; Dibutyl o-phthalate; n-Butyl phthalate; Phthalic acid di-n-butyl ester
Use and potential human exposure routes:	An estimated 76% of DBP manufactured is used as a polymer plasticiser, 14% in adhesives, 7% in printing inks and the remaining 3% for other applications (EFSA, 2005). Exposure to DBP may therefore arise from a large number of consumer products, such as food wrap and food packaging. Routes of exposure include via inhalation, dermal or dietary exposure. However, oral intakes are generally considered the most significant (EU, 2003). Total human intake via air, drinking water and food (EUSES) has been estimated at 3.59×10^{-4} mg/kg bw/d (EU, 2003) while an older estimate by MAFF (1996) suggested a mean UK DBP intake of 0.2 µg/kg based on a 60 kg adult. In a recent report, ECHA (2010) reviewed new scientific data on DBP, published since its use was restricted, to evaluate whether a re-examination of the existing restrictions was justified. ECHA reported that information available on the possible current uses and the extent of use of DBP in the EU was limited. However, as DBP is a phase-in substance under Reach Regulations, it is expected that one or more registration dossiers will be submitted to ECHA in due course, which will provide more comprehensive information on current levels of DBP use.
Established/Study-specific exposure limit:	TDI = 0.01 mg/kg bw/d (EFSA, 2005). A US Health-Based Recommended Occupational Exposure Level (HB-ROEL) of 5 mg/m^3 for respirable dust and 10 mg/m^3 for total inhalable dust. A TLV of 5 mg/m^3, was established by the ACGIH (EU, 2003).
Basis for exposure limit:	The TDI was based on effects on reproduction and development which were considered the most sensitive endpoints (EFSA, 2005). A dietary developmental study in the rat with animals fed diet containing DBP from gestation day 15 to postnatal day 21 showed developmental changes in germ cells and mammary glands at dose of 20 mg/kg feed (1.5 – 3.0 mg/kg bw/d) or above; since effects were reversible and NOAELs or LOAELs had been established in other reproductive toxicity studies, the TDI was based on a LOAEL of 20 mg/kg, applying an uncertainty factor of 200 (Lee *et al*., 2004).
Evidence for human relevant endocrine disrupting potential:	The oestrogenic activity of DBP *in vitro* in two human breast cancer cell lines (ZR-75 and MCF-7) was shown at at concentrations between 10-6 and 10-4 M. However, these figures could not be used to predict oestrogenic activity *in vivo* (Jobling *et al*., 1995).

	In humans, prenatal exposure to DBP (measured as MBP in urine) was inversely associated with anogenital distance in boys (Swan *et al*., 2005) while Hauser *et al.* (2006) found altered semen quality associations with urinary concentration of phthalate monoester- and oxidative-metabolites including a dose-related effect for mono-n-butylphthalate on sperm concentration and motility. In the by Lee *et al.* (2004) anogenital distance was significantly reduced in males fed 10000 mg/kg DBP, at PND 2. The incidence of retained nipples/areolas was slightly increased in all treated groups but only reached statistical significance at 1000 mg/kg. A dose-related decrease in numbers of spermatocytes was noted at20 mg/kg or above. Significant reductions in germ cell development occurred at 2000 mg/kg and above. Mahood *et al.* (2007) demonstrated infertility, cryptorchidism and indicators of foetal testis dysgenesis in rats at 20 mg/kg bw/d.
Other significant toxic effects:	Studies in human volunteers confirmed oral absorption of DBP While, in animals, DBP is rapidly absorbed and excreted following oral administration (>90% is excreted in urine rats or hamsters within 24-48h; faecal excretion is low at 1.0-8.2%). Acute oral toxicity in rodents is low: Oral LD_{50} in rats = 6,300 to 8,000 mg/kg bw, and in mice = 4,840 to 5,289 mg/kg bw. Based on available data , DBP is considered a non-genotoxin. No robust long-term toxicity or carcinogenicity studies on DBP are available (EU, 2003).
Primary mode of endocrine activity	Oestrogenic.
Study estimate of anticipated exposure via drinking water	Intake via drinking water is predicted to occur following both conventional and advanced treatment processes. Intakes (µg/kg/d) based on conventional treatment are: Infant 2.398; Toddler 1.599; Adult 0.533. Intakes (ug/kg/d) based on advanced treatment: Infant 0.052; Toddler 0.035; Adult 0.012
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	The TDI for DBP is 0.01 mg/kg bw/d, established based on an endocrine-relevant reproductive and developmental effects. Given that the 'worst case' predicted intake via drinking water using conservative assumptions if supplied via a conventional treatment processes is 2.398 µg/kg bw/d, this would suggest a margin of safety of around only 4.8 times for infants. If the estimated total intake of 0.36 µg/kg bw/d from food (EU 2003) is also taken into consideration (total 2.75 ug/kg bw/d) this reduces the margin of safety to 3.6 times. It should be noted that removal rates for DBP are higher under advanced treatment processes, bringing the margin of safety to around 200 times for infants; at this level there would be much less cause for concern.
References:	ECHA (2010) Evaluation of new scientific evidence concerning the restrictions contained in Annex Xvii to regulation (EC) No 1907/2006 (REACH): Review of new available information for dibutylphthalate (DBP). Available at: http://echa.europa.eu/doc/reach/.../dbp_echa_review_report_2010_6.pdf (accessed Dec 2011). EFSA (2005) Opinion of the Scientific Panel on Food Additives, Flavourings,

	Processing Aids and Material in Contact with Food (AFC) on a request from the Commission related to; Di-butylphthalate (DBP) for use in food contact materials. The EFSA Journal **242:** 1 – 7. EU (2003) European Union Risk Assessment Report. Institute for Health and Consumer Protection. European Chemical Bureau: Existing Substances: Dibutyl Phthalate. Vol. 29. Hauser, R., Meeker, J. D., Duty, S., Silva, M. J., Calafat, A. M. (2006) Altered semen quality in relation to urinary concentrations of phthalate monoester and oxidative metabolites. Epidemiolog **17** (6): 682 – 691. Jobling S *et al*. (1995) A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ. Health Perspect. **103** (6), 582-587, as cited in EU (2003). Lee, K. Y., Shibutani, M., Takagi, H., Kato, N., Shu, T., Unemaya, C., Hirose, M. (2004) Diverse developmental toxicity of di-n-butyl phthalate in both sexes of rat offspring after maternal exposure during the period from late gestation through lactation. Toxicology **203:** 221 – 238. MAFF (1996) Ministry of Agriculture, Fisheries and Food. Survey of plasticiser levels in food contact materials and in foods. Food Surveillance Papers No. 21. 1996. Mahood, I. K., Scott, H. M., Brown, R., Hallmark, N., Walker, M., Sharpe, R. M. (2007) In utero exposure to di(n-butyl) phthalate and testicular dysgenesis: comparison of fetal and adult end points and their dose sensitivity. Environ Health Perspect **115** (1): 55 – 61. Swan, S. H., Main, K. M., Liu, F., Stewart, S. L., Kruse, R. L., Calafat, A. M., Mao, C. S., Redmon, J. B., Ternand, C. L., Sullivan, S., Teague, J. L. (2005) Study for Future Families Research Team; Decrease in anogenital diseance among male infants with prenantl phthalate exposure. Environ Health Perspect **13** (8): 1056 – 1061.

Hazard Assessment

Substance name:	**2,4-Dichlorophenoxyacetic acid (2,4-D)** 
CAS No.:	94-75-7
Synonym(s):	(2,4-Dichloor-fenoxy)-azijnzuur; (2,4-Dichlorophenyloxy)acetic acid; 2,4-D acid; 2,4-Dichlorophenoxyacetic acid; Acetic acid, (2,4-dichlorophenoxy)-; Acide 2,4-dichloro phenoxyacetique; Agrotect; Amoxone; Chloroxone; Dicopur; Emulsamine; Estone; Fernesta; Fernimine; Ferzone; Hedonal; Tributon; Vergemaster; Verton 2D; Vidon 368; Weedone; Weedtrol; 2,3-Dichlorophenoxyacetic acid; Dichlorophenoxyacetic acid.
Use and potential human exposure routes:	2,4-Dichlorophenozyacetic acid (2,4-D) is a synthetic phenoxy herbicide which disrupts the hormone responses of plants leading to reproductive abnormalities, injury and reductions in plant growth. Used in post-emergence control of broad leaved weeds in cereal croplands, lawns, turfs, pastured, forests, non-cropland and broad-leaved aquatic weeds, 2,4-D can enter the environment through effluents and spills arising from manufacture and transport or as a consequence of direct environmental application. In the UK, 2,4-D is among the top six herbicides used by local authorities (PAN UK, 2011). The main routes of exposure of humans to 2,4-D are from ingestion of contaminated foods or drinking water. Dermal exposure may occur during application, however by-stander exposure to 2,4-D is also possible. Following environmental degradation of 2,4-D, the major product formed is 2,4-dichlorophenol. In drinking water, the taste threshold for 2,4-D is 3.13 mg/L (US EPA, 1987), however 2,4-dichlorophenol can be detected at a much lower level of 0.3 µg/L (IPCS, 1984).
Established/Study-specific exposure limit:	An ADI of 0.01 mg/kg bw/d (10 µg/kg bw/d) has been established by JMPR for 2,4-D from the NOAEL for dogs (see below) using an uncertainty factor of 10 for inter-species and 10 for inter individual differences (WHO, 2003).
Basis for exposure limit:	In a 52 week study of dogs fed diet containing 2,4-D at 5 or 7.5 mg/kg bw/d, decreased body weight gain and increased levels of blood urea nitrogen (BUN), creatine, alanine aminotransferase activity and cholesterol were noted; histopathology showed lesions in the kidney and liver; a critical endpoint NOAEL of 1 mg/kg bw/d was derived.
Evidence for human relevant endocrine disrupting potential:	The endocrine disruptive potential of 2,4-D has been studied for over 15 years but no conclusive evidence has yet been identified. Epidemiological studies of farmers exposed to 2,4-D have shown reduced sperm quality and the prevalence of circulatory/respiratory, urogenital and muscoskeletal/ integumental birth defects has been suggested to be higher in regions with high agricultural use of 2,4-D (Cox, 1999). Roman (2007) suggested a possible association between transient *in utero* hypothyroxinaemia and environmental antithyroid agents such as 2,4-D and a putative role in autism. Howdeshell (2002) also suggests that 2,4-D may affect serum protein-bound iodide levels perturbing the production, transport

	and metabolism of thyroid hormone. Mice fed 2,4-D at 0, 1, 15, 100 or 300 mg/kg bw/d for a 90 day period showed decreased thyroxine activity at 100 mg/kg bw/d or above; a NOAEL of 15 mg/kg bw/d was identified (PAN UK, 2011). Stürtz *et al.* (2010) report alteredmonoamine levels (dopamine and serotonin) in specific areas of the brain and changes in serum hormones involved in milk synthesis and milk ejection in rats given 2,4-D at 2.5, 5, 15, 25, 50 or 70 mg/kg bw/d; prolactin (PRL) and oxytocin (OT) were significantly reduced in treated females and administration of OT before lactation restored milk ejection. A dose-related increase in calcium-dependent and calcium-independent nitric oxide synthase (NOS) activity was also noted in the brain. The authors suggest a mechanism for these effects involving inhibition of suckling-induced hormone release by stimulation of hypothalamic NOS and dopamine operating via serotonin inhibition. Kim *et al*. (2002) report a positive response in a Hershberger assay, with evidence of inhibition of cytochromeP450 mediated metabolism of testosterone, following exposure to 2,4-D with its degradation product DCP. In a mammalian prostate cancer cell line, co-treatment with 10 nM 2,4-D or DCP and 10nM DHT resulted in a 1.6-fold increase in cell proliferation. In transient transfection assays, androgen-induced trans-activation was increased up to 32-fold ,or 1.28 fold by co-treatment of 2,4-D or DCP, respectively with DHTy (Kim *et al*., 2005). 2,4-D also inhibited ovulation in Xenopus oocytes (LaChapelle *et al*., 2007) but was inactive in an ovulation assay and oestrogenic yeast screen (Orton *et al.*, 2009).
Endocrine disrupting activities of concern:	Limited, inconclusive evidence of thyroid and androgen interaction, but potency not adequately characterised
Other significant toxic effects:	Acute exposure to 2,4-D in workers has been associated with eye and skin irritation, weakness, fatigue, respiratory tract irritations, chemical hypersensitivity and neurotoxic effects, including inflammation of nerve endings (PAN UK, 2011). 2,4-D and its amine salts and esters are slightly acutely toxic; rat oral LD_{50} = 400-2000 mg/kg; rat dermal LD_{50} = >2000 mg/kg. In rats exposed to 2,4-D at the maximum attainable concentration of <5.39 mg/L for 4 hours, no deaths occurred (PAN UK, 2011). In addition to the chronic dog study described above, in a 2-year mouse carcinogenicity study at 1, 15 or 45 mg/kg bw/d increased absolute and/or relative kidney weight and pathology were noted at the higher doses. However, no carcinogenicity was seen and a NOAEL for renal toxicity of 1 mg/kg bw/d was proposed (WHO, 2003). However, using *in vitro* human cell assays significant chromosomal damage has been reported via non-mutagenic and non-genotoxic mechanisms (Colborn *et al*., 1993). In pregnant Sprague-Dawley rats given 2,4-D orally at 0, 12.5, 25, 50, 75 or 88 mg/kg bw/d during days 6 – 15 of gestation, the maternal NOAEL was 88 mg/kg bw/d Foetotoxicity (reduced body weight) was noted at 50 mg/kg bw/d or above and a developmental toxicity NOAEL of 25 mg/kg bw/d was established. In a further study on pregnant Fischer 244 rats maternal and developmental toxicity was noted with a NOAEL of 25 mg/kg bw/d (PAN UK, 2011). In a 90-day mouse study at 0, 1, 15, 100 or 300 mg/kg bw/d decreased glucose levels in females and decrease thyroxine activity (reported above)

	and increased absolute and/or relative kidney weights in males were noted at 100 mg/kg bw/d or above; the NOAEL was 15 mg/kg bw/d (PAN UK, 2011). In a ND4 mouse developmental study on a commercial formula containing 2,4-D, mecoprop and dicamba, an inverted U-shaped dose-response was reported for litter size (PAN UK, 2011).
Study estimate of anticipated exposure via drinking water	Limited intake via drinking water is predicted to occur following both conventional and advanced treatment processes in all age groups. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.183; Toddler 0.122; Adult 0.041. Intakes (ug/kg bw/d) based on advanced treatment: Infant 0.059; Toddler 0.040; Adult 0.013
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	An ADI of 0.01 mg/kg/bw was established on the basis of the NOAEL of 1 mg/kg bw/d in the 1-year study of toxicity in dogs and the 2-year study in rats, using an uncertainty factor of 100. Given that the 'worst case' predicted intake (based on conservative assumptions) for infants drinking water from a conventional treatment process is 0.183 µg/kg bw/d, this would afford a margin of safety compared with the ADI of around 55 times, which would suggest there is little cause for concern. Worst case intake of 2,4-D from foods has been estimated as 0.3 - 2 µg/kg bw/d (3 – 20% of ADI for an adult; JMPR, 2001) which is between 1.6 - 10 times higher than the intake estimated from drinking water in this study.
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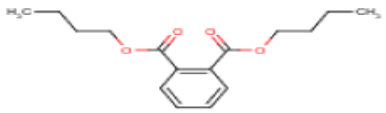
	uk.org/pestnews/Actives/24d.htm [Accessed 08.2011]. Roman, G. C. (2007) Autism: transient in utero hypothyroxinemia related to maternal flavonoid ingestion during pregnancy and to other environmental thyroid agents. *Journal of Neurological Sciences* **262:** 15 – 26. Stürtz, N., Jahn, G. C., Deis, R. P., Rettori, V., Duffard, R. O., Evangelista de Duffard, A. M. (2010) Effects of 2,4-dichlorophenoxyacetic acid on milk transfer to the litter and prolactin release in lactating rats. Toxicology 271: 13 – 20. WHO (2001) World Health Organisation. Pesticide residues in food: Report of the 2001 Joint FAO/WHO meeting of experts. Available at: http://www.fao.org/agriculture/crops/core-themes/theme/pests/pm/lpe/lpe-d/en/ (accessed Dec 2011). WHO (2003) World Health Organisation. 2,4-D in Drinking Water: Background document for development of WHO guidelines for drinking water quality. WHO/SDE/WSH/03.04/70.

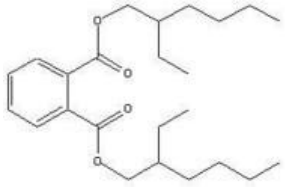
Hazard Assessment

Substance name:	**Diethyl phthalate (DEP)** 
CAS No.:	84-66-2
Synonym(s):	1,2-Benzenedicarboxylic acid, diethyl ester; Benzenedicarboxylic acid diethyl ester; DEP; Diethyl ester; Diethyl ester of 1,2-Benzenedicarboxylic acid; Diethyl-1,2-benzenedicarboxylate; Diethylester kyseliny ftalove; Diethyl-o-phthalate; Ethyl phthalate; Diethyl 1,2-benzenedicarboxylate; Diethylphthalate; o-Benzenedicarboxylic acid, diethyl ester; Phthalic acid; Phthalic acid, diethyl ester; Phthalsaeurediaethylester
Use and potential human exposure routes:	DEP is used as a plasticizer in consumer products including plastic packaging films, cosmetic formulations and toiletries and in medical treatment tubing. It is also used in cosmetics and personal care products, primarily as a solvent and vehicle, and in a wide range of industrial processing (NTP, 2006). The greatest human exposure arises from use of DEP-containing consumer products and ingestion of contaminated foods (e.g. from leaching from packaging materials) (NTP, 2006). In Europe, there has been a general move away from the use of phthalates in flexible food packaging but they are still used in plastic tubing and flexible hoses in food processing equipment (COT, 2011). DEP has been reported in drinking water in the US at 0.01-4.6 µg/L. Overall, daily intake in the US estimated at 13 µg/kg bw (women, 20-40 years) with a 95th percentile value of 90 µg/kg bw (NTP, 2006). Upper bound total dietary exposure of toddlers (>1.5 to 2.5 years) in the UK estimated at 0.3-0.8 µg DEP/kg bw/d; corresponding values for adults are 0.15-0.5 µg DEP/kg bw/d (COT, 2011).
Established/Study-specific exposure limit:	US EPA ambient water quality criteria = 350 mg/L, based on the protection of human health (NTP, 2006). US Food Tolerance Levels and Drinking Water Limits: Not available (NTP, 2006). Occupational Inhalation Exposure Limits: 5 mg/m^3 TWA (ACGIH TLV; NIOSH REL; OSHA PEL) (NTP, 2006) Oral TDI = 0.5 mg/kg bw/d (COT, 2011)
Basis for exposure limit	Rationale for US limits not specified. COT (2011) concluded that a TDI proposed by WHO was appropriate for DEP; this TDI value of 0.5 mg/kg bw/d was based on the NOAEL of 1600 mg/kg bw/d for developmental effects(from Tanaka et al, 1987, see below), with an uncertainty factor of 300 (3 for incomplete database, 10 each for intra- and inter-species variation)
Evidence for human relevant endocrine disrupting potential:	DEP was classified in the EU endocrine disrupters prioritisation exercise as Category 3b* (i.e. some data available but evidence considered insufficient even with additional data form industry; BKH, 2000). More recently, according to NTP (2006), DEP was not oestrogenic in a *in*

	vitro recombinant/receptor gene bioassay with HeLa cells or breast cancer cell line ZR-75 but was weakly activity in human MCF-7 cells and yeast cells with human oestrogen receptors (hER). It did not induce cell proliferation in MCF-7 cells and was not oestrogenic in immature female Wistar rats, a recombinant yeast strain assay or a *in vitro o*estrogen receptor-binding assay using rat uterine cytosol. In a study by Tanaka et al (1987), intra-peritoneal injection (i.p.) administration of DEP at 500, 1600 or 5600 mg/kg bw/d to pregnant ICR mice on gestation days 0 to 17 resulted in a significant reduction in thymus weight and non-significant (7%) reduction in the spleen weight (not considered adverse) in all treated female groups and an increase in adrenal and kidney weights in high dose dams; the NOAEL was 1600 mg/kg bw/d for maternal and offspring toxicity. Fujii S et al (2005) identified a similar NOAEL of 1500 mg/kg bw/d for reproductive and developmental endpoints. ACC (2001 & 2006) and COT (2011) report that a lower NOAEL (750 mg/kg bw/day) had been identified in an oral study (Grey et al, 2000) but as only a single dose level was used, questioned the studies robustness
Endocrine disrupting activities of concern:	Equivocal evidence of weak oestrogenicity.
Other significant toxic effects:	Oral DEP is readily absorbed and subject to initial metabolism to a monoester derivative by the small intestine and liver; quantitative species differences exist with, for the liver, hydrolase activity decreased as follows: baboon (516 µmol/hour/g) > rat (231 µmol/hour/g) > ferret (45.9 µmol/hour/g) and, for intestines, baboon (4.33 µmol/hour/g) > rat (0.648 µmol/hour/g) > ferret (0.053 µmol/hour/g). For humans, rates were 31.2-153 nmol/hour. Monoester derivatives are then further hydrolysed to phthalic acid and excreted or conjugated to glucuronide and excreted via the urine (NTP, 2011). Acute toxicity is low (LD_{50} for Rat - oral = 9200-9500 mg/kg bw and dermal= >22,400 mg/kg bw; for mouse - oral = 8600 mg/kg bw; for Guinea pig - oral = 8600 mg/kg; NTP, 2011). Repeat dose dietary NOAEL in rats is 750 mg/kg/d in males and 150 mg/kg/d in females. At higher doses (5% diet, equivalent to ~ 3750 mg/kg/d in males), decreased bodyweight and organ weights noted (Lehman, 1955). DEP is not genotoxic (ACC, 2001) and did not elicit chronic toxicity or carcinogenic effects in a 1-year dermal initiation-promotion study in male mice (NTP, 1995).
Study estimate of anticipated exposure via drinking water	Intakes (µg/kg/d) from drinking water from advanced treatment process: Infant 0.003; Toddler 0.002; Adult 0.001. Intakes (µg/kg/d) based on conventional treatment: Infant 0.029; Toddler 0.019; Adult 0.006
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	The evidence for the oestrogenicity of this substance appears to be limited, conflicting and, hence, not particularly robust. Nonetheless, a TDI of 0.5 mg/kg bw/d that is based on reproductive and developmental endpoints has recently been confirmed by COT (2011) and is, therefore, considered an appropriate comparator for this study. Comparing the TDI value to the maximum estimated drinking water intake of 0.029 µg/kg/d (for infants drinking water from a conventional treatment plant), indicates that the margin of safety is > 1700-times, and hence should not be regarded as of concern.

	It may also be noted that the estimated overall dietary intake of 0.8 µg DEP/kg bw/d for the population subgroup under particular consideration here – a level considered acceptable by COT (2011) – is itself > 20-times the worst case amount estimated as being derived from drinking water in this study.
References	ACC (2001) High production volume (HPV) chemical challenge program Test plan for Phthalate esters category. December 10, 2001. American Chemical Council . Available at Internet site http://www.epa.gov/hpv/pubs/summaries/benzene/c13467tp.pdf ACC (2007) High production volume (HPV) chemical challenge program Revised test plan for Phthalate esters category. December 10, 2001. American Chemical Council . Available at Internet site http://www.epa.gov/hpv/pubs/summaries/benzene/c13467rt3.pdf BKH (2000) Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption. Bkh consulting engineers. Available at Internet site http://ec.europa.eu/environment/endocrine/strategy/substances_en.htm. COT (2011) *COT statement on dietary exposure to phthalates - data from the Total Diet Study (TDS).* Committee on Toxicity, UK. Available at Internet site *http://cot.food.gov.uk/cotstatements/cotstatementsyrs/cotstatements2011/cot201104*. Fujii S et al (2005) J. Toxicol. Sci., 30, 97-116, as cite din COT, 2011. Gray L et al. (2000) Toxicological Sciences, 58, 350-365, as cited in COT, 2011. Lehman AJ. (1955) Food and Drug Officials of the United States, Quarterly Bulletin, 19, 87-99, as cited in ACC, 2001. NTP (1995) NTP TR429, NIH Publication No 95-3356, as as cited in ACC, 2001. NTP (2006) Supporting Nomination for Toxicological Evaluation by the National Toxicology Program, December 18, 2006. National Toxicology Program, NIEHS. Available at Internet site http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/Diethyl_phthalate.pdf. NTP (2011) Testing status of Agents at NTP. CAS Registry Number: 84-6-2 Toxic Effects. National Toxicology Program, Department of Health and Human Services. Available at Internet site http://ntp.niehs.nih.gov/index.cfm?objectid=E883B613-BDB5-82F8-F1C5F2AD88F02743 Tanaka C et al. (1987) Oyo Yakuri , 33, 387–392, as cited in COT, 2011

Hazard Assessment

Substance name:	**Bis(2-ethylhexyl) phthalate (DEHP)** 
CAS No.:	117-81-7
Synonym(s):	Di(2-ethylhexyl) phthalate; 1,2-Benzenedicarboxylic acid; Bis(2-ethylhexyl) ester; Bis(2-ethylhexyl) 1,2-benzenedicarboxylate; Bis(2-ethylhexyl) o-phthalate; Bis(2-ethylhexyl) phthalate; Di(2-ethylhexyl) phthalate; Dioctyl phthalate; DOP; Phthalic acid dioctyl ester; Phthalic acid.
Use and potential human exposure routes:	DEHP is a plasticised polymeric material commonly used in consumer products such as flooring, toys, cables, medical products sealants and paints. DEHP is known to migrate slowly from polymer products during their entire lifetime (EC, 2008). In the European Union, DEHP is permitted as a plasticiser in repeated use materials and articles containing non-fatty foods with a specific migration limit (SML) of 1.5 mg/kg food stimulant, and is allowable in technical support agents in concentrations up to 0.1% of the final product. DEHP may be present in food due to the migration from food contact materials or as a result of its widespread presence as an environmental contaminant in air, water, soil and food. In an early study, MAFF (1996) demonstrated contamination of food products, estimating a mean exposure of 0.15 mg/ person/day, equivalent to 2.5 µg/kg bw/day (adult), attributable to four main food groups, meat, eggs, poultry and milk. More recently, a study in Denmark estimated the total daily oral intake to be 4.5 µg/kg bw/day in adults, 26 µg/kg bw/day in children (aged 1-6 years) and 11 µg/kg bw/day in adolescents (aged 7 to 14 years). Using a modelling approach, the main sources of DEHP exposure have been suggested to be leaf crops (53%), root crops (13%), milk (12%) and fish (10%), (Müller *et al.*, 2003).
Established/study-specific exposure limit:	Oral TDI = 0.05 mg/kg bw/d (EFSA, 2005). Occupational exposure limit for inhalation: 5mg/m^3 TWA in several EU Member states (EC, 2008).
Basis for exposure limit:	A NOAEL of 4.8 mg/kg bw/day (5 mg/kg bw/d) based on testicular toxicity and developmental toxicity was established by a rodent multi-generation study to OECD Guideline 416. Sprague-Dawley rats showed a NOAEL for testicular atrophy of 100 mg/kg (equivalent to 8 mg/kg bw/d) in F0 animals but only 5 mg/kg bw/d in F1 and F2 animals. The LOAEL for this endpoint was 300 mg/kg (23 mg/kg bw/d in F0 animals and 14 mg/kg bw/d in F1 and F2 rats). An uncertainty factor of 100 was applied to the NOAEL to derive the TDI (Wolfe and Layton, 2003). In a recent draft report by the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (2011), the working group concluded that none of the relevant *in vivo* DEHP studies reported since 2005 (Andrade *et al.*, 2006; Grande *et al.*, 2007; Gray *et al.*, 2009) indicate any need to modify the established TDI.
Evidence for human	A two generation study in rats given DEHP showed reductions in

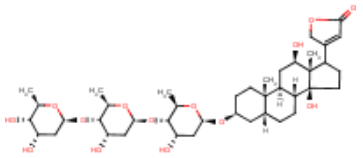
Di(2-ethylhexylphthalate (DEHP)

relevant endocrine disrupting potential:	reproductive performance and fertility in F0 and F1 animals at 1088 mg/kg bw/d and adverse developmental toxicity at 340 mg/ kg bw/d (Schilling *et al*., 1999). In a further multi-generation study in rats, Wolfe and Layton (2003) reported a NOAEL for reproductive toxicity of 1000 mg/kg (77 mg/kg bw/d in F0, and 48 and 46 mg/kg bw/d in F1 and F2 generations, respectively) and a NOAEL for developmental toxicity of 100 mg/kg (equivalent to 8 mg/kg bw/d in F0 and 5 mg/kg bw/d in F1 and F2 generations). DEHP has been shown to be embryotoxic and teratogenic in mice at oral dose levels below those producing observable toxicity in dams; continuous breeding studies identified NOAELs for maternal and developmental toxicity of 600 and 20 mg/kg bw/d respectively (Lamb *et al*., 1987). *In vivo* mechanistic studies have indicated a mechanism involving Leydig cell disruption, as measured by decreased testosterone output, indicating that DEHP may exert its toxicity due to its anti-androgenic potential (EFSA, 2005; Gray *et al*., 1999; Mylchreest and Foster, 1999). The lowest identified NOAEL for testicular effects following dietary DEHP exposure is 3.7 mg/kg bw/day in rats, as identified by a high incidence (7/9) of Sertoli cell vacuoliation at the next dose level (37.6 mg/kg bw/day) in a 13-week guideline study (Poon et al., 1997).
Endocrine disrupting activities of concern:	**Anti-androgen**
Other significant toxic effects:	Studies in both human volunteers and animals show ingested DEHP is generally rapidly absorbed and distributed in the body but there is no evidence of accumulation. DEHP is metabolised via several pathways followed by urinary excretion (with some excretion by bile seen in rodents only). In addition, DEHP has been shown in humans and animals to be transferred to milk, raising concern regarding possible lactational transfer. Acute toxicity of DEHP is low via the oral (LD_{50} rat = >20,000 mg/kg bw; LD_{50} mice = > 10,000 mg/kg bw) and inhalation route (LD_{50} rat = 10,600 mg/m^3 for 4 hours; JRC, 2008). A NOAEL for nephrotoxicity of 28.9 mg/kg bw/d in males and 36.1 mg/kg bw/d in females (based on organ weight change) has been reported in a 2-year chronic toxicity study in rats (Moore, 1996). Increased hepatocellular tumours were noted in rats and mice following long-term dietary administration of DEHP. However, the mechanism of action is activation of PPAR-α, a mechanism not considered relevant to human carcinogenesis and, hence, DEHP is not classed as a human carcinogen (IARC, 1995).
Study estimate of anticipated exposure via drinking water	Intakes via drinking water are predicted to occur following both conventional and from advanced treatment processes in infants and toddlers but only in association with the conventional treatment processes for adults. Intakes (µg/kg/d) based on conventional treatment: Infant 0.015; Toddler 0.010; Adult 0.003. Intakes (µg/kg/d) based on advanced treatment: Infant 0.002; Toddler 0.001
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	The established TDI for reproductive and developmental toxicity is 0.05 mg/ kg bw/d (i.e. 50 µg/kg bw/d). Given that the 'worst case' predicted intake via drinking water based on a conventional treatment processes (and conservative assumptions) is 0.015 µg/kg bw/d for infants, this would suggest a margin of safety of >3000-fold.

	Even combining this with the historic estimated total intake of 2.5 µg/kg bw/d estimated to arise from foodstuffs (MAFF, 1996), giving a total intake of 2.515 ug/kg bw/d, this would still represent a margin of safety of 19-times the TDI.
References:	Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006) *Toxicology* **228** (1): 85 – 97 (as cited in COT, 2011). COT (2011) Committee on Toxicology of Chemicals in Food, Consumer Products and the Environment. Second Draft COT Statement on Dietary Exposure to Phthalates – Data from the Total Diet Survey. TOX/2011/04. EFSA (2005) Opinions of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to Bis(2-ethylhexyl)phthalate (DEHP) for use in food contact materials. The EFSA Journal **243:** 1 – 20. Grande *et al*. (2007) *Toxicology* **229**: 114 – 122 (as cited in COT, 2011). Gray, J. L. E., Barlow, N. J., Howdeshell, K. L., Ostby, J. S., Furr, J. R., Gray, C. L. (2009) *Toxicological Sciences* **110** (2): 411 – 425 (as cited in COT, 2011). Gray *et al*., (1999) *Toxicol. Ind. Health* **15** (1-2): 94 – 118 (as cited in EFSA, 2005). Lin, H., Ge, R.., Chen, G., Hu, G., Dong, L., Lian, Q., Hardy, D. O., Sottas, C. M., Li, X. K., Hardy, M. P. (2008) Involvement of testicular growth factors in fetal Leydig cell aggregation after exposure to phthalate in utero. *PNAS* **105** (20): 7218 – 7222. IARC (1995) International Agency for Research on Cancer. Peroxisome Proliferation and its role in Carcinogenesis, views and expert opinions of an IARC Working Group Lyon, 7 – 11 Dec 1994. IARC Technical Report No. 24 Lyon, 1995. EC (2008) European Commission Joint Research Council: Institute of Health and Consumer Protection (ICHP). Bis-(2-ethylhexyl)phthalate (DEHP) Summary of Risk Assessment Report. EUR 23384 EN/2. Lamb, I. V., Chapin, R. E., Teague, J., Lawton, A. D., Reel, J. R. (1987) Reproductive effects of four phthalic acid esters in the mouse. *Toxicol Appl Pharmacol* **88:** 255 – 269. MAFF (1996) Ministry of Agriculture, Fisheries and Food. Survey of plasticiser levels in food contact materials and in foods. Food Surveillance Papers No 21. 1996. Moore, M. R. (1996) Oncogenicity study in rats with Di(2-ethylhexyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses. Orning Hazleton Inforporated (CHV), 9200 Leesburg Pike, Vienna, Virginia 22182-1699. Laboratory Study Identification: CHV 663-134; Sponsor: Eastman Chemical Company, First America Centre, P.O. Box 1994 Kingsport, Tennessee 37662-5394. Müller, A. M., Nielsen, A., Ladeforged, O. (2003) Ministeriet for Fødevarerm Landbrug og Fiskeri Veterunær- og Fødevaredirektoratet. Human exposure to selected phthalates in Denmark, rapport 2003: 15. Mylchreest *et al.,* (1999) *Toxicology and Applied Pharmacology* **156**: 81 – 95 (as cited in EFSA, 2005).

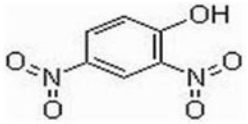
	Poon, R., Lecavalier, P., Mueller, R., Valli, V. E., Procter, B. B., Chu, I. (1997) Subchronic oral toxicity of di-n-octyl phthalate and di(2-ethylexyl) phthalate in the rat. *Food Chem. Toxicol.* **35**: 225 – 239. Schilling, K., Deckardt, K., Gemnardt, Chr., Hildebrand, B. (1999) Di-2-ethylhexyl phthalate – two-generation reproduction toxixity range-finding study in Wistar rats. Continous dietary administration. Department of Toxicology of BASF Aktiengesellschaft, D-67056 Ludwigshafen, FRG. Laboratory Project Identification 15R049/97096. Wolfe, G. W., Layton, K. A. (2003) Multigeneration reproduction toxicity study in rats (unaudited draft): Diethylhexylphthalate: Multigenerational reproductive assessment when administered to Sprague-Dawley rats in the diet. TherImmune Research Corporation (Gaithersburg, Maryland), TRC Study No. 7244-200

Hazard Assessment

Substance name:	**Digoxin** 
CAS No.:	20830-75-5
Synonym(s):	(3β, 5β, 12β)-3-((O-2, 6-Dideoxy-β-D-ribohexapyranosyl-(1,4)-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1-4)-2,6-dideoxy-β-D-ribo-hexopyranosyl)oxy), 12, 14-dihydroxycard-20(22)-enolide; 12β-Hydroxydigitoxin; 3β, 12β, 140Trihydroxy-5 β, 14 β-card-20(22)-enolid-3-tridigitoxosid; Acygoxin; Cardigox; Cardioxin; Digacin; Digomal; Digos; Digosin; Digoxine; Silanacin; Dimecip; Dixina; Hemigoxine Nativelle; Homolle's digitalin; Lanacrist; Lanatilin; Lanicorl Lanoxin; Lenoxin; Longdigox; Novodigal; Purgoxin; Rougoxin; Saroxin; Stillacor; Vanoxin.
Use and potential human exposure routes:	Digoxin is a purified cardiotonic glycosilide extracted from the foxglove plant (*Digitalis lanata*). The positive inotropic and negative chronotropic activity of digoxin has led to its use to control ventricular rate in atrial fibrillation and in the management of congestive heart failure (HSDB, 2011). Digoxin preparations are commonly available as a 0.05 mg/mL oral solution or 0.25 mg/mL and 0.5 mg/mL injectable solution.
Established/Study-specific exposure limit:	**Therapeutic dose by age group:** <1 month : 6 µg/kg/ bw 1 month to 12 years : 10 µg/kg/ bw 12 to 18 years : 62.5 µg/kg/ bw (62.5 – 250 µg/kg/ bw) < 18 years : 125 µg/ kg/ bw (125 – 250 µg/kg/ bw) **Study-specific Exposure Limit** = 0.06 µg/ kg bw/ d
Basis for exposure limit:	Assuming an uncertainty factor of 100 from the minimum human therapeutic dose.
Evidence for human relevant endocrine disrupting potential:	None Identified
Other significant toxic effects:	In a study on the effects of digoxin on the autonomic nervous system of male Wistar rats, the levels of acethylcholine, a parasympathetic marker, and norepinephrine, a sympathetic marker, were used as markers of myocardial function. Rats subcutaneously exposed to 0.75 mg/kg and 2.5 mg/kg demonstrated toxic symptoms and weight loss, 0.35 mg/kg was considered to be a sub-toxic dose, and 0.1 mg/kg did not exhibit any toxicity (Watanabe *et al.*, 1989).
Primary mode of endocrine activity	None Identified
Study estimate of anticipated exposure via drinking water	Limited intake via drinking water is predicted to occur in all age groups following conventional treatment processes. No intake was predicted in any age group following advanced treatment processes.

	Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.016; Toddler 0.011; Adult 0.004.
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	No evidence to either prove or disprove endocrine disrupting activity per se has been identified for this substance. Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes and conservative assumptions is 0.016 µg/kg bw/d for infants, this would suggest a margin of safety of around 4 times against the study-specific exposure limit. These figures were based on modelled levels in drinking water calculated with usage figures obtained from UK prescription data, undergoing conventional water treatment; as such they are conservative in nature. It should be noted that margins of safety following advanced water treatment were significantly increased with a predicted intake of zero in all age groups. An intake level below a margin of safety of only 10 times, as seen here with respect to toddlers and infants, is of potential concern. Nonetheless since no evidence of endocrine disrupting potential has been found and in the light of the highly conservative assumptions used throughout the modelling process, significant concern is not considered to be warranted in this case.
References:	HSDB (2011) Digoxin. Hazardous Substances Data Bank (HSDB). UK National Library of Medicine. Available at Internet site http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB [Accessed 12.09.2011] Watanabe Y., et al. (1989) J Cardiovas Pharmacol 13 (5): 702 – 708 (as cited in HSDB, 2011).

Hazard Assessment

Substance name:	**2,4-Dinitrophenol** 
CAS No.:	51-28-5
Synonym(s):	1'alpha-2,4-Dinitrophenol; 1-Hydroxy-2,4-dinitrobenzene; 2,3-DNP; 2,4-Dinitrofenol; Aldifen; DNP; EPA Pesticides Chemical Code 037509; Nitrophen; Nitrophene; alpha-Dinitrophenol
Use and potential human exposure routes:	2,4-Dinitrophenol is chemically similar to trinitrophenol (picric acid) and is commonly used in the chemical synthesis of dyes, picramic acid, wood preservatives, diaminophenol dihydrochlorinde (a photograph developer), explosives, insecticides and as a pH indicator (Coulter *et al.*, 1969; HSDB, 1994). In addition, 2,4-DNP uncouples oxidative phosphorylation and is subsequently used in many biochemical studies of oxidative processes. DNP is present in industrial wastewaters (dye manufacturers have reported effluent containing an average of 3.2 mg/L), while levels in ground water adjacent to industry have been reported to be as high as 30.6 mg/L. General population exposure may result from proximity to landfill and waste sites, which may contaminate dietary and air sources with 2,4-DNP. Dermal exposure is also possible through contact with contaminated water.
Established/study-specific exposure limit:	A study-specific exposure limit of 0.002 mg/kg bw/d (2 µg/kg bw/d) was established based on the current RfD value (COT, 2003). Using human data the US EPA set a Minimum Risk Level (MRL) of 0.01 mg 2,4-DNP kg^{-1} bw/day for acute oral exposure, and a reference dose (RfD) of 0.002 mg 2,4-DNP kg^{-1} bw/day for chronic exposure and protection against cataracts (EPA, 1995; FSA, 2003; COT, 2003). The US EPA lists 2,4-DNP as a hazardous air pollutant under the Clean Air Act, recommending that concentrations in water matrices such as, lakes and rivers should not exceed 0.07 mg/L for swimming purposes, or 0.765 mg/L where no swimming is permitted.
Basis for exposure limit:	The MRL of 0.01 mg/kg bw/d was derived from a LOAEL of 1.2 mg/kg bw/d in 37 humans who took 2,4-DNP for weight reduction. Toxicity endpoints included a sensation of warmth, increased perspiration and body weight loss, however, no dermal effects, cataracts, haematological effects or symptoms of peripheral neuritis occurred at this dose level (Tainter *et al*., 1935). From the same human study a LOAEL of 2 mg/kg bw/d for cataract formation (Horner, 1942) was used to derive the RfD of 0.02 mg/kg bw/d for 2,4-DHP by applying an uncertainty of 10 for human variation and 10 for extrapolation of a LOAEL to a hypothetical NOAEL.
Evidence for human relevant endocrine disrupting potential:	An autopsy performed on a women who had consumed 1.03 mg 2,4-DNP daily for 46 days, showed extensive vascularisation of the spleen and pituitary, with goiter in the thyroid; these endpoints may however be coincidental (Goldman and Haber, 1936). MacBryde and Taussig (1935) reported decreased glucose tolerance in 5 out of 8 clinical study participants

	following treatment with 2,4-DNP at a dose of 4.3 mg/kg/bw. Furthermore, 11 humans exposed to 6.4 mg/kg/bw over 2 days showed a 21% decrease in serum protein bound iodine (Castor and Beierwaltes, 1956). *In vivo* rat studies have identified disruption of the hypothalamic-pituitary-thyroid axis as a result of dietary 2,4-DNP exposure (0.2% feed concentration; Bakke and Lawrence, 1965; England *et al*., 1973).
Other significant toxic effects:	Several clinical studies have reported death as a result of treatments with doses of 2,4-DNP of between 3.5 – 5.27 mg/kg bw/d (Cutting *et al*., 1934; Grant and Schube, 1934). Dermal contact has also been shown to be a significant pathway for exposure in humans. Jiunun *et al.* (2010) reported two occupationally related deaths following dermal exposure to 2,4-DNP. 2,4-DNP has been also been shown to have neurotoxic effects in human females at levels of 4.4 mg/kg bw/day, affecting the central and peripheral nervous systems (Dintenfass, 1934).
Primary mode of endocrine activity	Thyroid axis.
Study estimate of anticipated exposure via drinking water	Intake via drinking water is predicted to occur following both conventional and advanced treatment processes in infants and toddlers and following conventional treatment processes in adults. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.018; Toddler 0.012; Adult 0.004. Intakes (ug/kg bw/d) based on advanced treatment: Infant 0.002; Toddler 0.001.
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	A study-specific exposure limit of 0.002 mg/kg bw/d (2 µg/kg bw/d) was established. Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes and conservative assumptions is 0.018 µg/kg bw/d, this would suggest a margin of safety of around 110 times.
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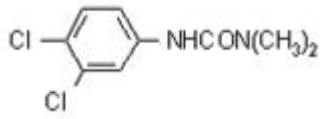
MacBryde, C. M., Taussig, B. L. (1935) Functional changes in liver, heart and muscles and loss of dextrose tolerance due to dinitrophenol. JAMA **105**: 13 – 17.

Tainter, M. L., Stockton, A. B., Cutting, W. C. (1935) Dinitrophenol in the treatment of obesity: Final Report. JAMA **105:** 332 – 337.

Diuron

Hazard Assessment

Substance name:	**Diuron** 
CAS No.:	330-54-1
Synonym(s):	AF 101; Cekiuron; Crisuron; Dailon; Dcmu; Diater; 3-(3,4-Dichloor-Fenyl)-1,1-Dimethylureum; Dichlorfenidim; 3-(3,4-Dichlorophenol)-1,1-Dimethylurea; 3-(3,4-Dichlorophenyl)-1,1-Dimethylurea ; 1-(3,4-Dichlorophenyl)-3,3-Dimethyluree ; 3-(3,4-Dichlor-Phenyl)-1,1-Dimethyl-Harnstoff; 3-(3,4-Dicloro-Fenyl)-1,1-Dimetil-Urea; 1,1-Dimethyl-3-(3,4-Dichlorophenyl)Urea; Di-On; Direx 4l; Diurex; Diurol; Diuron; Diuron 4l; DMU; Drexel; Drexel Diuron 4l ;Duran; Dynex; Farmco Diuron; Herbatox; Hw 920; Karmex; Karmex Dw; Marmer; Na 2767; N'-(3,4-Dichlorophenyl)-N,N-Dimethylurea; Sup'r Flo; Telvar; Unidron; Urea, 3-(3,4-Dichlorophenyl)-1,1-Dimethyl-; UROX D; USAF P-7; USAF XR-42; Vonduron
Use and potential human exposure routes:	Diuron is a urea-based herbicide that has been widely used on agricultural and ornamental plants. It has moderate solubility in water, where it slowly degrades. It is lipophilic and shows strong adsorbancy to sediment. In soil it very persistent (half-life 90-180 d; Lintelmann et al., 2003). The theoretical maximum daily intake (TMDI), excluding that from water or products of animal origin, is estimated to be only 1.6% of the established ADI for a 60kg adult in Europe; any additional intake from water is not anticipated to be of concern (EU, 2008b)
Established/Study-specific exposure limit:	Occupational inhalation exposure limit = 10 mg/m^3 TWA by NIOSH (CDC, 1995). Dietary ADI = 0.007 mg/kg/day (EU, 2008). Aquatic EQS – AA-QS all surface water = 0.2 µg/L; Abstraction of water intended for Human consumption (AWIHC) = <1 µg/L; Water intended for human consumption (WIHC) = 0.1 µg/L (EC, 2005). Maximum acceptable concentration (MAC) in drinking water = 150 µg/L (Health Canada, 1989)
Basis for exposure limit	The ADI for Europe is based on data from a 2-year rat study (EFSA, 2011); effects of concern relate to the blood and urothelial systems with a NOAEL of 1.0 mg/kg bw/d established for males and LOAELs of 10 and 1.7 mg/kg bw/d noted in males and females, respectively. The female LOAEL formed the basis for establishing an ADI; an uncertainty factor of 2.5 was applied to standard inter-species extrapolation factors (EC, 2005). The drinking water standard was based on the established limit for individual pesticides under 98/83/EC (EC, 2005). Identified occupational exposure limit was based on concern regarding irritancy (CDC, 1995)
Evidence for human relevant endocrine disrupting potential:	Diuron was identified in the EU Endocrine Disrupter prioritisation exercise as a Category 2 substance (i.e. potential for endocrine disruption; in vitro data were considered to indicate a potential for endocrine disruption in intact organisms; BKH, 2000). It was also considered of concern by IUPAC on the

Diuron

	basis of its structural similarity to linuron (Lintelmann et al., 2003). While apparently not an endocrine disrupter itself, the main metabolite, DCA, is believed to be anti-androgenic (IMF, 2003; Mnif et al, 2011). Although reproductive parameters were unaffected in a rat dietary 3-generation study at levels equivalent to 6 mg/kg bw/d, slight foetotoxicity (reduced bodyweight) was noted at the high dose in F2 and F3 litters. It was not teratogentic but was foetotoxic at 250 mg/kg bw/d (low foetal weight and minor rib and bone anomalies noted); effects were apparent but not statistically significant at 125 mg/kg bw/d. The LOAEL of 125 mg/kg bw/d was established for developmental endpoints (Health Canada, 1989)
Endocrine disrupting activities of concern:	Anti-androgen
Other significant toxic effects:	Diuron is absorbed from the gastrointestinal and respiratory tracts. In humans it is metabolized within hours by hydroxylation and N-dealkylation and is excreted via the urine (Health Canada, 1989). The main metabolite of diruon is 2,4-DCA (Lintelmann et al., 2003). Acute exposure causes mild dermal irritation and narcosis in animals; LD_{50} = 2500 mg/kg for rabbit (dermal) and 1017 mg/kg rat (oral; CDC, 1995). Juveniles and animals on protein-deficient diets are more susceptible than adults (Health Canada, 1989). Rats given diuron via the diet for 42 days showed reduced RBC count and haemoglobin level at 2000 ppm or above, growth impairment at 4000 ppm or above, and increased mortality at 8000 ppm. Similar findings, together with splenic enlargement and methaemoglobinaenemmia, were noted with 5000 ppm for 90 days. In rats fed 250 ppm or above for 2 years, effects included impaired growth, reduced haemoglobin levels, effects on the spleen and increased mortality. Rib anomalies were noted in the fetuses of rat dams orally dosed at 250 mg/kg/d (80% diuron) for days 6-15 of gestation (CDC, 1995). Long-term feeding studies have identified the critical effects as damage to the blood system and urothelial system; in rats, increased neoplasia was noted in the urothelium and, in mice, of the mammary gland. Thresholds of effect (as mg/kg/d) in various species over 2 years are: Rat NOAEL = 1.0 in males but not established for females, LOAEL = 10 in males and 1.7 in females; Mice NOAEL = 50.8 in males/77.5 in females, LOAEL = 111 in males, 203 in females; and Dog NOAEL = 6 i both sexes, LOAEL = 12 in both sexes (EC, 2005). However, diuron is not mutagenic in most microbial tests with/without metabolic activation but was clastogenic in an in vivo rat test (Health Canada, 1989). Under Reg. No. 1271/2008, diuron is classified as: Carc. Cat. 3; R40, Xn; R22, Xn; R48/22, N; R50/53, Acute Tox. 4 - H302, STOT RE 2 - H373, Carc. 2 - H351 (EU, 2008a)
Study estimate of anticipated exposure via drinking water	Intake (µg/kg bw/d) from drinking water from advanced treatment process: Infant 0.005; Toddler 0.003; Adult 0.001. Intake (µg/kg bw/d) based on conventional treatment: Infant 0.042; Toddler 0.028; Adult 0.009

Diuron

Assessment of endocrine disruption potential at modelled drinking water concentration(s)	Although the main metabolite is a suspected anti-androgen, the available database on this substance appears to be limited to in vitro assays and no robust data on dose-response relationships has been identified. Nonetheless, diuron is known to cause minor developmental changes (associated with fetotoxicity), with a LOAEL of 125 mg/kg bw/d. The ADI recently established by European Authorities (0.007 mg/kg/day; EU, 2008) is based of the identification of the critical effect as the chronic toxicity/neoplasia – not developmental effects (i.e., repeat dose LOAEL of 1.7 mg/kg bw/d is >70-times less than the developmental LOAEL of 125 mg/kg bw/d). Hence, it is reasonable to assume that the established ADI would provide an adequate level of protection from any risks arising from the substance that might associate with the endocrine activity of its metabolite. In this study, the highest estimated drinking water intake is 0.042 µg/kg bw/d (infants drinking conventionally treated water). Comparing this to the established ADI (0.007 mg/kg/day; i.e. 7 µg/kg bw/d) demonstrates a satisfactory safety margin of 166, and hence even the worst case predicted levels in drinking water are not considered of concern
References	BKH (2000) Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption. Bkh consulting engineers. Available at Internet site http://ec.europa.eu/environment/endocrine/strategy/substances_en.htm. CDC (1995) Occupational Safety and Health Guideline for Diuron. Centres for Disease Control and Prevention, National Institute for Occupational Safety and Health, US Department of Health and Human Services, USA. Available at Internet site http://www.cdc.gov/niosh/docs/81-123/pdfs/0247.pdf. EC (2005) Common Implementation Strategy for the Water Framework Directive. Environmental Quality Standards (EQS) Substance Data Sheet. Priority Substance No. 13 Diuron. CAS-No. 330-54-1. Final Version Brussels, 15 January 2005. European Commission. Available at Internet site http://www.efsa.europa.eu/en/panels/pesticides.htm. EFSA (2011) Reasoned Opinion - Review of the existing maximum residue levels (MRLs) for diuron according to Article 12 of Regulation (EC) No 396/20051. Euroepan Food Standards Agency. Available at Internet site http://www.efsa.europa.eu/en/efsajournal/doc/2324.pdf. EU (2008a) EU Pesticides database – Diruon Available at Internet site http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=activesubstance.detail. EU (2008b) Review report for the active substance diuron finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 11 July 2008 in view of the inclusion of diuron in Annex 1 of Directive 91/414/EEC. SANCO/2184/2008 REV 3. European Commission Health & Consumer Protection Directorate-General. IMF (2003) Final Report ENV.D.1/ETU/2000/0083 Study on Endocrine Disrupters in Drinking Water. Fraunhofer Institute for Molecular Biology and Applied Ecology, Germany and EWSE Institute for Water Research and Water Technology (JOGU-ESWE), Germany. Available at Internet site http://ec.europa.eu/research/endocrine/pdf/drinking_water_en.pdf. Health Canada (1989) Guidelines For Canadian Drinking Water Quality -

Diuron

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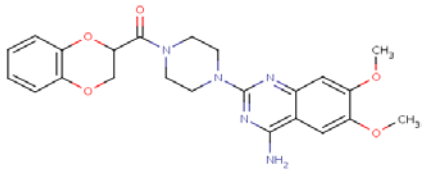
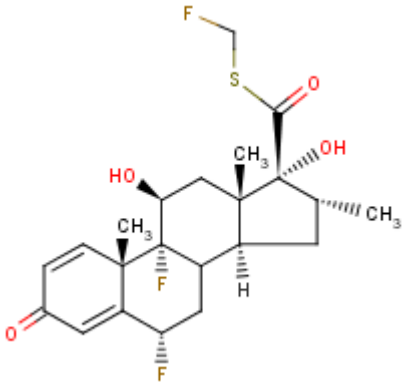
Hazard Assessment

Substance name:	**Doxazosin** 
CAS No.:	74191-85-8
Synonym(s):	Doxazosin mesilate; Doxazosin mesylate
Use and potential human exposure routes:	Used for the treatment and management of mild to moderate hypertension and urinary obstruction symptoms caused by benign prostatic hyperplasia (BPH). Doxazosin acts by inhibiting the postsynaptic alpha(1)-adrenoceptors on vascular smooth muscle. This inhibits the vasoconstrictor effect of circulating and locally-released catecholamines (epinephrine and norepinephrine), resulting in peripheral vasodilation. Doxazosin is a quinazoline-derivative that selectively antagonizes postsynaptic α_1-adrenergic receptors (Drug Bank, 2011). Occupational exposure to doxazosin may occur through inhalation and dermal contact with this compound at workplaces where doxazosin is produced or used. Direct exposure to doxazosin among the general population would be limited to those administered medications that contain doxazosin or indirectly through release into the environment (HSDB, 2003). Literature reporting doxazosin levels in water sources is limited. Langford and Thomas (2009) reported doxazosin in the influent and effluent of a wastewater treatment works in Oslo, Norway, at < 10 ng/L. No U.K. data could be identified.
Established/Study-specific exposure limit:	**Therapeutic doses by age range:** < 1month – 6 years : not used 6- 12 years: 500 µg/ d (min dose) 12 to18 years: 1000 µg/ d (min dose) > 18 years: 1000 µg/d No ADI or TDI has been established for doxazosin. **Study-specific exposure limit:** 1 µg/ kg bw/day
Basis for exposure limit:	Applying an uncertainty factor of 100 to the minimum human therapeutic dose would suggest a study-specific exposure limit of 1 µg/kg bw/d for a 5kg infant. However, using the lowest dose associated with a reversible reduction in male fertility (20 mg/kg bw/day) for rats (Micromedex Thompson Health Care, 2002) with an uncertainty factor of 1000 (100 for intra- and inter-species variation and 10 for the use of a reversible LOAEL) would give a value of 20 µg/kg bw/d. Adopting the most precautionary of these approaches, suggests that the study-specific exposure limit should be 1 µg/kg bw/d.

Evidence for human relevant endocrine disrupting potential:	Rat reproductive toxicity studies have identified a reversible reduction in male fertility with a LOAEL of 20 mg/kg bw/day (no further details identified). Doxazosin is able to cross the placenta in rats but no evidence of foetotoxicity was noted at 20 or 40 mg/kg/d. Reduced foetal survival was seen at 82 mg/kg/d in rabbits (Micromedex Thompson Health Care, 2002). Developmental studies in rats given 40 or 50 mg/kg/d perinatally resulted in delayed postnatal development (lower body weight gain and slight delays in developmental milestones) (Micromedex Thompson Health Care, 2002).
Endocrine disrupting activities of concern:	This substance was identified as of possible concern as QSAR modelling by the Study Team indicated that it may have protein binding properties (indicating the possibility of androgenic effects). However, no evidence suggestive of endocrine disrupting activity per se was identified for doxazosin in subsequent literature review.
Other significant toxic effects:	Doxazosin is well absorbed from the GI-tract, showing a bioavailability of around 65% (Micromedex Thompson Health Care, 2002). It undergoes extensive hepatic metabolism and has an estimated half-life of about 20 hours (Hardman et al, 2001). Several active and inactive metabolites have been identified (2-piperazinyl, 6' and 7'-hydroxy,6' and 7'-O-desmethyl, and 2-amino) (Micromedex Thompson Health Care, 2002). Elimination is mainly via the faeces, with around 5% as unchanged drug and 63 to 65% metabolites. Renal elimination amount to only around 9% (HSDB, 2003). Doxazosin has low acute oral toxicity in rats and mice (LD_{50} = >1000 mg/kg; Drug Bank, 2011). Chronic exposure of humans to doxazosin is generally well tolerated. Adverse effects include most frequently dizziness, headache, drowsiness, lack of energy (e.g, lethargy, fatigue), nausea, oedema and rhinitis. In patients receiving the drug for benign prostatic hyperplasia (BPH), the most frequent adverse effects are dizziness, headache, fatigue, oedema, dyspnoea, abdominal pain, and diarrhoea (McEvoy, 2002). Diazosin is not considered to be carcinogenic (Micromedex Thompson Health Care, 2002)
Study estimate of anticipated exposure via drinking water	Limited intake via drinking water is predicted to occur following conventional treatment processes in all age groups. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.036; Toddler 0.024; Adult 0.008. No exposure is anticipated following advanced water treatment.
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	Doxazosin is known to be a reproductive (male infertility) and developmental (delays in developmental milestones) toxin, although clear evidence of any endocrine-disrupting mechanism of action has not been identified. Comparing the worst case exposure (infants given water from conventional treatment process) with the highly precautionary study-specific exposure limit of 1 µg/kg bw/day identified for this substance, suggests a margin of safety of >100-times, indicating that there is no appreciable cause for concern
References:	Drug Bank (2011). Doxazosin. Available at: http://drugbank.ca/drugs/DB00590 Hardman, J.G., Limbird, L.E., Gilman A.G. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw-Hill, 2001., p. 246

	Hazardous Substances Data Base (2003). Doxazosin. Available at: http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~GXylR0:1 McEvoy, G.K. (ed.). American Hospital Formulary Service- Drug Information 2002. Bethesda, MD: American Society of Health-System Pharmacists, Inc. 2002 (Plus Supplements)., p. 1819] MICROMEDEX Thomson Health Care. USPDI - Drug Information for the Health Care Professional. 22nd ed. Volume 1. MICROMEDEX Thomson Health Care, Greenwood Village, CO. 2002. Content Reviewed and Approved by the U.S. Pharmacopeial Convention, Inc., p. 1278

Hazard Assessment

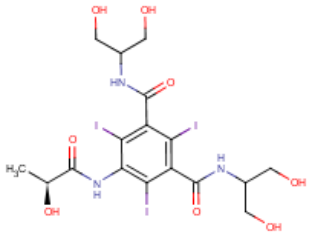
Substance name:	**Fluticasone** 
CAS No.:	80474-14-2
Synonym(s):	(6alpha,11beta,16alpha,17alpha)-6,9-difluoro-11-hydroxy-16-methyl-3-oxo-17-(1-oxopropoxy)androsta-1,4-diene-17-carbothiolic-S-(fluoromethyl)ester; S-fluoromethyl6alpha,9alpha-difluoro-11beta-hydroxy-16alpha-methyl-17alpha-propionyloxy-3-oxoandrosta-1,4-diene-17beta-carbothioate; CCI-18781; Cutivate; Flixonase; Flixotide; Flonase; Flovent; Flunase
Use and potential human exposure routes:	Fluticasone may be applied dermally to relieve inflammatory and pruritic symptoms of dermatoses and psoriases, intranasally to manage symptoms of allergic and non-allergic rhinitis or orally for the treatment of asthma (Wishart et al, 2009). Fluticasone propionate nasal spray is indicated for use in adults and pediatric patients 4 years of age and older. Fluticasone propionate cream may be used in adults and with caution in pediatric patients 3 months of age or older. Fluticasone propionate inhalation powder is indicated for therapy in adults and pediatric patients 4 years of age and older (US NIH, 2009). Occupational exposure to fluticasone may occur through inhalation and dermal contact with this compound at workplaces where fluticasone is produced or used. Direct exposure to fluticasone among the general population will most probably be limited to those administered medications that contain fluticasone (HSDB, 2009). The production of fluticasone may result in release to the environment through various waste streams. If released to air, fluticasone is expected to exist solely in the particulate phase in the ambient atmosphere with subsequent removal by wet or dry deposition (Bidleman, 1988). If released to soil, fluticasone is expected to have moderate mobility; however biodegradation data is not currently available (Meylan et al, 1992). If released to water, fluticasone is expected to adsorb to suspended solids with a low potential for bioconcentration in aquatic organisms (Meylan et al, 1999). Literature reporting fluticasone levels in water sources is limited. Salgardo et al (2010) reported levels of fluticasone in influent, effluent and sludge of 5 Portugese wastewater treatment plants (WWTPs) in spring and autumn. Fluticasone was identified in WWTP influent in 3/5 samples, in WWTP

	effluent in 3/9 samples and in 2/9 WWTP secondary sludge samples. The concentration of fluticasone detected was; 196 ± 1 – 1298 ± 82 ng/L in influent; 33 ± 0.3 – 2848 ± 14 ng/L in effluent; 1473 ± 17 - 2330 ± 42 ng/L in secondary sludge.
Established/Study-specific exposure limit:	**Therapeutic doses by age range:** < 1month – 5 years : not used 5- 12 years: 100 µg/ d (min dose) 12 to18 years: 100 µg/ d (min dose) > 18 years: 100 µg/d No authoritative TDI has been identified for fluticasone. **Study-specific exposure limit :** 0.1 µg/kg bw/day
Basis for exposure limit:	Reproductive and developmental toxicity studies have reported foetal toxicity in rats at doses of 100 µg/kg bw/d fluticasone when administered daily via subcutaneous route (HSDB, 2009). Applying an uncertainty factor of 100 to the minimum human therapeutic dose would suggest a study-specific exposure limit of 0.2 µg/kg bw/d for a 5kg infant. However, an uncertainty factor of 1000 applied to the foetal toxicity LOAEL of 100 µg/kg bw/d would suggest a study-specific exposure limit of 0.1 µg/kg bw/d. Adopting the most conservative assumption, the low value of 0.1 µg/kg bw/d was adopted for the purposes of this study.
Evidence for human relevant endocrine disrupting potential:	Fluticasone propionate is a highly selective agonist of the human glucocorticoid receptor but has negligible activity for androgen, oestrogen and mineralocorticoid receptors. In preclinical studies, fluticasone propionate reportedly exhibited weak progesterone-like activity but as plasma concentrations of fluticasone propionate are very low following intranasal administration of the drug at recommended doses, the clinical importance of this finding is not uncertain (American Society of Health System Pharmacists, 2009). Fluticasone propionate is teratogenic and embryotoxic in mice or rats when given subcutaneously at daily dosages of 45 or 100 ug/kg respectively (approximately equivalent to 4 times the maximum recommended daily intranasal dosage in adults based on surface area). Foetal toxicity was characteristic of potent glucocorticoid compounds, including embryonic growth retardation, omphalocele, cleft palate, and retarded cranial ossification (American Society of Health System Pharmacists, 2009). Reproduction studies in rats using subcutaneous fluticasone propionate at daily dosages of 50 ug/kg (approximately 2 times the maximum recommended daily intranasal dosage in adults on a $\mu g/m^2$ basis) have shown no evidence of impaired fertility although prostate weight was significantly reduced at 50 µg/kg (American Society of Health System Pharmacists, 2009).
Endocrine disrupting activities of concern:	Glucocorticoid agonist

Other significant toxic effects:	Fluticasone propionate is poorly absorbed from the respiratory and GI tracts following nasal inhalation of the drug as an aqueous spray, with a systemic bioavailability of less than 2%. A major portion of intranasal doses are swallowed and will be subject to extensive first-pass metabolism in the liver (American Society of Health System Pharmacists; 2009). Dermal absorption of fluticasone is also poor, with studies in humans and rats showing less than 5% of the dose absorbed systemically via the skin (US Natl Inst Health, 2009). The majority of fluticasone propionate that is delivered to the lung is systemically absorbed with bioavailability of fluticasone propionate in humans being around 18%.(US Natl Inst Health, 2009 Fluticasone propionate is widely distributed following iv administration. However the extent of distribution following intranasal administration is unknown. Fluticasone propionate is approximately 91% bound to human plasma proteins but not appreciably bound to human transcortin (corticosteroid-binding globulin; American Society of Health System Pharmacists; 2009). Fluticasone propionate can cross the placenta of rats or rabbits. It is not known if it is present in human milk following maternal intranasal administration but other corticosteroids do pass into human milk and it is transferred to milk in lactating rats (American Society of Health System Pharmacists, 2009). Fluticasone propionate undergoes rapid hepatic metabolism by CYP3A4, with the principal metabolite being an inactive 17β-carboxylic acid derivative (American Society of Health System Pharmacists, 2009). Total faecal excretion ranges from 87-100% of oral dose (American Society of Health System Pharmacists, 2009). Fluticasone has low mammalian acute toxicity orally (LD_{50} rat = >2000 mg/kg) and dermally (LD_{50} rat = >1000 mg/kg) but higher inhalation toxicity (LC_{50} rat = >40.77 mg/m^3/h) ((American Society of Health System Pharmacists, 2009). Chronic exposure of humans to fluticasone is well tolerated. There is no evidence of carcinogenicity in human or animal studies and no evidence for genotoxicity from *in vivo* or *in vitro* studies (American Society of Health System Pharmacists, 2009).
Study estimate of anticipated exposure via drinking water	Limited intake via drinking water is predicted to occur following both conventional and advanced treatment processes in all age groups. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.024; Toddler 0.016; Adult 0.005. Intakes (ug/kg bw/d) based on advanced treatment: Infant 0.005; Toddler 0.003; Adult 0.001
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes and conservative assumptions is 0.024 µg/kg bw/d for infants, this would suggest a margin of safety of around 4 times against the proposed study-specific exposure limit.
References:	American Society of Health System Pharmacists; AHFS Drug Information 2009. Bethesda, MD. (2009), p. 2897 Bidleman, T.F. (1988). Atmospheric processes. Wet and dry deposition of

	organic compounds are controlled by their vapour-particle partitioning. Environ Sci Technol 22: 361-367 (1988). Hazardous Substances Data Base (2009). Futicasone. Available at: http://toxnet.nlm.nih.gov/cgi-bin/sis/search/a?dbs+hsdb:@term+@DOCNO+7740 Meylan, W.M et al (1992). Molecular topology/fragment contribution method for predicting soil sorption coefficients. Environ Sci Technol 26: 1560-67. Meylan, W.M et al (1999). Improved method for estimating bioconcentration/bioaccumulation factor from octanol/water partition coefficient. Environ Toxicol Chem 18: 664-72. Salgado, R et al (2010). Analysis of 65 pharmaceuticals and personal care products in 5 wastewater treatment plants in Portugal using a simplified analytical methodology. Water Sci. & Technol, 2862-2871. US Natl Inst Health; DailyMed. Current Medication Information for Cutivate (fluticasone propionate) cream (April 2008). Available from, as of June 29, 2009: http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?id=7340 Wishart DS et al; DrugBank: a comprehensive resource for in silico drug discovery and exploration. Nucleic Acids Res. 2006 1;34. Available from, as of Apr 22, 2009: http://www.drugbank.ca

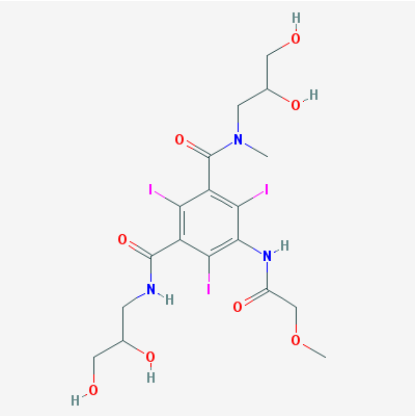
Hazard Assessment

Substance name:	**Iopamidol** 
CAS No.:	60166-93-0
Synonym(s):	(S)-N,N'-bis(2-Hydroxy-1-(hydroxymethyl)ethyl)-2,4,6-triiodo-5-lactamidoisophthalamide; 1,3-Benzenedicarboxamide, N,N'-bis(2-hydroxy-1-(hydroxymethyl)ethyl)-5-((2-hydroxy-1-oxopropyl)amino)-2,4,6-triiodo-, (S); Gastromiro; Iopamidolum; Iopamiro; Iopamiron; Isovue; Jopamiron; L-(+)-N,N'-Bis(2-hydroxy-hydroxymethylethyl)-2,4,6-triiodo-5-lactamide isophthalamide; L-5alpha-Hydroxypropionylamino-2,4,6-triiodoisoftal-di(1,3-diidrossi-2-propilamide); Niopam; Oypalomin; Solutrast.
Use and potential human exposure routes:	Iopamidol is a non-ionic, water soluble contrast agent used in myelography, arthrography, nephroangiography, arteriography and other radiological procedures. More than 600 million X-ray examinations are conducted yearly and around 75 million of these procedures are performed with the use of a contrast medium, such as iopamidol (Amersham Health, 2002). Occupational exposure to iopamidol may occur through inhalation and dermal contact with this compound at workplaces where iopamidol is produced and distributed. The general population may be exposed via the i.v. route through medical administration of this compound.
Established/Study-specific exposure limit:	**Therapeutic doses by age:** < 1month : 10 ml of 76% Iopamidol 1 month to 12 years : 15 ml of 76% Iopamidol (15 – 100 ml) 12 years to 18 years : 125 ml of 76% Iopamidol > 18 years : 250 ml of 41% Iopamidol Each 1 ml of Isovue-370 (Iopamidol injection 76%) provides 755 mg Iopamidol with 1 mg tromethamine and 0.48 mg edentate calcium disodium. The solution contains approximately 0.053 mg (0.002 mEq) sodium and 370 mg organically bound iodine per ml. Therefore the lowest therapeutic dose = 7550 mg of iopamidol (10ml of 755 mg/ ml) for a 5 kg infant (1510 mg/kg bw/day). **Study-specific Exposure Limit**= 1.510 mg/kg bw/day (1510 µg/kg bw/day).
Basis for exposure limit:	Assuming an uncertainty factor of 1000 from the minimum human therapeutic dose (uncertainty factor of 100 for protection of infants and an extra uncertainty factor of 10 included as there is no chronic toxicity data available).
Evidence for human relevant endocrine disrupting potential:	None Identified.

Other significant toxic effects:	Adverse drug reactions include: Arrhythmias, arterial spasm and flushing (MIMS, 2011). Iopamidol has been demonstrated to increase the risk of renal toxicity in patients with known or suspected hepatic or billary disorder given concurrent oral use. (Gale *et al*., 1984). However, the risk is not considered to be greater than for other currently used contrast agents. *In vitro* haematology studies have shown that radiopaque contrast agents, such as iopamidol, may depress plasma coagulation factors, including prothrombin time, partial thrombopalstin time and fibrinogenen, while increasing platelet and red blood cell aggregation. Transitory changes may occur in red cell and leucocyte counts, serum calcium, serum creatinine, serum glutamic oxaloacetic transaminase (SGOT) and uric acid in urine; transient albuminuria may occur. **Pregnancy Category B** Reproduction studies in rats and rabbits at doses up to 2.7 and 1.4 times the maximum recommended human dose (1.48 g/kg in a 50 kg individual), respectively, have shown no evidence of impaired fertility or embryo toxicity. However, as there are no adequate and well-controlled studies in pregnant women and animal reproduction studies are not always predictive of human responses, this drug is only recommended for use during pregnancy if required.
Primary mode of endocrine activity	None Identified
Study estimate of anticipated exposure via drinking water	Limited intake via drinking water is predicted to occur in all age groups following conventional treatment processes. No intake is predicted to occur following advanced treatment processes in any age group. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.036; Toddler 0.024; Adult 0.008.
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	No evidence to either prove or disprove endocrine disrupting activity per se has been identified for this substance. Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes and conservative assumptions is 0.036 µg/kg bw/d for infants, this would suggest a margin of safety of >10,000 times against the study-specific exposure limit. These figures were based on modelled levels in drinking water calculated using measured data from a non-UK study, undergoing conventional water treatment; as such they are conservative in nature. However, with such a large predicted margin of safety it is unlikely that there is cause for concern regarding exposure to iopamidol via drinking water.
References:	Amersham Health (2002) Annual Report (as cited in Christiansen, 2005). Christiansen, C. (2005) X-ray contrast media – an overview. *Toxicology* **209**: 185 – 187. Gale, M. E., Robbins, A. H., Hamburger, R. J., Widrich, W. C. (1984) Renal Toxicity of Contrast Agents: Iopamidol, Iothalamate, and Diatrizoate. *AJR* **142**: 333 – 335. Orgyn (undated) Iopamidol http://www.orgyn.com/resources/genrx/D001567.asp [Accessed 06.03.2012] Medscape Reference (undated) Iopamidol http://reference.medscape.com/drug/isovue-scanlux-iopamidol-343763 [Accessed 06.03.2012]

	MIMS (2011) USA Drugs Information: Iopamidol. http://www.mims.com/USA/drug/info/iopamidol/?type=full&mtype=generic [Accessed 18.09.2011]

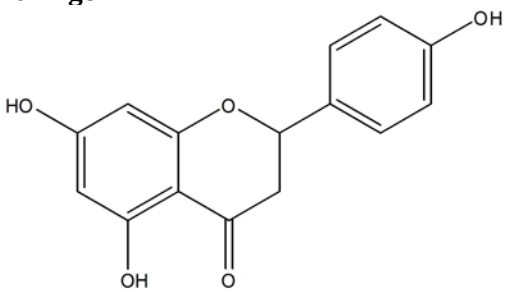
Hazard Assessment

Substance name:	**Iopromide** 
CAS No.:	73334-07-3
Synonym(s):	Ultravist 150; Ultravist 240; Ultravist 300; Ultravist 370
Use and potential human exposure routes:	Iopromide is a nonionic, water soluble x-ray contrast agent for intravascular administration. Iopromide is indicated in cerebral arteriography; coronary arteriography and left ventriculography; in aortography and visceral angiography; peripheral arteriography and venography; excretory urography; brain and body imaging (drugs.com). Occupational exposure to iopamidol may occur through inhalation and dermal contact with this compound at workplaces where iopromide is produced or used. The general population may be exposed via the i.v. route through medical administration of this compound.
Established/Study-specific exposure limit:	**Therapeutic doses by age:** < 1month : not used 1 month to 12 years : 1mL/kg bw/d 12 years to 18 years : 1 mL/kg bw/d or 50mL > 18 years : 50 ml ULTRAVIST Injection is available in three strengths: 240 mg I/mL provides 498.72 mg/mL iopromide, 300 mg I/mL provides 623.4 mg/mL iopromide, 370 mg I/mL provides 768.86 mg/mL iopromide. Therefore the lowest therapeutic dose (1mL/kg bw/d) of the lowest strength preparation (498.72 mg/mL iopromide) would result in a dose of 2494 mg of iopromide for a 5kg infant. **Study-specific Exposure Limit**= 0.499 mg/kg bw/day (499 µg/kg bw/day).
Basis for exposure limit:	Assuming an uncertainty factor of 1000 from the minimum human therapeutic dose (uncertainty factor of 100 for protection of infants and an extra uncertainty factor of 10 included as there is no chronic toxicity data available).
Evidence for human relevant endocrine disrupting potential:	Using a precautionary approach, this substance was identified as of possible concern following expert advice from the clinician within the project team. However, no evidence suggestive of endocrine disrupting activity per se was identified for iopromide in subsequent literature review.

Iopromide

Other significant toxic effects:	Adequate and well-controlled studies in humans have not been carried out. **FDA Pregnancy Category B** Reproduction studies in rats and rabbits have not shown that iopromide, administered in doses up to 2.2 times the maximum recommended dose for a 50–kg human, causes harm to the foetus. Long-term animal studies to evaluate the carcinogenic potential of iopromide have not been performed. Iopromide demonstrated no mutagenic effects in the Ames test, in an *in vitro* human lymphocyte test for chromosomal aberrations, and in *in vivo* mouse studies, including the micronucleus test and dominant lethal assay. (source drugs.com; dailymed.nlm.nih.gov)
Primary mode of endocrine activity	None Identified
Study estimate of anticipated exposure via drinking water	Limited intake via drinking water is predicted to occur in all age groups following conventional treatment processes. No intake is predicted to occur following advanced treatment processes in any age group. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.105; Toddler 0.07; Adult 0.023.
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	No evidence to either prove or disprove endocrine disrupting activity per se has been identified for this substance. Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes and conservative assumptions is 0.105 µg/kg bw/d for infants, this would suggest a margin of safety of >10,000 times against the study-specific exposure limit. These figures were based on modelled levels in drinking water calculated from measured data obtained from non-UK study, undergoing conventional water treatment; as such they are conservative in nature. However, with such a large predicted margin of safety it is unlikely that there is cause for concern regarding exposure to iopromide via drinking water.
References:	Drugs.com: available at http://www.drugs.com/mmx/iopromide.html (accessed Dec 2011) Dailymed: available at http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=d96a7883-d160-40a1-bdbd-2e8d7877cd18#section-16 (accessed Dec 2011)

Hazard Assessment

Substance name:	**Naringenin**  5,7-Dihydroxy-2-(4-hydroxy-phenyl)-chroman-4-one (**2S-naringenin**, flavanone)
CAS No.:	67604-48-2; 480-41-1
Synonym(s):	4',5,7-Trihydroxyflavanone; (2S)-5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-4H-chromen-4-one; 4H-1-benzopyran-4-one, 2,3-dihydro-5,7-dihydroxy-2-(4-hydroxyphenyl)-, (2S)-; (S)-5,7-Dihydroxy-2-(4-hydroxy-phenyl)-chroman-4-one
Use and potential human exposure routes:	Naringenin, a precursor to genistein, occurs naturally in some citrus fruits particularly grapefruit, where it is mainly found in glycoside-forms such as naringenin-7-rhamnoglucoside or naringenin-7-glucoside (CDC, 2009; Nahmias et al., 2008). Although no data on the specific intake of naringenin has been identified it is noted that the daily intake of mixed flavonoids with a Western-type diet is of the order 0.5-1 g, although actual daily intake is frequently lower (Felgines et al., 2000)
Established/Study-specific exposure limit:	As a naturally-occurring constituent of some plant foods, no environmental limits or acceptable daily intakes have been considered to be appropriate.
Basis for exposure limit	Not applicable
Evidence for human relevant endocrine disrupting potential:	Although naringenin was not specifically identified as of concern by IUPAC (Lintelmann, 2003), its flavonone structure qualifies it as a potential phytoestrogen. Given its nature as a naturally-occurring substance, it was specifically excluded from consideration under the EU prioritisation exercise (BKH, 2000). Furthermore, it was not considered by FSA (2003) although some related prenylated flavonoids which occur predominately in beer hops, were considered. Naringenin (Nar) has been suggested to have specific protective benefits relating to osteoporosis, cancer and cardiovascular diseases, which have been suggested as attributable to its anti-oestrogenic and oestrogenic activities. Examples of its reported effects include inhibition of ERα signaling by interference with ERα-mediated ERK activation and of phosphoinositide 3-kinase signaling pathways, in the absence of effects at a transcriptional level. Studies have also suggested that it induces ERα-depalmitoylation more rapidly than 17β-oestradiol and impedes ERα binding and signalling. However, naringenin has also been suggested to induce ER-dependent (palmitoylation-independent) activation of p38 kinase resulting in an anti-proliferative effect on cancer cells (Galluzo et al., 2008). There is, however, in vitro evidence of species differences in ER responsiveness with no response being seen in a medaka sex reversal/*vtg* gene expression assay while mammalian in vitro assays showed a positive response (Zierau et al., 2004).

	Kuiper et al. (1998) estimated that the oestrogenic activity of naringenin compared with 17 β-oestradiol was $1x10^{-4}$ to $1x10^{-3}$ for ERα and $<2x10^{-3}$ for ERβ. In contrast, Ruh et al (1995) report that doses of up to 40 mg/rat, did not cause significant increase in any 17β-oestrodiol-induced responses but that co-administration of 17β-oestrodiol at 0.5 µg/rat with naringenin at 30 mg/rat significantly inhibited the oestrogen-induced uterine wet weight, DNA synthesis, PR binding, and peroxidase activity. Further, while in vitro 1 nM oestradiol increased (3- to 4-fold) growth of MCF-7 cells, 1-1000 nM naringenin had no effect on cell proliferation. However, co-administration of 1 nM oestradiol with 1000 nM naringenin resulted in a significant decrease in oestrogen-induced cell growth. Similar effects were also noted in MCF-7 cells transiently transfected with a pS2 promoter-regulated luciferase reporter gene in which naringenin alone exhibited weak oestrogenic activity but inhibited the action of oestradiol when co-administered
Endocrine disrupting activities of concern:	Weak oestrogen; anti-oestrogen
Other significant toxic effects:	Flavanones are efficiently absorbed with their bioavailability being influenced by the glycosidic moiety. For example, in rats, the rate of naringenin appearance in plasma varied depending upon the form the substance was presented in (aglycone, glucoside or rhamnoglucoside) with more rapid uptake seen with naringenin or the 7-glucoside. However, the maximum plasma levels obtained were similar. After absorption, naringenin is largely esterified to sulfate groups (~90%) with circulating metabolites being glucurono- and sulfoconjugated derivatives. It may also be metabolized into phenolic acids (Felgines et al., 2000). As a constituent of plants considered appropriate for human nutrition, no hazard profile has been developed for naringenin, other than in relation to its hormonal activity. Citrus flavonoids have been investigated for biological activity, and anti-inflammatory, anticarcinogenic, and antitumor activities have been reported (Felgines et al., 2000). These proposed activities properties are generally attributed to their antioxidant activity (Benavente-García & Castillo, 2008). However, the impact of naringenin exposure on survival and morphogenesis of amphibian embryos has been evaluated in an AMPHITOX assay. Effects were noted on mortality and malformation incidence which showed concentration-dependence with, for example, a level of 10 mg/L causing 100% malformation and 30% mortality. The main changes seen comprised reduced body size, axial curves, microcephaly, abdominal oedema and underdeveloped gill. The teratogenic index (TI) was reported as '2' indicating a high hazard potential. Overall, it was concluded that amphibian early life stages were most susceptible, especially at >5 mg/L (Perez-Coll & Herkovitis, 2004).
Study estimate of anticipated exposure via drinking water	No intake anticipated from drinking water from advanced treatment process. Intake (µg/kg bw/d) based on conventional treatment: Infant 0.029; Toddler 0.019; Adult 0.006
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	Although data from the study by Perez-Coll & Herkovitis (2004) suggest a high embryo-toxic potential may exist for naringenin in amphibian species, available data on the oestrogenic activity of the substance - including studies on rodent-based assays - suggest that in mammalian species it is only a weak

	oestrogen that may, in some circumstances, show some anti-oestrogenic potential. Together with its presence in long-established food sources and the known activities of flavinoids in general, it therefore appears unlikely that the amphibian data should be considered of any relevance to assessing risks that may be posed to humans from exposure either in food or via drinking water. Even so, it is instructive to consider the 'worst case' estimated drinking water level for this substance of 190.57 ng/L (for conventional treatment) and compare it with the levels at which amphibian embryo toxicity was noted to be apparent >5 mg/L (Perez-Coll & Herkovitis, 2004) since this indicates that the level at which amphibian effects can be clearly discerned are >25,000-times those predicted. An alternative approach to informing on the level of concern that might be warranted is to consider the evidence regarding daily dietary intakes of the substance. As noted above, intake of all flavonoids for individuals on a Western-type diet has been estimated at 0.5-1 g. Even if only 1% of this was found to be attributable to naringenin, this would equate to a potential daily intake of the order of 5 mg/d/person, equivalent to – say – 1000 µg/kg bw/d for a 5 kg infant. Comparing this to the highest predicted intake for this study, 0.029 µg/kg bw/d for infants given drinking water from conventional treatment plants, show a difference of the order of >30,000 between dietary and drinking water intake. Hence, any exposure that might occur via drinking water is not considered to be of concern
References	Benavente-García O. & Castillo J. (2008) Update on uses and properties of citrus flavinoids: new findings in anticancer, cardiovascular and anti-inflammatory activity. J. Agric. Food Chem., 56, 6185-6205. BKH (2000) Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption. Bkh consulting engineers. Available at Internet site http://ec.europa.eu/environment/endocrine/strategy/substances_en.htm. CDC (2009) Fourth National Report on Human Exposures to Environmental Chemicals - . Chemical Information Phytoestrogens. Centers for Disease Control and Prevention, Atlanta, GA, USA. Available at Internet site http://www.cdc.gov/exposurereport/data_tables/Phytoestrogens_ChemicalInformation.html; Felgines C. et al. (2000) Bioavailability of the flavanone naringenin and its glycosides in rats. American Journal of Physiology – Gastrointestinal and Liver Physiology, 279, 1148-1154. Available at Internet site http://ajpgi.physiology.org/content/279/6/G1148.abstract FSA (2003) COT Report - Phytoestrogens and Health. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, Food Standards Agency, UK. Available at Internet site http://www.food.gov.uk/multimedia/pdfs/phytoreport0503. Galluzo P. et al. (2008) The Nutritional Flavone Naringenin triggers Antiestrogenic Effects by Regulating Estrogen Receptor α-Palmitoylation. Endocrinology, 149, 2567-2575. Available at Internet site http://endo.endojournals.org/content/149/5/2567.short

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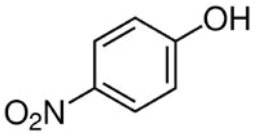
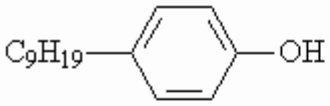
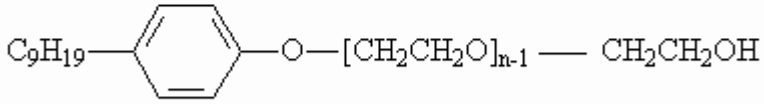
Hazard Assessment

Substance name:	**4-Nitrophenol** 
CAS No.:	100-02-7
Synonym(s):	p-Nitrophenol; Phenol, 4-nitro; Niphen; 4-Hydroxynitrobenzene; Paranitrophenol; Phenol, p-nitro; p-Nitrofenol; Mononitrophenol; Paranitrophenol; PNP; EPA Pesticide Chemical Code 056301
Use and potential human exposure routes:	4-Nitrophenol is used in the manufacture of drugs (e.g. acetaminophen), fungicides, consumer dyes and methyl and ethyl parathion insecticides (ATSDR, 1990). 4-Nitrophenol is also present in light-duty gasoline and diesel exhaust fumes. Exposure of humans to 4-Nitrophenol may occur via exposure to contaminated air, water and soil. Inhalation and oral exposures are considered the most relevant pathways for human toxicity.
Established/Study-specific exposure limit:	Study-specific exposure limit: 0.1 µg/kg bw/d There are no current regulatory limits for environmental exposure in the EU or US.
Basis for exposure limit:	Immature male rats implanted with a silastic tube containing crystalline testosterone and subcutaneously dosed with 0.1 mg/kg bw/d 4-Nitrophenol for 5 days showed significant decreases in weights of seminal vesicles, ventral prostate, levator ani plus bulbocavernosus muscle, and gland penis. Plasma FSH and LH levels were significantly increased. No adverse effects were observed at 0.01 mg/kg bw/d and a NOAEL for testicular toxicity of 0.01 mg/kg bw/d was derived (Li *et al*., 2006). An uncertainty factor of 100 was applied to the NOAEL reported by Li *et al*. (2006) to derive a endocrine-relevant study-specific exposure limit value
Evidence for human relevant endocrine disrupting potential:	The study by Li *et al*. (2006) discussed above showed a series of effects in male reproductive organs. In addition this study also reports that ovarectomised immature female rats given 10 or 100 mg kg^{-1} 4-Nitrophenol/day subcutaneously showed a significant increase in uterine weight; no effects were noted at 1 mg/kg/d. *In vitro*, 4-Nitrophenol has shown affinity for both oestrogen (ER) and androgen (AR) receptors *in vitro* (Taneda *et al*., 2004); no comparison with E_2 was made.
Endocrine disrupting activities of concern:	**Oestrogenic and anti-androgenic** (sexual dimorphism)
Other significant toxic effects:	After oral, dermal, intravenous or intraperitoneal dosing with 4-Nitrophenol to rats, mice, dogs or rabbits, most of the dose (up to 95%) was excreted as the glucuronide and sulphate conjugates via the urine within 24–48 h. Only small amounts were eliminated via faeces (about 1%) or as unchanged substance (about 2–7%; WHO, 2002). 4-Nitrophenol has moderate acute oral toxicity (LD_{50} rat =220 - 620 mg/kg bw; LD_{50} mice = 380 – 470 mg/kg bw). Chronic inhalation of 4-Nitrophenol has been shown to lead to methaemoglobinaemia in humans; this endpoint is also relevant for oral

	exposures. Other effects include decrease in body weight gain, changes in organ weight and focal fatty degeneration of the liver and haematological changes (WHO, 2002). In a 28-day study to OECD Test Guidelines 407, significant increases in locomotor inhibition and hepatotoxicity were reported in Sprague-Dawley rats given 4-Nitrophenolat 70, 210 or 630 mg/kg bw/d by gavage relative to controls. A slight increase in leukocyte count was also seen at 210 and 630 mg/kg bw/d (Andrae *et al*., 1981). 4-Nitrophenol is not considered carcinogenic. In some *in vitro* assays, 4-Nitrophenol has been shown to be positive for mutagenicity, however, no data are available on the mutagenic potential *in vivo* (WHO, 2002).
Study estimate of anticipated exposure via drinking water	Some intake via drinking water is predicted to occur for infants and toddlers following either conventional or advanced treatment processes, and for conventional treatment in adults. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.020; Toddler 0.013; Adult 0.004. Intakes (ug/kg bw/d) based on advanced treatment: Infant 0.002; Toddler 0.001
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	The study-specific exposure limit derived for 4-Nitrophenol is 0.1µg/kg bw/d, based on endocrine-relevant reproductive and developmental NOAEL of 0.01 mg/kg bw/d and an uncertainty factor of 100. Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes and conservative assumptions, is 0.02 µg/kg bw/d for infants, this would suggest a margin of safety of only 5-times against the study-specific exposure limit. Such a low margin of safety may give cause for concern, especially for susceptible groups such as infants and toddlers. However, removal rates following advanced water treatment processes are considerably higher, affording margins of safety against the study-specific exposure limit of 50 and 100 in infants and toddlers respectively.
References*:*	Andrae, U., Bieniek, D., Freitag, D., Goeggelmann, W., Huber, W., Klein, W., Kotzias, D., Lahaniatis, E., Monsour, M., Parlar, H., Politzki, G., Rohleder, H., Rott, B., Scheunert, I., Spieser, H., Biswanathan, R. (1981) Feasibility of test guidelines and evidence of the base-set testing accordin to the chemicals legislation. Muenchen, Gesellschaft für Strahlen and Umweltfoschung mbH (in German) (as cited in WHO, 2000). ATSDR (1990) Agency for Toxic Substances and Disease Registry: Toxicological Profile for Nitrophenols (Draft). Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA. Li, C. M., Tandeda, S., Suzuki, A. K., Furuta, C., Watanabe, G., Taya, K. (2006) Estrogenic and anti-androgenic activities of 4-Nitrophenol in diesel exhaust particles. *Toxicology and Applied Pharmacology* **217**: 1 – 6. Taneda, S., Mori, Y., Kamata, K., Hayashi, H., Furuta, C., Li, C., Seki, K., Sakushima, A., Yoshino, S., Yamaki, K., Watanabe, G., Taya, K., Suzuki, A. K. (2004) Estrogenic and anti-androgenic activity of nitrophenols in diestel exhaust particles (DEP). *Biol. Pharm. Bull.* **27**: 835 – 837. WHO (2002) Concise International Chemical Assessment Document 20: Monoditrophenols. ISBN 9241530200.

Hazard Assessment

Substance name:	**Nonylphenol***;* **4-Nonylphenol, branched** **4-Nonylphenol monoethyoxycarboxylate (NP1EC);** **4-Nonylphenol diethyoxycarboxylate (NP2EC);** **4-Nonylphenol monoethoxylate (NP1EO); 27980-36-3** **4-Nonylphenol diethoxylates (NP2EO); and** **4-Nonylphenoltriethoxylate (NP3EO)**  Nonylphenol  Nonylphenol ethoxylate, generalised formula n = number of ethyle oxide units
CAS No.:	Nonylphenol – **25154-52-3** 4-Nonylphenol, branched **- 84852-15-3** NP1EC – **3115-49-9** NP2EC – **106807-78-7** NP1EO – **27980-36-3** NP2EO – **9016-45-9** NP3EO – **51437-95-7**
Synonym(s):	Multiple including Nonylphenol; NP; isononylphenol; phenol, nonyl-, branched; para-nonylphenol; monoalkyl (C_{3-9}) phenol; Nonylphenol ethoxylate: NPE; nonylphenol polyoxyethylene ether; nonylphenol polyethylene glycol; nonylphenol polyethylene glycol ether; polyoxyethylene nonylphenol ether
Use and potential human exposure routes:	The term "nonylphenol" (NP) has historically been applied to a large number of isomeric substances of general formula $C_6H_4(OH)C_9H_{19}$. These vary in structure with respect to the substitution position of the nonyl groups on the phenol, and the degree of branching of the nonyl group (EU, 2002). NP is manufactured by reacting mixed nonenes with phenol (EU, 2002). The majority of production is used as a starting material for synthesis of nonylphenol ethoxylates (NPEs). Most of the rest is used as monomer in polymer production. In turn, NPEs are manufactured by reacting NP with ethylene oxide to form polyethylene oxide chains of the desired length. NPEs are non-ionic surfactants used widely (e.g. ingredients in cleaners and detergents, household cleaning, personal-care products and for emulsion polymerisation and polymer stabilisation, textile processing, in agricultural chemicals, pulp and paper processing, metal and mineral processing, latex paints, wetting agents and emulsifiers, foaming agents, inks, adhesives, and in pharmaceuticals). Usage of NP in some sectors is associated with

	particular environmental concerns but usage appears to be falling in Europe due to a Voluntary Agreement by industry. However, NPE production/use appears relatively stable (EU, 2002) with NPEs believed to account for 90-95% of all APE production. NPEs are subject to microbial degredation in STWs and may also undergo environmental degradation into nonylphenol; the process involves cleaved off the ethylene oxide chain until only short-chain NPEs remain (typically mono- and diethylene oxides). Oxidation of the oligomers creates a corresponding carboxylic acid and NP. The forms of NP are not readily biodegradable showing negligible abiotic degradation in water (half-life in surface water = about 30 days). A similar half-life mainly due to biodegradation occurs in soil (EU, 2002). NP bioconcentrates to a significant extent in aquatic species despite relatively rapid excretion/metabolism (EU, 2002); e.g. fresh water species BCFs = 1.4-230. The environmental toxicity of longer chain forms of NP is significantly lower than shorter hydrophobic chains (e.g. Fish LC_{50} = 110 mg/L for NP16EO, 11.2 mg/L for NP9EO and 1.4 mg/L for NP; Lintelmann et al., 2003). Historical levels of NP in rivers in England and Wales were generally in the range 0.2-12 µg/L (180 µg/L in the Aire; Lintelmann et al, 2003; OSPAR, 2004). Levels of about 0.08-5.2 µg/L are reported for a UK estuary while liver total nonylphenol ethoxylate content of 9.5 mg/kg/d (dry weight) was reported for river fish (OSPAR, 2004). River levels in a Swiss river have been reported at 0.3-45 µg/L for NP and 0.3-99 µg/L for NPEs (OSPAR, 2004). As a consequence of the very wide use pattern of these substances, possible routes of human exposure include: dermal contact and inhalation for workers involved in manufacture and use; dermal contact and inhalation for consumers of household pesticide products; and orally via various environmental sources for the general population (EU, 2002). The extent of human exposure from background environmental sources (i.e. excluding the effect of any potential local point source such as an industrial facility) was, estimated at only 5.31 µg/kg/day based on historic data(EU, 2002). Canadian Authorities have estimated exposure to be mainly attributable to surface water (0.39 µg/kg/day), food packaging (17 µg/kg/day) and meat (17 µg/kg/day); other sources only contributing a minimal additional load (UNEP, 2004).
Established/Study-specific exposure limit:	Swedish recommended limit for NP in sludge for agricultural use = 50 mg/kg bw/d (EC, 2002). UK Operational EQS were established based on non-hormone related endpoints (RPA, 1999) for NP release of 1 µg/L for fResh and marine waters (annual average) of 1 ug/L with a freshwater maximum acceptable concentration of 2.5 ug/L. However, under the Water Framework Directive, stricter UK standards are now in place of: Inland surface waters AA-EQS = 0.3 ug/L; Other surface waters AA-EQS = 0.3 ug/L Inland surface waters MAC-EQS = 2.0 ug/L Other surface waters MAC-EQS = 2.0 ug/L (EU, 2002; EA, 2012). This was based on concerns expressed by SCHER (2001) on the potential environmental levels of NP.

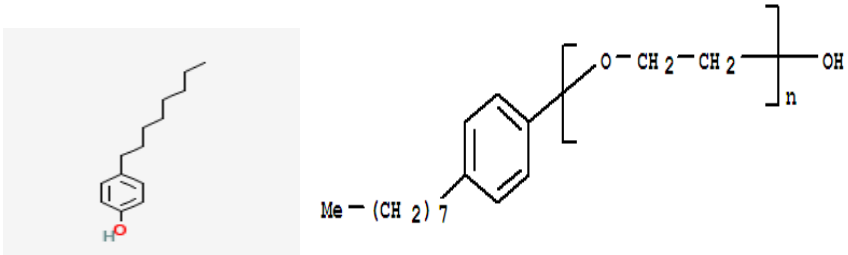
	Under REACH Regulation 552/2009, there is a Annex XVII restriction on the manufacture, placing on the market and use of nonylphenol and its ethoxylates for certain uses (EA, 2010).
Basis for exposure limit:	UK EQS initially based on generic control requirements but now controlled under the Water Framework Directive (EC, 2002; EA, 2012).
Evidence for human relevant endocrine disrupting potential:	In the EU endocrine prioritisation exercise (BKH, 2000), nonylphenol was categorised as a Category 1 endocrine disrupter (*at least one study providing evidence of endocrine disruption in an intact organism. Not a formal weight of evidence approach*) based on reports of it causing increased uterine weight and inducing vitellogenin levels and reducing testes weight in animal models and was positive for oestrogenicity in a range of in vitro assays. IUPAC (Lintelmann et al, 2003) also drew attention to alkylphenol ethoxylates in general as a cause for concern, and reports that it is the degredation products arising from microbial processes (rather than the parent forms) that possess oestrogen activity. 4-NP has been shown to be oestrogenic in a wide range of in vitro and rodent and fish in vivo assays; its potency relative to 17β-oestradiol was estimated at 10^{-4} in an E-screen (Lintelmann et al, 2003). The considerable body of evidence (in vivo and in vitro) demonstrating the oestrogenic potential of NP was further acknowledged by EC (2002) and UNEP (2004); overall, the estimated potency of NP was considered to be about 3-6 orders of magnitude less than oestradiol. NP is also an established reprotoxin with effects reported for a range of fertility and reproductive performance endpoints; a NOAEL of 15 mg/kg/day was proposed and the established LOAEL for testicular toxicity is 100 mg/kg/day. Developmental studies conducted to standard designs suggested maternal and foetal NOAELs of 75 and 300 mg/kg bw/d respectively; some non-standard studies have reported developmental effects at lower levels. The identified reproductive/developmental effects are generally attributable to NP's oestrogenic properties. Given this, a NOAEL of 1.5 mg/kg bw/d - based on reproductive toxicity - is identified as the critical effect for risk characterisation (EC, 2002). A more conservative LOAEL of 12 mg/kg/d was identified by Canadian authorities based on changes in a rat multigeneration study (UNEP, 2004).
Endocrine disrupting activities of concern:	Oestrogenic
Other significant toxic effects:	Nonylphenols were classified in Annex 1 of Directive 67/548/EEC (EU, 2002) as: Xn; R22 – Harmful if swallowed C, R34 – Causes burns N, R50-53 Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment. In the US, NP4,5,6,and 9E forms are classified as slight to non-toxic to mammals; EPA Category III or IV but that of NP is higher. A common acute and chronic RfD for nonylphenol ethoxylates of 0.1 mg/kg/d has been proposed in the US (Bakke, 2003). Absorption of NP from the GI-tract is rapid and extensive, and is followed by metabolism (via glucuronidation or sulphation) which includes a first-pass effect that results in only <10% becoming systemically available via the oral route. Major routes for excretion are the faeces and urine (EU, 2002).

	NP has moderate mammalian acute toxicity (rat oral LD_{50} = 1200-2400 mg/kg (male) & 1600 – 1900 mg/kg (female)). Liquid NP may be irritant but is probably not a sensitizer (EC, 2002). For comparison, Bakke (2003) reported mammalian LD_{50} = 580-1620 mg/kg for NP and 4290-7400 mg/kg for NP4E. NP is not considered mutagenic and is of low carcinogenic potential. The EU risk assessment (2002) identified a oral repeat dose LOAEL of 15 mg/kg bw/d, based on renal changes though noting that liver toxicity may occur at higher doses. Bakke (2003) suggested a chronic NOAEL of 10 mg/kg bw/d based on liver and kidney effects in rats, and notes this as protective of teratogenic and reproductive effects. Bakke (2003) also suggests the sub-chronic and chronic NOAEL for NP9E in rats and dogs as falling into the range 10-40 mg/kg/d
Study estimate of anticipated exposure via drinking water	No intake of NP is predicted to occur from drinking water produced using advanced treatment process. Intake (µg/kg bw/d) of NP from drinking water from conventional treatment: Infant 0.041; Toddler 0.027; Adult 0.009. Predicted intakes of the individual NPEs considered range between 0 and 0.255 µg/kg bw/d based on the advanced treatment process, and between 0.004 and 10.67 µg/kg bw/d based on the conventional treatment process
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	A number of rigorous assessments have clearly established that the principle cause for concern with regard to nonylphenol and its ethoxylates (i.e. NP and NPEs) is the level of exposure to NP, since the NPEs are of much lower mammalian toxicity and not of concern with regard to endocrine or reproductive effects. Furthermore, although possessing some oestrogenic (10^{-3} to 10^{-6} that of oestradiol) and showing both reproductive and development toxicity, the assessments have established that the principle basis for assessment is the sub/chronic toxicity of NP, and that control on this basis is adequately protective of the other hazardous effects. Given this, the identified repeat dose LOAEL of 15 mg/kg bw/d for NP that was identified by the EC (2002) is considered to be an acceptable basis against which exposure to these substances via drinking water might be assessed. If a precautionary study-specific uncertainty factor of 1,000 (10 for use of a LOAEL as the basis, 10 for interspecies extrapolation and 10 for inter-individual variation) were to be applied to the repeat dose LOAEL, this would suggest a study-specific standard of 15 µg/kg bw/d. However, given the uncertainties with regard to the extent to which other forms of NP (e.g. NP1EC, NP2EC) might conceivably contribute to the overall hazard posed, it is propsed to introduce an additional uncertainty factor of 10 (i.e. 10,000 in all) giving a study-specific exposure limit for NP of 1.5 µg/kg bw/d. Comparing this value to the highest intake predicted in this study of 0.041 µg/kg bw/d for infants given drinking water from a conventional treatment plant, suggests a margin of safety of >35, indicating that there should be little cause for concern. It should also be noted that the modelled predictions of intakes for NP are based on a PEC_{intake} of 0.3 µg/L resulting in drinking water concentrations of only 2 and 270 ng/L, for advanced and conventional treatments respectively. These values also compare favourably with the established UK Operational

	EQS for NP release of 1 µg/L. Further reassurance regarding exposure to NP and its ethoxylates is provided by the restrictions on the use of NPEs in various applications that are now in place across the EU (UNEP, 2004; EA, 2010) which indicate that future levels of exposure of the European population would be confidently expected to show continued reduction and hence any concern would be low.
References	Bakke D (2003) Human and Ecological Risk Assessment of Nonylphenol Polyethoxylate-based (NPE) Surfactants in Forest Service Herbicide Applications. USDA Rorest Service, Pacific Southwater Region (Region 5) USA. BKH (2000) Towards the establishment of a priority list of substances for further evaluation of their role in endocrine. Bkh consulting engineers. Available at Internet site http://ec.europa.eu/environment/endocrine/strategy/substances_en.htm. EA (2010) REACH Annex XVII Restrictions. Nonylphenol and its ethoxylates. Guidance Note December 2010. Environment Agency, UK. Available at Internet site http://www.environment-agency.gov.uk/static/documents/Business/Guidance_pack__NP_NPE.pdf. EA (2012) Chemical Standards Report: Nonylphenol CAS RN: 25154-52-3. Environment Agency, UK. Available at http://evidence.environment-agency.gov.uk/ChemicalStandards/loginpage.aspx?id=user. EU (2002) European Union Risk Assessment Report. 4-NONYLPHENOL (BRANCHED) AND NONYLPHENOL CAS Nos: 84852-15-3 and 25154-52-3; EINECS Nos: 284-325-5 and 246-672-0. European Communities, Office for Official Publications of the European Communities, Luxemburg. Available at Internet site http://esis.jrc.ec.europa.eu/doc/existing-chemicals/risk_assessment/REPORT/4-nonylphenol_nonylphenolreport017.pdf. IPCS (2004) Integrated Risk Assessment: Nonylphenol Case Study. Report Prepared for the WHO/UNEP/ILO International Programme on Chemical Safety (WHO/IPCS/IRA/12/04). United Nations Environment Programme, International Labour Organisation and World Health Organisation Lintelmann J. et al (2003) Endocrine Disruptors in the Environmnet. Pure Appl. Chem, 75, 631-681. OSPAR (2004) Hazardous Substances Series. Nonylphenol/ Nonyphenolethoxylates 2001 (2004 Update). OSPAR Commission. RPA (1999) Risk Reduction Strategy for Nonylphenol. Risk & Policy Analysts Ltd, UK. Available at http://archive.defra.gov.uk/environment/quality/chemicals/documents/nonylphenol_rrs.pdf. SCHER (2001) Opinion on the results of the Risk Assessment of: 4-NONYLPHENOL (Branched) AND NONYLPHENOL - CAS No.: 84852-15-3, 25154-52-3 - EINECS No.: 284-325-5, 246-672-0. Report version (Human Health effects) : November 2000 carried out in the framework of Council Regulation (EEC) 793/93 on the evaluation and control of the risks of existing substances1. Opinion expressed at the 22nd CSTEE plenary

	meeting, Brussels, 6/7 March 200. Available at http://ec.europa.eu/health/scientific_committees/environmental_risks/opinions/sctee/sct_out91_en.htm.

Hazard Assessment

Substance name:	**Octylphenol** (OP)*;* **4--Octylphenol-mono-ethoxylate** (OP1EO) **4-tert-octylphenol tri-ethoxylate** (OP3EO) **OP** **OPE** 
CAS No.:	4-tert-Octylphenol - **140-66-9** 4-Octylphenol-mono-ethoxylate (OP1EO) – **Not available** 4-Octylphenol tri-ethoxylate (OP3EO) - **Not available** (A mixed form of octylphenol ethoxylates has CA No. 9036-19-5)
Synonym(s):	For OP - octylphenol PT; para-(or p-)*tert*-octylphenol; p-(1,1,3,3-tetramethylbutyl)phenol; 4-(1,1,3,3-tetramethylbutyl)phenol. For OPEs - Octylphenol ethoxylate; Alkylaryl polyether alcohols; Octylphenoxypolyethoxyethanol; Poly(oxy-1,2-ethanediyl); TRITON™ nonionic surfactants; Octylphenyl polyethylene glycol; Octylphenol polyoxyethylene ether surfactants; α-[(1,1,3,3-Tetramethylbutyl) phenyl]-ω-hydroxy; Polyoxyethylene octylphenol; DOW™ octylphenyl ethoxylate surfactants; Polyethoxylate; Polyoxyethylene; Octylphenyl ether; Polyethylene glycol; Octylphenyl ether; Octoxynol
Use and potential human exposure routes:	The term "octylphenol" (OP) represents a large number of isomeric compounds of the general formula C8H17.C6H4(OH) of which the octyl group (C8H17) may be branched in various ways or form a straight chain. Of the potential isomers, 4-*tert*-octylphenol (CAS No. 140-66-9) is most important commercially (OSPAR, 2004). The main use of 4-*t*-OP is as an intermediate in producing phenol/ formaldehyde resins (98%) and octylphenol ethoxylates (OPEs; 2% of use; OSPAR, 2004); production of OPEs is achieved through addition of ethylene oxide to OP under pressure (RPA, 2008). Minor quantities are also used to produce ether sulphates (OSPAR, 2004). The resultant products are employed in a range of uses such as: tackifier in tyre rubber; ethoxylated resins; electrical insulating varnishes; paper and foundry industries; printing inks; pesticide formulations; water-based paints; and textile auxiliaries (OSPAR, 2004). OP may also occur as impurity in commercial grade nonylphenol. EU production of 4-t-OP has been estimated at around 23,000 tonnes per annum while OPE use amounted to about 1,050 t/a in 2001 (OSPAR, 2004). Other estimates suggest, for OPEs, a EU market of >2,000 t/a in 2005, of which about 100 t were sold in the UK (RPA, 2008). As a consequence of the very wide use pattern of OPs and OPEs, multiple routes of entry of OP and OPEs into the environment may occur. Importantly, OPEs break down sequential to the non-ethoxylated OP form; this is associated with a gradually increase in toxicity, particularly to some

	aquatic species (SEPA, undated). According to the EC PBT criteria, 4-t-OP meets that for 'P' or 'vP' and has a low NOEC of 6,1 µg/L, meeting the T criteria. However, it has a predicted BCF of 297 in fish which is below the B cut-off so cannot be regarded as a PBT. Since 1976, the UK government has had voluntary agreements with industry that OPEs should not be added to domestic detergent while at a European level, OPEs/OP are regulated by Directives 793/93, 76/464/EEC and are a "priority hazardous substance" under the Water Framework Directive (SEPA, undated). River levels of 4-t-OP of <0.02-0.43 µg/L were been reported in several UK rivers in the 1990's and raised levels of 4-t-OP have all so been reported in sediment associated with industrial and STW discharges. 4-t-OP has also been found in European estuarine and coastal waters while OP1EO levels in German rivers were reported to be 0.6-6.3 µg/L (OSPAR, 2004). However, Lintelmann et al. (2003) report levels of OP in rivers in England and Wales to be below the limit of detection (1 µg/L) although levels of up to 5.2 µg/L were reported for estuaries.
Established/Study-specific exposure limit:	OP is a Priority Substance under Annex II of the Directive 2008/105/EC (EC, 2011); annual average EQS values have been established at 0.1 µg/L for inland surface waters and 0.01 µg/L for other surface waters (EU, 2008). These limits are reflected in UK controls, for example, in Scotland (SEPA, 2010; Scottish Government, 2008) and the establishment of SPRI emission reporting thresholds (1.00 kg/yr to water and 1.00 kg/yr to waste water; SEPA, undated). Octylphenol has also recently been proposed as a Substances of Very High Concern by ECHA under REACH Article 57(f) as of equivalent concern based on its environmental endocrine disrupting properties, the first substance to be suggested as warranting SVHC status on this basis (ECHA, 2011).
Basis for exposure limit:	Environmental limits established under the EU WFD
Evidence for human relevant endocrine disrupting potential:	In the EU endocrine prioritisation exercise (BKH, 2000), 4-*t*-OP was categorised as a Category 1 endocrine disrupter (at least one study providing evidence of endocrine disruption in an intact organism. Not a formal weight of evidence approach) and was considered of medium concern; the categorisation was based on reports of effects in fish cell assays and, in rats, on a positive uterotrophic response and an association with developmental delay (vaginal opening). IUPAC (Lintelmann et al., 2003) also drew attention to the alkylphenol ethoxylates in general (and nonyl phenol and 4-*t*-OP in particular) as a cause for concern, and notes that it is the degradation products of the APEs formed through microbial processes (rather than the parent forms) that possess oestrogen activity. 4-*t*-OP has been shown to be oestrogenic in a range of in vitro assays in which it generally shows a potency greater than nonylphenol. The degree of potency found varies considerably between assays, with some reporting a potency of 1000-times less than 17β-oestradiol and others finding a potency of 100,000-fold less. It elicits oestrogen receptor-mediated itellogenin induction in fish and amphibian in vivo (IEH, 1995 & 1999; ECHA, 2011), and it is considered by ECHA to be an endocrine disrupter in fish, amphibian and molluscs, based on the IPCS definition (ECHA, 2011). Limited effects have been reported on mammalian fertility but only at doses at which significant systemic toxicity (including 75% male and 33% female mortality in 1 study) occurred. There was some evidence of developmental

	delay (e.g. in vaginal patency) in offspring but these correlated with low pup bodyweight. Overall, it appears that mammalian reproductive effects only occur at doses causing significant systemic toxicity. Available no-guideline developmental studies employed parenteral routes of administration suggest effects on female sexual maturation and fertility. However, these should not be considered of relevance to humans. ECHA (2011) conclude that the potency of 4-t-OP is 10^{-5} to 10^{-6} that of 17β-oestradiol in vitro while, in vivo, about **2000-fold higher doses are required to elicit a similar response to reference oestrogens.** Although there are some suggestions of other endocrine (non-oestrogen) related activities these are not well established and not considered of concern (EC, 2011).
Endocrine disrupting activities of concern:	Possible weak mammalian oestrogen but not considered of concern in vivo. The main regulatory concern for OP relates to its ecotoxic and persistence potential.
Other significant toxic effects:	OP was classified in Annex 1 of Directive 67/548/EEC (EU, 2002) as: Xi; R38-41 N; R50-53 R38: Irritating to skin; R41 Risk of serious damage to eyes R50/53 – very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment. After oral dosing, 4-t-OP is rapidly absorbed reaching a C max within 20 min to 2 hrs in rodents while systemic levels may be 2- to 4-times higher under conditions of repeat dosing than those achieved with a single exposure. However, no tissue-specific accumulation occurs. Metabolic pathways include hydroxylation, glucuronidation and sulphation with 38% biliary excretion reported for rats (ECHA, 2011). According to the IUCLID datasheet (EINECS, 2000), OP shows: low acute oral and dermal toxicity (LD_{50} rat oral = 1,000-4,040 mg/kg; dermal = >2,000 mg/kg); is a dermal irritant; is negative in Ames mutagenicity assays; has a 3-month dietary NOAEL of 30 ppm and LOAEL of 300 ppm (based on growth impairment) in rats while, in a 60 day oral repeat dose study the NOAEL was 50 mg/kg bw/d and LOAEL was 125 mg/kg bw/d for systemic endpoints. When potential endocrine-related endpoints are considered, altered oestrous cyclicity occurred at 200, but not 100, mg/kg/d over 25 days although, in another study, a dose of 100 mg/kg/d was well tolerated for <60 days (ECHA, 2011).
Study estimate of anticipated exposure via drinking water	No intake of NP is predicted to occur from drinking water produced using advanced treatment process. Intake (µg/kg bw/d) of OP from drinking water from conventional treatment: Infant 0.015; Toddler 0.010; Adult 0.003. Predicted intakes of the individual OPEOs considered here range between 0 and 0.033 µg/kg bw/d based on the advanced treatment process, and between 0.007 and 0.1 µg/kg bw/d based on the conventional treatment process.
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	A number of assessments by authoritative bodies have established that the principle cause for concern for 4-*t*-OP and its ethoxylates relates to the level of exposure to the OP, since the OPEs are of much lower toxicity and are not of particular concern per se. Also, although OP is recognised to be a

	particular concern to the ecosystem because of its high ecotoxicity - particularly in sediment-dwelling organisms - significant concerns have not been identified with regard to the risk posed to mammalian species, including humans. It has been established that the in vitro potency of 4-t-OP is only of the order of 10^{-5} to 10^{-6} that of 17β-oestradiol and ECHA (2011) estimated that it's in vivo potency is about 2000-fold lower than that of reference (natural) oestrogens; these aspects are considered of more relevance in this risk assessment. However, against this, it must be noted that experimental evidence clearly demonstrates that the threshold for endocrine-related responses in mammals in vivo lies considerably above that at which frank toxicity occurs, demonstrating that endocrine-related effects should not be considered to constitute a good basis for assessing the risk posed by this substance. For the purposes of this study, applying an uncertainty factor of 100 (10 for interspecies and 10 for inter-individual variability) to the rodent NOAEL (based on general toxic effects) of 50 mg/kg/day from a 60 day oral repeat dose study, would suggest a possible study value of 0.5 mg/kg bw/d might be considered. However, to ensure that adequate cognisance is taken of the high level of public concern regarding the endocrine activity of the substance and potential uncertainties regarding the extent to which other form of OP (i.e. OP1EO & OP3EO) might contribute to the overall hazard posed, application of a further factor of 10 could be considered; this would give a study-specific value of 50 µg/kg bw/d for comparison with predicted intakes. The highest predicted intake was, however, only 0.015 µg/kg bw/d for infants given drinking water from conventional treatment. This would equate to margin of safety of > 3000-times when based solely on the general toxic concerns rather than the less sensitive endocrine-related responses seen in rodents. Furthermore, it should be noted that – driven by the environmental concerns (as opposed to direct human health concern) – there are established restrictions on the levels of emission of OPEs and OP to the environment across the EU. Also its status as a OSPAR priority substance and the recent proposal to consider it a SVHC, will result in ongoing pressure to remove OP and its source mateirals (i.e. OPEs/OPEOs) from uses that could conceivable result in environmental contamination. Together ongoing measures can be anticipated to not only control the levels present in the aqueous environment (the source for drinking waters) but also place ongoing pressure on the extent to which OPEs are used.
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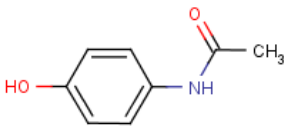
Scottish Government (2008): Annex 9: Surface Water Quality – Environment Quality Standards for Priority Substances and other Dangerous Substances. Table A: Environmental quality standards for priority substances and other dangerous substances (indicated in bold font) for which standards have been set at EU-level. The Scottish Government, UK Available at Internet site http://www.scotland.gov.uk/Publications/2008/06/26111138/16

Hazard Assessment

Substance name:	Paracetamol (Acetaminophen) 
CAS No.:	103-90-2
Synonym(s):	4'-Hydroxyacetanilide; 4-(Acetylamino)phenol; 4-Acetamidophenol; Abensanil; Acarnol; Acenol; Acetofen; Acetamide; Acetaminofen; Aceteminophen; Actron; Bacatamol; Cadafen; Calpol; Captin; Cefalex; Codoliprane; Cosutone; Cofamol; Curpol; Duracetamol; Durapan; Fluparmol; Ketaprin; Ildamol; Lemsip; N-(4-Hydroxyphenyl)acetamide; N-(4-Hydroxyphenyl)acetanilide; N-Acetyl-p-aminophenol; Naprinol; Neo-Fepramol; Neodol; Neuridon; Pacemol; Panadiene; Panadol; Panamax; Paracenol; Paracemol; Paracet; Paracodol; Parake; Phenaphen; Rubophen; Rupemol; Sanicopyrine; Setamol; Sinedol; Sinmol; Tazamol; Tricoton; Valadol; Verpol; Viruflu; Volpan; Zatinol; p-Hydroxyacetanilide
Use and potential human exposure routes:	Paracetamol (Acetaminophen) is a widely available 'over the counter' medication for pain relief (analgesia) and reducing body temperature during episodes of fever (antipyretic). Literature reporting paracetamol levels in water sources is fairly abundant. In the UK, Kasprzyk-Horden et al (2007) reported concentrations of paracetamol in the River Taff, in Wales, of between 216 – 1388 ng/L, and of paracetamol in the River Ely, also in Wales, of up to 1534 ng/L (Kasprzyk-Horden et al, 2009). Occupational exposure to paracetamol may occur through inhalation of dust and dermal contact with this compound at workplaces where paracetamol is produced or dispensed. The general population may be exposed via the oral route to paracetamol through medical administration of this compound.
Established/Study-specific exposure limit:	**Therapeutic doses by age range:** < 1 month : 30 mg/kg bw/d 1 month to 12 years : 30 – 500 mg/d 12 to 18 years : 500 – 2000 mg/d >18 years : 500 – 2000 mg/d **Study-specific Exposure Limit** = 300 µg/kg bw/d.
Basis for exposure limit:	Assuming an uncertainty factor of 100 from the minimum human therapeutic dose.
Evidence for human relevant endocrine disrupting potential:	Paracetamol is a potent inhibitor of prostaglandin synthesis and has recently been implicated in endocrine pathology due to interaction with anti-androgens. Paracetemol has been shown to cross the placenta. A prospective birth cohort study of mild analgesic use in Danish (n=2251) and Finnish (n=2728) pregnant women assessed 2570 male newborns for variances in anogenital distance and testicular position. In the Danish birth cohort the use of mild analgesis was shown to be associated with incidence of congenital

	cryptorchidism (OR=1.43; 0.73, 2.79) in a dose-dependent manner; this was particularly apparent following exposure in the second trimester (OR = 2.3; 1.12, 4.73) (Kristensen et al., 2010). To verify these findings, a mechanistic study was conducted in Wistar rats subchronically exposed to paracetamol (150, 250 and 350 mg/kg bw/d) *in utero* from gestational day 13 to 21. Intrauterine exposure was associated with a decrease in anogenital distance and in vitro examination demonstrated reduction in testosterone secretion, leading to the conclusion that intrauterine exposure to mild analgesics, including paracetamol, is a risk factor for the development of male reproductive disorders. Developmental and reproductive toxicity, potentially indicative of an endocrine mechanism, has been observed at doses of acetaminophen (400 mg/kg bw) administered by i.p. in B6C3/FI/BOM mice. Reductions in testicular weight and significant decreases in thymine levels in testis were observed following the first 3 hours of a single dose of acetaminophen (100 to 400 mg/kg). Flow cytometric analysis revealed significant reductions in tetraploid populations of pachytene and spermatocyte testicular cells at days 5 and 10. Thus, acetaminophen may delay spermiogensis. Furthermore, exposure was shown to predispose susceptibility to increased DNA denaturation *in situ*, altering sperm chromatin structure (Wiger *et al*., 1995).
Other significant toxic effects:	Acute oral toxicity in rats is low (LD_{50} Rat oral= 2400 mg/kg) and in mice is moderate (LD_{50} Mouse oral=338 mg/kg; Lewis, 2004). In healthy adults an acute dose of 150 mg/kg (approximately 7.5g in total) has been shown to be hepatotoxic, however, as susceptibility to paracetamol toxicity varies between individuals doses in excess of 350 mg/kg may be expected to cause severe liver damage in all cases (Prescott, 1983). Young children appear to be less susceptible to hepatatoxicity following paracetamol overdose, possibly due to a more efficient detoxification pathway as a result of an increased glutathionate or sulphate conjugation rate (Rumack, 1985; Penna and Buchanan., 1991; Miller *et al*., 1976). Chronic oral toxicity study in rats administered paracetemol at concentrations between 800 and 25000 ppm (mg/L), demonstrated increased mortality in the high dose range. Acetaminophen (paracetamol) related lesions were observed in the liver (necrosis, chronic active inflammation, hepatocytomegaly), kidney (tubule case, tubule necrosis, tubule regeneration), reproductive organs (atrophy of testis, ovary and uterus), thymus and lymph nodes (lymphoid depletion) of rats receiving 12500 ppm (mg/L), and of the liver and testis of male rats receiving 12500 ppm (mg/L). Liver toxicity, indicated by hepatocytomegaly, focal calcification, pigmentation and necrosis, was observed in males that received 6200, 12500 and 25000 ppm (mg/L), and females receiving 12000 or 25000 ppm (mg/L). A NOAEL of 3200 ppm (mg/L) was derived for hepatotoxicity. In a 2-year carcinogenicity study in Fischer F344/N rats orally exposed to 0, 600, 3000 or 6000 ppm acetaminophen, no evidence of carcinogenic activity was demonstrated in males. However, there was equivocal evidence for carcinogenicity in female rats based on an increased incidence of mononuclear cell leukaemia. In a study of male and female B6C3F1 mice receiving the same dose range, there was no evidence for carcinogenicity (NTP, 1993). Paracetamol is not considered to be genotoxic.

Primary mode of endocrine activity	**Uncertain** Paracetamol showed weak oestrogenic activity in recombinant yeast cells containing the human oestrogen receptor (hERα). Furthermore, the YES-assay showed induction of beta-galactosidase expression following paracetamol exposure (Fent *et al.*, 2006). However, Dowdy *et al.* (2003) demonstrated weak anti-oestrogenic activity in human endometrial adenocarcinoma (Ishikawa) cells. Paracetamol inhibited both basal and oestradiol-induced alkaline phosphatise activity in a dose dependent manner, without inhibiting the enzyme. Competition binding assays with human ERα and ERβ demonstrated that 106-fold molar excess of paracetamol did not significantly interact with the ligand-binding domain of either receptor, suggesting weak anti-oestrogenic activity in Ishikawa cells without directly binding to the nuclear receptors (Dowdy *et al.*, 2003).
Study estimate of anticipated exposure via drinking water	Limited intake via drinking water is predicted to occur in all age groups following conventional treatment processes. Following advanced treatment processes intakes are predicted to be zero in all age groups. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.025; Toddler 0.017; Adult 0.006.
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	There is some evidence to suggest that paracetamol, among other non-steroidal anti-inflammatory drugs, may be a human endocrine disruptor (at particular life stages). Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes and conservative assumptions is 0.025 µg/kg bw/d for infants, this would suggest a margin of safety of >10,000 times against the study-specific exposure limit. These figures were based on modelled levels in drinking water calculated with usage figures obtained from UK prescription data, undergoing conventional water treatment; as such they are conservative in nature. However, with such large predicted margins of safety, this substance is not considered as being of concern.
References*:*	Dowdy, J., Brower, S., Miller, M. R. (2003) Acetaminophen exhibits weak antioestrogeni activity in human endometrial adenocarcinoma (Ishikawa) cells. *Toxicological Sciences* **72**: 57 – 65. Kasprzyk-Hordern, B., Dinsdale, M., Guwy, A.J. (2007) Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra performance liquid chromatography–positive electrospray ionisation tandem mass spectrometry *Journal of Chromatography A* **1161** :132 – 145. Kasprzyk-Hordern, B., Dinsdale, M., Guwy, A.J. (2009) The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. *Water Research* **43** : 363–380. Kristensen, D.M., Hass, U., Lesné, L., *et al.* (2011) Intrauterine Exposure to Mild Analgesics is a Risk Factor for Development of Male Reproductive Disorders in Human and Rat. *Human Reproduction*, 26(1), 235-244. [English (eng)]. Fent, K., Escher, C., Caminada, D. (2006) Estrogenic activity of pharmaceuticals and pharmaceutical mixtures in a yeast reporter gene system. *Reproductive Toxicology* **22:** 175 – 185. Lewis, R. J. Sr. (2004) Sax's Dangerous Properties of Industrial Materials.

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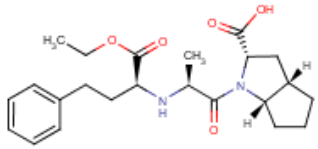
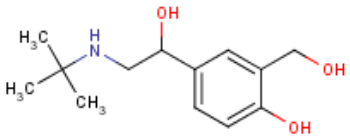
Hazard Assessment

Substance name:	**Ramipril** 
CAS No.:	87333-19-5
Synonym(s):	2S,3aS,6aS)-1-((S)-N-((S)-1-Carboxy-3-henylpropyl)alanyl)octahydrocyclo-penta(b)pyrrole-2-carboxylicacid,1-ethylester; (2S,3aS,6aS)-1-((S)-N-((S)-1-Ethoxycarbonyl-3-phenylpropyl)alanyl)octahydrocyclopenta(b)pyrrol-2-carbonsaeure; Acovil; Altace; Carasel; Cardace; Cyclopenta(b)pyrrole-2-carboxylicacid,1-(2-((1-(ethoxycarbonyl)-3-phenylpropyl)amino)-1-oxopropyl)octahydro-,(2S-(1(R*(R*)),2alpha,3abeta,6abeta))-;Delix; HOE 498; Hytren; Lostapres; Naprix; Pramace; Quark; Ramace; Ramipril; Ramiprilum; Ramiprilum [Latin]; Ramipro; Triatec; Tritace; UNII-L35JN3I7SJ; Vesdil.
Use and potential human exposure routes:	Ramipril is a prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor class of medications. It is metabolized to ramiprilat in the liver and, to a lesser extent, kidneys. Ramipril is a potent, competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system (RAAS). Ramipril may be used in the treatment of hypertension, congestive heart failure, nephropathy, and to reduce the rate of death, myocardial infarction and stroke in individuals at high risk of cardiovascular events (DrugBank.com). Literature reporting measurement of ramipril in UK waters was not identified. Occupational exposure to ramipril may occur through inhalation of dust and dermal contact with this compound at workplaces where it is produced or dispensed. The general population may be exposed via the oral route to ramipril through medical administration of this compound.
Established/Study-specific exposure limit:	**Therapeutic doses by age range:** <1month : not used 1 month to 12 years : not used 12 to 18 years : not used > 18 years : 1.25 mg/day (1.25 - 10 mg/day) **Study-specific Exposure Limit** = 0.0025 mg/kg bw/d (2.5µg/kg bw/d)
Basis for exposure limit:	Assuming an uncertainity factor of 100 from the minimum human therapeutic dose (1.25 mg/ day) for a 5 kg infant.
Evidence for human relevant endocrine disrupting potential:	Using a precautionary approach, this substance was identified as of possible concern following expert advice from the clinician within the project team. However, no evidence suggestive of endocrine disrupting activity per se was identified for ramipril in subsequent literature review.
Other significant toxic	Ramipril has an oral LD_{50} of >10,000 mg/kg in the rat; >10,500mg/kg in the

effects:	mouse and >1000 mg/kg in dogs (Sanofi-Aventis, 2011). In a 90 day chronic toxicity study in mice administered 1000 mg/kg/d by the oral route, reduced erythrocytes, haemoglobin, haematocrit, increased reticulocytes were reported. Nephrotoxicity was also noted as hyperplasia of juxtaglomerular apparatus, (Sanofi-Aventis 2011). An 18 month oral toxicity study in which rats were administered doses of ramipril between 0 and 500 mg/kg bw/d reported fibromuscular pads in gastric fundus mucosa and focal atrophies in renal cortex, partly with cysts at the lowest dose of 3.25 mg/kg bw/d. At 40 and 500 mg/kg bw/d, anaemia, increased BUN and serum creatinine, urinary epithelial cells, reduced heart weight and increased kidney and adrenal weights were observed (Sanofi-Aventis 2011). Dogs administered ramipril by the oral route for 12 months at doses of 2.5, 25, 250 mg/kg bw/d showed reduced body weight at all doses. In addition, at 25 and 250 mg/kg bw/d anaemia and leukopenia, impaired erythropoiesis, increased haemosiderin deposition in liver and spleen, and juxtaglomerular hyperplasia were also noted. At the highest dose of 250 mg/kg bw/d increased BUN and serum creatinine were reported (Sanofi-Aventis 2011). A 6 month oral study in monkeys at doses of 0.5, 16, 500 mg/kg bw/d ramipril reported increased BUN and juxtaglomerular hyperplasia and reduced body weight at 16 and 500 mg/kg bw/d. At 500 mg/kg bw/d, diarrhoea, anaemia, increased serum creatinine, some urinary casts, leukocytes and epithelial cells were noted (Sanofi-Aventis 2011). Due to adverse events observed in some animal studies, ramipril is considered pregnancy category C during the first trimester. Based on human data, ramipril is considered pregnancy category D if used during the second and third trimesters (Merck Manuals). Ramipril is not considered to be carcinogenic or mutagenic.
Primary mode of endocrine activity	Not identified
Study estimate of anticipated exposure via drinking water	Limited intake via drinking water is predicted to occur in all age groups following conventional treatment processes and in infants and toddlers following advanced treatment processes. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.047; Toddler 0.031; Adult 0.010. Intakes (ug/kg bw/d) based on advanced treatment: Infant 0.002; Toddler 0.001.
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	No evidence to either prove or disprove endocrine disrupting activity per se has been identified for this substance. Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes and conservative assumptions is 0.047 µg/kg bw/d for infants, this would suggest a margin of safety of around 53 times against the study-specific exposure limit. These figures were based on modelled levels in drinking water calculated with usage figures obtained from UK prescription data, undergoing conventional water treatment; as such they are conservative in nature. It should be noted that margins of safety following advanced water treatment were significantly increased to a minimum of 1000 times (for infants). As there is currently no evidence to support ED activity of ramipril, the pharmaceutical is not considered of concern under the specific remit of this project, and will not be assessed further.

References*:*	DrugBank.com: http://drugbank.ca/drugs/DB00178 (accessed Dec 2011) Merck Manuals: http://www.merckmanuals.com/professional/lexicomp/ramipril.html (accessed Dec 2011). Sanofi-Aventis (2011) PRODUCT MONOGRAPH: ALTACE® (ramipril capsules, Mfr Std.). Available at: www.sanofi.ca/products/en/altace.pdf

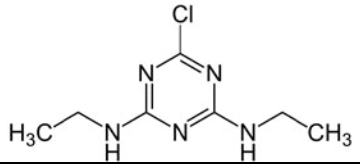
Hazard Assessment

Substance name:	**Salbutamol (Ventolin)** 
CAS No.:	35763-26-9
Synonym(s):	(+-)-Albuterol; (+-)-Salbutamol; (+-)-alpha(sup 1)-(((1,1-dimethylethyl)amino)methyl)-4-hydroxy-1,3-benzenedimethanol; R,S-Albuterol; Racemic-Salbutamol
Use and potential human exposure routes:	Salbutamol (Ventolin HFA) is a β_2-adrenergic agonist; the β_2-adrenoceptor is involved in the relaxation of bronchial smooth muscle, the gastrointestinal tract, blood vessels and the uterus. Thus, salbutamol is used in the treatment and prevention of bronchospasm, in patients in excess of 4 years of age with reversible obstructive airway disease, prevention of exercise-induced bronchospasm and in premature birth (Waldeck, 2002). Salbutamol is also used in combination with other drugs as a growth promoter in livestock. This β2-adrenergic drug enhances lipolysis and the rate at which fatty acids are oxidised producing leaner animals (Hernàndez-Carrasquilla, 2003).
Established/Study-specific exposure limit:	**Therapeutic dose by age range:** 1 < month : 100 µg/day (100 µg to 20 mg) 1 month to 12 years : 100 µg/day (100 µg to 20 mg) 12 years to 18 years : 100 µg/day (100 µg to 30 mg) > 18 years : 100 µg/day (100 µg to 30 mg) Australian Guidelines for Water Recycling (2008) ADI = 3 µg/ L. **Study-specific Exposure Limit** = 0.2 µg/kg bw/day
Basis for exposure limit:	Assuming an uncertainty factor of 100 from the minimum human therapeutic dose (100 µg/ day) for a 5 kg infant.
Evidence for human relevant endocrine disrupting potential:	In a case-control study of pregnant women treated with oral salbutamol (n=20) or not exposed to β-blocking agents (n=18), cord blood concentrations of insulin, growth hormone (GH), triiodothyronine (T3), thyroxine (T4), and thyroid stimulating hormone (TSH) were measured. No significant difference was found in circulating insulin, T3, T4, and TSH between both groups. However, GH levels were significantly higher in the treated group (36.5 ± 17.4 ng/mL) than in the control group (17.4 ± 6.6 ng/mL; P < .001). The authors suggest that the unexpected increase in GH levels in the exposed group could reflect either a fluctuation in foetal blood glucose in response to episodic betamimetic administration or direct foetal pituitary production through adrenergic stimulation (Desranges *et al*., 1987). A significant up-regulation of CYP17 gene expression (13-fold) was observed following exposure to salbutamol (0.05 µg/L) in the H295R cell bioassay. The H295R assay is considered effective in identifying chemicals that may potentially effect the steroidogenic pathway, the induction of

	CYP17 observed in this study would ensure that oestradiol (E2) was not produced despite small concentrations of substrate, affecting hormone homeostasis (Gracia *et al*., 2007).
Other significant toxic effects:	There are no adequate, well-controlled studies of salbutamol exposure in pregnant women. However, various congenital anomalies including cleft palate and limb defects have been reported in the offspring of patients. No consistent pattern of defects can be discerned and affected patients were taking multiple medicines. A relationship between salbuterol use and congenital anomalies has not been established; however there is some animal evidence to suggest teratogenicity. In a CD-1 mouse reproduction study, subcutaneously administered salbuterol sulphate produced cleft palate formation in 5 of 111 (4.5%) foetuses at exposures approximately equal to the maximum recommended human dose (MRHD) for adults (0.025 mg/kg) and 10 of 108 (9.3%) foetuses at approximately 8 times the MRHD (2.5 mg/kg). A teratogenicity NOAEL can be set at approximately one eleventh of the MRHD (0.0025 mg/kg). In a rabbit reproduction study, orally administered albuterol sulphate produced cranioschisis in 7 of 19 foetuses (37%) at approximately 680 times the MRHD. In another rabbit study, albuterol sulphate/HFA-134 formulation administered by inhalation exposure produced enlargement of the frontal portion of the foetal fontanelles at approximately one third of the MRHD. In a 2-year reproduction and developmental toxicity study, salbutamol (>2.0 mg/kg) exposure was associated with an increase in the incidence of benign leiomyomas of the mesovarium, in Sprague-Dawley rats. However, no evidence of impaired fertility at oral doses of albuterol sulphate up to 50 mg/kg. A reproduction study in Stride Dutch rabbits revealed cranioschisis in 7 of 19 foetuses (37%) following oral administration of salbutamol (50 mg/ kg). Salbutamol exposure (16 µg/kg bw or 3 mg/kg bw, twice daily for 5 days) has also been shown to produce significant increases in muscle hypertrophy, defined as an increase in muscle to body mass ratio, in male Wistar rat, skeletal muscles (soleus, gastrocnemius and plantaris), in a dose dependent manner (Mersmann, 1998). Taneka *et al.* (2005) demonstrated a significant dose dependent increase in plasma IL-6 levels, but not IL1β or TNF-α following intravenous salbutamol (60 mg/kg) exposure. The toxic dose of β2-adrenoceptor agonists induced the expression of stress-inducible proteins in the major tissues of rats (heart, lung, liver and spleen). The expression of MT-1, HO-1 and iNOS were altered tissue-dependently by salbutamol, furthermore, the suppressive effect of a lower dose in the LPS-treated inflammatory model was mediated by β2-adrenoceptor changes in iNOS and HO-1. The authors suggest salbutamol has biphasic actions with a tissue-protective and inflammatory effect. CD-1 mice tumourigenesis NOAEL = 500 mg/kg (dietary dose). Golden Hamster tumourigenesis NOAEL = 50 mg/kg (dietary dose).
Primary mode of endocrine activity	None Identified
Study estimate of anticipated exposure via drinking water	Limited intake via drinking water is predicted to occur in all age groups following conventional treatment processes. Intake in all age groups following advanced treatment processes is predicted to be zero. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.070; Toddler 0.046; Adult 0.015.

Assessment of endocrine disruption potential at modelled drinking water concentration(s)	Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes and conservative assumptions is 0.07 µg/kg bw/d for infants, this would suggest a margin of safety of around 3 times against the study-specific exposure limit. These estimates were based on modelled levels in drinking water derived from UK prescription data assuming conventional water treatment. Predicted exposure in the case of advanced water treatment was zero for all age groups considered. An intake level below a MOS of 10 as seen here for toddlers and infants would be of some potential concern given the possibility of endocrine disrupting potential. However, given the highly conservative nature of the exposure estimate, it is considered that there is no appreciable need for concern.
References:	Cepero, M., Perex-Pertejo, Y., Cubria, J. C., Reguera, R., Balana-Fouce, R., Ordonez, C., Ordonez-Escudero, D. (2000) Muscle and serum changes iwht salbutamol administration in aerobically exercised rats. Comparative Biochemistry and Physiology Part C 126: 45 – 51. Desranges, M. F., Moutquin, J. M., Peloquin, A. (1987) Effects of maternal oral salbutamol therapy on neonatal endocrine status at birth. *Obstetrics and Gynecology* **69** (4): 582 – 584. http://us.gsk.com/products/assets/us_ventolin_hfa.pdf Gracia, T., Hilscherova, K., Jones, P. D., Newsted, J. L., Higley, E. B., Zhang, X., Hecker, M., Murphy, M. B., Yu, R. M. K., Lam, P. K. S., Wu, R. S. S., Giesy, J. P. (2007) Modulation of steroidogenic gene expression and hormone production of H295R cells by pharmaceuticals and other environmentally active compounds. *Toxicology and Applied Pharmacology* **225:** 142 – 153. Hernàndez-Carrasquilla, M. (2003) Gas chromatography-mass spectrometry analysis of β2-agonists in bovine retina. *Anal. Chim. Acta*. **408:** 285 – 290. Mersmann, H. J. (1998) Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. *J. Anim. Sci*. **76:** 160 – 172. Tanaka, S., Momose, Y., Tsutsui, M., Kishida, T., Kuroda, J., Shibata, N., Yoshida, T., Tamagishi, R. (2004) Quantitative estimation of myocaria fibrosis based on receptor occupancy for β2-adrenergic receptor agonists in rats. *J. Toxicol. Sci*. **29:** 179 – 186. Tanaka, S., Yamagishi, R., Tsutsui, M., Kishida, T., Murakami, M., Kuroda, J., Yoshida, T. (2005) Tissue- and dose-dependent alteration of stress-inducible proteins by β2-adrenoceptor agonist, Salbutamol, in rats. *The Journal of Toxicological Sciences* **30** (4): 305 – 314. Waldeck, B. (2002) β-Adrenoceptor agonists an asthma – 100 years of development. *Eur. J. Pharmacol*. **445**: 1 – 12.

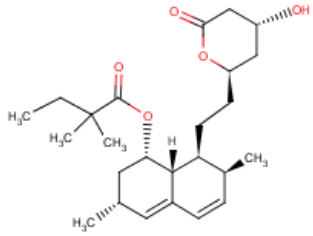
Hazard Assessment

Substance name:	**Simazine** 
CAS No.:	122-34-9
Synonym(s):	1-Chloro-3,5-bis(ethylamino)-2,4,6-triazine; 2,4-Bis(aethylamino)-6-chlor-1,3,5-triazin; 6-Chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine; 6-Chloro-N2,N4-diethyl-1,3,5-triazine-2,4-diamine; Aquazine; Amizine; Azotop; Batazina; Bitemol; Herbazin-50; Herboxy; Premazine; Princep; Simazin; Primatel; Radacon; Tafazine; Taphazine; Yrodazine
Use and potential human exposure routes:	Simazine is a pre-emergence triazine herbicide for control of broad-leaved and grass weeds in artichokes, asparagus, berries, broad beans, citrus fruits, coffee, cocoa, hops, maize, oil palms, olives, orchards, ornamentals, sugar-cane, tea, tree nurseries, turf and vineyards, as well as in non-crop areas (BCPC, 1991). Although subject to a ban in the majority of the EC due to concerns over environmental persistence, it continues to be used under derogation in the UK (Pan UK, 2003). Worker exposure to simazine may occur directly during mixing and application of the herbicide while the general population may be indirectly exposed via consumption of contaminated drinking water and foods (Kim *et al*., 2003).
Established/Study-specific exposure limit:	A TDI of 0.005 mg/kg bw/d has been established by WHO (2003). WHO have derived a drinking water guideline value of 2 µg/L for simazine assuming 10% of TDI comes from drinking water for a 60 kg human consuming 2 l of water per day (WHO, 2003).
Basis for exposure limit:	The TDI was based on a NOAEL of 10 mg/kg (0.52 mg/kg bw/d) from a rat carcinogenicity study (described below) and an uncertainty factor of 1000 (100 to account for intra- and interspecies variation and a further 10 for non-genotoxic carcinogenicity; WHO, 2003).
Evidence for human relevant endocrine disrupting potential:	Indirect evidence of ER- interaction was generated in a study on rats co-exposed to simazine and oestradiol in which a statistically significant increase in uterine peroxidise activity was noted (Connor *et al.,* 1996). Effects on uterine weight were noted in ovariectomised Spraque-Dawley rats when a single dose of simazine at 0, 1, 10, 50, 100 or 300 mg/kg bw/d together with oestradiol but not when simazine was given alone (Tennant *et al.,* 1994). The authors suggest that 50 mg/kg oral simazine blocked 1 µg/kg of oestradiol for thymidine incorporation. Furthermore, 300 mg/kg simazine was shown to block approximately half of the progesterone receptor response to 1µg/kg of oestradiol, indicative of weak antagonism. In immature female animals, thymidine uptake was reduced by up to 30% when simazine given at 50, 100 or 300 mg/kg bw/d but not at t 0, 1 or 10 mg kg^{-1}/day (Tennant *et al.*, 1994). *In vivo* effects on weights of levator ani/bulbocavernosus muscles and adrenal glands noted in castrated testosterone-treated Wistar rats and simazine inhibit the androgen-responsive gene PBP C3. However, *in vitro* androgen receptor R1881-induced translational activity was not influenced (Birkhoj *et al.,* 2004).

	Jowa and Howd (2011) have suggested a possible mechanism for action via the pituitary and hypothalamus
Endocrine disrupting activities of concern:	Antioestrogen
Other significant toxic effects:	Simazine shows slight to no acute oral toxicity; oral LD_{50} in rats and mice = >5000 mg/kg; dermal LD_{50} is 3100 mg/kg in rats and >10,000 mg/kg in rabbits. A 4-hour inhalation LC_{50} in rats = > 2 mg/L (Kidd, 1991). In male C57BI/6 mice fed simazine at 600 mg/kg diet for 4 weeks, increased spleen CD4+ cells and thymus CD8+ cells were noted and inhibition of IgM plaque-forming cell numbers, lowered IgG level and significantly decreased proliferation of mitogen-stimulated B and T cells. Macrophage cytokine production was also impaired. Collectively, results indicate simazine to be immunosuppressant (Kim *et al.*, 2003). In a 2 year dietary dog study using doses of 0, 20, 100 or 150 mg/kg diet, cachexia was observed at the highest dose and exposure was associated with reduced erythrocyte parameters in males and females and increased thrombocytes in males; the NOAEL was 20 mg/kg diet (0.8 mg/kg bw/d). In a 2 year carcinogenicity study, Sprague-Dawley rats fed diet containing simazine at 0, 10, 100 ore 1000 mg/kg diet showed alterations in body weight and haematological parameters and increased mammary tumours at levels above 10mg/kg; the NOAEL was 10 mg/kg diet (0.52 mg/kg bw/d) (WHO, 2003). Simazine was not tumorigenic in mice at a maximum tolerated dose of 215 mg/kg bw/day for 18-months. Other studies are suggested to have found effects as low as 5 mg/kg/day for thyroid and mammary tumours in females (Stevens, 1991) but their robustness is unclear.
Study estimate of anticipated exposure via drinking water	Intake via drinking water is predicted to occur following both conventional and advanced treatment processes in infants and toddlers and following conventional treatment processes in adults. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.019; Toddler 0.013; Adult 0.004. Intakes (ug/kg bw/d) based on advanced treatment: Infant 0.002; Toddler 0.001.
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	The predicted maximum concentration for simazine in drinking water according to this study of 0.13 ug/L is well below the WHO (2003) drinking water guideline of 2 µg/L. Also, there is an established authoritative TDI for simazine of 0.52 µg/kg bw/d, based on a NOAEL of 0.52 mg/kg bw/d and appropriate uncertainty factors for a non-genotoxic carcinogen. Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes and conservative assumptions is 0.019 µg/kg bw/d, this would suggest a margin of safety of 27 times when compared to the TDI, and hence is considered of low concern. Furthermore, since the TDI incorporates a significant uncertainty factor, it is considered that this should be protective for any endocrine-related properties that this substance may poses.
References:	BCPC (1991) British Crop Protection Council. The Pesticide Manual, 9th Ed. Worthing CR, Ed. Farnham. Birkhoj, M., Nellemann, C., Jarfelt, K., Jacobsen, H., Anderson, H. R., Dalgaard, M., Vingaard, A. M. (2004) The combined anti-androgenic effects

	of five commonly used pesticides. *Toxicol Appl Pharmacol* **201**: 10 – 20. Connor, K., Howell, J., Chen, I., Liu, H., Berhane, K., Sciarretta, C., Safe, S., Sacharewski, T. (1996) Failure of chloro-s-triazine-derived compounds to induce oestrogen receptor-mediated responses *in vivo* and *in vitro*. *Fundam Appl Toxicol* **30** (1): 93 – 101. Kidd, H. and James, D. R., Eds. The Agrochemicals Handbook, Third Edition. Royal Society of Chemistry Information Services, Cambridge, UK, 1991 (as updated).8-7 Kim, K. R., Son, E. W., Hee-Um, S., Kim, B. O., Rhee, D. K., Pyo, S. (2003) Immune alterations in mice exposed to the herbicide simazine. *Journal of Toxicology and Environmental Health, Part A* **66***:* 1159 – 1173. Pan UK (2003) Advisory Committee on Pesticides: Atrazine and Simazine: Restrictions now effective. Available at: http://www.pan-uk.org/pestnews/Issue/pn21/PN21P19a.htm [First appeared in Pesticide News No. 21 September 1993, page 19]. Stevens, J. T. and Sumner, D. D. Herbicides. In Handbook of Pesticide Toxicology. Hayes, W. J., Jr. and Laws, E. R., Jr., Eds. Academic Press, New York, NY, 1991.8-4 Tennant, M. K., Hill, D. S., Eldridge, J. C., Wetzel, L. T., Breckenridge, C. B., Stevens, J. T. (1994) Chloro-s-triazine antagonism of oestrogen action: limited interaction with estrogen receptor binding. *J Toxicol Environ Health* **43** (2): 197 – 211. WHO (2003) World Health Organisation, Simazine in Drinking Water: Background document for development of WHO guidelines for drinking-water quality. WHO/SDE/WSH/03.04/42.

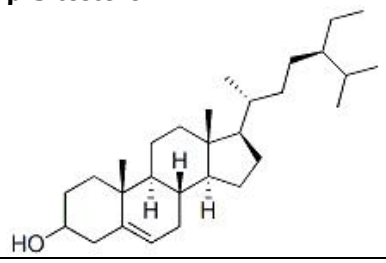
Hazard Assessment

Substance name:	**Simvastatin** 
CAS No.:	79902-63-9
Synonym(s):	2,2-Dimethylbutanoic acid (1S,3R,7S,8S,8aR)-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-[(2R,4R)-tetrahydro-4-hydroxy-6-oxo-2H-pyr an-2-yl]ethyl]-1-naphthalenyl ester; 2,2-dimethylbutyric acid 8-ester with (4R,6R)-6-[2-[(1S,2S,6R,8S,8aR)-1,2,6,7,8,8a-hexahydro-8-hydroxy-2,6-dimethyl-1-naphthyl]ethyl]tetrah ydro-4-hydroxy-2H-pyran-2-one; Synvinolin; Zocor
Use and potential human exposure routes:	Simvastatin is used for the treatment of hypercholesterolemia. A derivative of lovastatin and potent competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (hydroxymethylglutaryl COA reductases), which is the rate-limiting enzyme in cholesterol biosynthesis. It may also interfere with steroid hormone production. Due to the induction of hepatic LDL receptors, it increases breakdown of LDL cholesterol (DrugBank.com). Simvastatin has been reported to be present in UK water sources at levels <50 ng/L (Kasprzyk-Horden et al., 2009). Occupational exposure to simvastatin may occur through inhalation of dust and dermal contact with this compound at workplaces where it is produced or dispensed. The general population may be exposed via the oral route to simvastatin through medical administration of this compound.
Established/Study-specific exposure limit:	**Therapeutic doses by age range:** <1month : not used 1 month to 12 years : not used 12 to 18 years : 10mg/d (10-40 mg/d) > 18 years : 10 mg/day (10 – 80 mg/day) **Study-specific Exposure Limit** = 0.02 mg/kg bw/d
Basis for exposure limit:	Assuming an uncertainty factor of 100 from the minimum human therapeutic dose (10 mg/ day) for a 5 kg infant.
Evidence for human relevant endocrine disrupting potential:	Decreased fertility was noted in rats given simvastatin for 34 weeks at 15 times the maximum human exposure level. However, this effect was not observed in another study in rats given simvastatin for 11 weeks at the same dosage level. Seminiferous tubule degeneration was observed in rats given simvastatin at a dose of 180 mg/kg/day (44 times the exposure level of humans given 40 mg/day). Testicular atrophy, decreased spermatogenesis, spermatocytic degeneration, and giant cell formation were observed in dogs given simvastatin at a dose of 10 mg/kg/day (7 times the human exposure level at a dose of 40 mg/day)(Thomson.Micromedex 2004. Overall, no significant evidence of endocrine disrupting activity was noted in

	current literature.
Other significant toxic effects:	Simvastatin has an oral LD_{50} of 4438 mg/kg and an intraperitoneal LD_{50} of 705 mg/kg in the rat (PubChem). In humans, simvastatin use has been associated with development of pancreatitis (McDonald et al., 2002). Chronic exposure (2 years) of female rats to 50 and 100 mg/kg/d simvastatin was shown to be associated with development of hepatocellular adenomas and carcinomas; in male rats similar end points were seen at doses of 100 mg/kg daily. Rats also exhibited an increased incidence in thyroid follicular adenomas, regardless of gender at both doses, and female rats receiving 100 mg/kg daily exhibited an increased incidence of thyroid follicular cell carcinoma (McEvoy, 2004). Simvastatin is not teratogenic in rats or rabbits (Thomson Micromedex, 2004). Simvastatin did not exhibit mutagenic potential in vitro in microbial mutagen (Ames) tests using mutant strains of Salmonella typhimurium with or without rat or mouse liver metabolic activation, the alkaline elution assay using rat hepatocytes, a V-79 mammalian cell forward mutation study, a chromosome aberration study in Chinese hamster ovary cells, or in vivo in a chromosomal aberration assay in mouse bone marrow (McEvoy, G.K., 2004).
Primary mode of endocrine activity	Not identified
Study estimate of anticipated exposure via drinking water	Limited intake via drinking water is predicted to occur in all age groups following conventional treatment processes and in infants and toddlers following advanced treatment processes. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.082; Toddler 0.054; Adult 0.018. Intakes (ug/kg bw/d) based on advanced treatment: Infant 0.002; Toddler 0.001.
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	There is limited in vivo evidence to support endocrine disrupting activity for this substance. Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes and conservative assumptions is 0.082 µg/kg bw/d for infants, this would suggest a margin of safety of around 240 times against the study-specific exposure limit. These figures were based on modelled levels in drinking water calculated with measured data obtained from a UK based study, undergoing conventional water treatment; as such they are conservative in nature. It should be noted that margins of safety following advanced water treatment were significantly increased to a minimum of 10,000 times (for infants). Due to the large margin of safety predicted and the limited evidence to support ED activity of simvastatin, the pharmaceutical is not considered of concern for further assessment here. However as the use of simvastatin is becoming more widespread in the UK should more literature on either its toxicity or measured levels in UK waters becomes available, it may be prudent to re-evaluate this pharmaceutical.
References:	DrugBank.com: http://drugbank.ca/drugs/DB00641 (accessed Dec 2011) Kasprzyk-Horden, B., Dinsdale RM., Guwy, AJ. (2009) The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters.

	Water Res., 43, 363 – 380. McDonald, KB., Garbe,r BG., Perreault MM. (2002) Pancreatitis associated with simvastatin plus fenofibrate. *Ann Pharmacother*., 36 (2), 275-9. McEvoy, G.K. (ed.). American Hospital Formulary Service- Drug Information 2004. Bethesda, MD: American Society of Health-System Pharmacists, Inc. 2004 (Plus Supplements)., p. 1641] PubChem. Available at: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=6419910 (accessed Dec 2011) Thomson.Micromedex. Drug Information for the Health Care Professional. 24th ed. Volume 1. Plus Updates. Content Reviewed by the United States Pharmacopeial Convention, Inc. Greenwood Village, CO. 2004., p. 1553.

Hazard Assessment

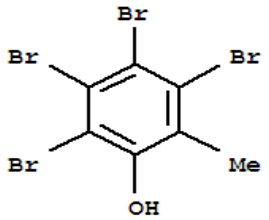
Substance name:	**β-Sitosterol** 
CAS No.:	83-46-5
Synonym(s):	B-Sitosterol; Beta-Sitosterol; A-Phytosterol; Cinchol; 22,23-Dihydrostimasterol; 22,23-Dihydrostigmasterol; 24-Alpha-Ethylcholesterol; 24beta-Ethylcholesterol
Use and potential human exposure routes:	β-Sitosterol is a plant sterol found in plant oils (from corn, subflower, soybean and rapeseed) , legumes and wood. It has been reported in STP effluent at <402 ng/L, while levels of 20-56 ng/L have been reported in rivers and tap-waters in Germany and it may occur at high levels in the manure of cattle. It is also used medically as a anti-lipaedemic agent (Lintelmann, 2003; SCF, 2002). Phytosterols are present in normal Western diets in amounts similar to dietary cholesterol (i.e. 150-400 mg/d) while vegetarian diets may contain about 50% higher levels. However, the introduction of plant sterol rich diary-substitutes may raise this to the order of 2-3 g/d while in infant formulae based on cow's milk, levels may be 0.08-0.2mmol/L. Phytosterols do not appear to play a nutritional role (SCF, 2000 & 2002).
Established/Study-specific exposure limit:	The use of phytosterol enriched fat spreads at up to 8% is considered safe for humans (SCF, 2000)
Basis for exposure limit:	The SCF (2000) conclusion was based on the established safety profile at this level of intake
Evidence for human relevant endocrine disrupting potential:	β-Sitosterol **i**s recognised as a phytoestrogen by IUPAC (Lintelmann, 2003) but, being a naturally-occurring substance, it was specifically excluded from consideration under the EU prioritisation exercise (BKH, 2000). No phytosterols were considered by FSA (2003) as data on the oestrogenic properties of these substances were considered equivocal. There are reports that it can induce vitellogenin production in male fish at µg/L levels and is oestrogenic in MCF-7 and T4TD cell. Biotransformation may also occur environmentally, for example in paper mill waste water, to produce androgenic metabolites (Lintelmann, 2003). Furthermore, oral dosing at 6.2 µg/L for 30 days resulted in increased uterine weight in rats. However no such effects were noted at 12.4 and 18.6 µg/L. Also no effects were noted in an immature rat uterotrophic assay on phytosterols (47.9% β-sitosterol) at 5-500 mg/kg bw/d for 3 days; this sterol mixture was also negative in a range of in vitro assays for oestrogenicity (SCF, 2000). It was also reported that use of phytosterol preparations for the treatment of benign prostatic hyperplasia at a daily dose of 20 mg, 3 times per day resulted in no adverse side effects SCF, 2000)
Endocrine disrupting activities of concern:	Oestrogenicity (equivocal/conflicting evidence)

Other significant toxic effects:	The rate of absorption of β-sitosterol by humans is <5% of daily intake, and results in plasma levels of 0.3-1.02 mg/100 ml for individuals consuming a typical Western-style diet. About 20% undergoes metabolism to cholic and chemodeoxycholic acids with the remainder excreted via the bile as free sterol (Salen et al., 1970). The safety of β-sitosterol – along with other plant sterols - has been extensively reviewed in relation to its subchronic, genotoxic and reproductive effects and in terms of potential oestrogenicity (SCF, 2000). The SCF review noted that in a 13-week dietary rat study on a mixture of phytosterol esters (48.7% β-sitosterol) at up to 8.1% of diet (equivalent to 4.1g phytosterol/kg bw/d) was reported to cause only minro haematological and blood chemistry changes while a phytosterol mixture containing 47.3% β-sitosterol was negative in a *Salmonella typimurium assay* and was not clastogenic in vitro to human lymphocytes. Also, no evidence of induction of unscheduled DNA synthesis or micronuclei formation was noted in in vivo studies in rodents. Subcutaneous treatment of rats has been associated with anti-androgenic changes in testes weight and sperm levels, but in a 2-generation rat study at levels similar to the above 13-week investigation no adverse effects were found; a NOAEL of 2.5-9 g phytosterol/kg bw/d (at different study stages) was established. There is evidence suggesting that plant sterols in general may act to reduce blood cholesterol (via a mechanisms thought to involve competitive inhibition of intestinal cholesterol absorption) thereby acting protectively against coronary heart disease and other cardiovascular conditions related to atherosclerosis. However, at high levels of consumption reductions in blood levels of carotenoids and other essential fat-soluble nutrients may occur (SCF, 2002)
Study estimate of anticipated exposure via drinking water	Intakes (µg/kg/d) from drinking water from advanced treatment process: Infant 0.001; Child 0; Adult 0. Intakes (µg/kg/d) based on conventional treatment: Infant 0.027; Child 0.018; Adult 0.006
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	A NOAEL of 2.5-9 g phytosterol/kg bw/d has been established from rodent studies, and the food use of phytosterol (in enriched fat spreads) has been approved in Europe at levels of up to 8% (SCF, 2000). Although no authoritative drinking water intake limit has been identified, applying an Uncertainty Factor of 1000 (10 for limited access to database, 10 for interspecies extrapolation and 10 for inter-individual variation) to the rodent NOAEL, would suggest a study-specific value of 2.5 mg/kg/d. Thus, even with the worst-case predicted intake via drinking water of 0.027 µg/kg bw/d for an infant given conventionally treated water, the margin of safety would be in excess of 90,000, indicating that there is no basis for concern
References	BKH (2000) Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption. Bkh consulting engineers. Available at Internet site http://ec.europa.eu/environment/endocrine/strategy/substances_en.htm. FSA (2003) COT Report - Phytoestrogens and Health. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, Food Standards Agency, UK. Available at Internet site http://www.food.gov.uk/multimedia/pdfs/phytoreport0503. Lintelmann J. et al. (2003) Endocrine Disruptors in the Environment (IUPAC

	Technical Report). Pure Appl. Chem, 75, 631-681. *Salen G. et al (1970) Metabolism of β-Sitosterol in Man. The Journal of Clinical Investigation, 49, 952-967. Available at Internet site http://www.ncbi.nlm.nih.gov/pmc/articles/PMC535768/pdf/jcinvest00221-0116.pdf* SCF (2000) Opinion on a request for the safety assessment of the use of phytosterol esters in yellow fat spreads. Opinion adopted by the Scientific Committee on Food on 6 April 2000. Scientific Committee on Food, European Commission, Health and Consumer Protection Directorate-General. A available at Internet site http://ec.europa.eu/food/fs/sc/scf/out56_en.pdf. SCF (2002) General view of the Scientific Committee on Food on the long-term effects of the intake of elevated levels of phytosterols from multiple dietary sources, with particular attention to the effects on β-carotene. SCF/CS/NF/DOS/20 ADD 1 FINAL 3 October 2002. Scientific Committee on Food, European Commission, Health and Consumer Protection Directorate-General. A available at Internet site http://ec.europa.eu/food/fs/sc/scf/out143_en.pdf

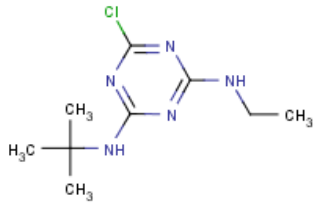
Tetrabromo-o-cresol

Hazard Assessment

Substance name:	**3,4,5,6-Tetrabromo-o-cresol** 
CAS No.:	576-55-6
Synonym(s):	o-Cresol, 3,4,5,6-tetrabromo- (6CI,7CI,8CI); 2,3,4,5-Tetrabromo-6-methylphenol; 2-Methyl-3,4,5,6-tetrabromophenol; 3,4,5,6-Tetrabromo-2-methylphenol; 3,4,5,6-Tetrabromo-o-cresol; Deodorant Richter/K; NSC 4866; Remanol; 2,3,4,5-Tetrabrom-6-methylphenol; 2,3,4,5-tetrabromo-6-methylphenol; 2,3,4,5-Tétrabromo-6-méthylphénol; Phenol, 2,3,4,5-tetrabromo-6-methyl-
Use and potential human exposure routes:	3,4,5,6-Tetrabromo-o-cresol is a brominated phenolic, used as an antiseptic and fungicide. For example, it has been used for hand disinfection and for topical preparations for the treatment of fungal infections (Daughton & Ternes, 1999; Pharmacopoeia Site, undated).
Established/Study-specific exposure limit:	No established environmental standards or guide values have been identified
Basis for exposure limit:	Not available
Evidence for human relevant endocrine disrupting potential:	This substance was identified as of possible concern because of its use profile and a report of its presence in relevant water bodies (Kasprzyk-Hordern et al., 2009). Subsequent QSAR modelling by the Study Team indicated that it may show protein binding properties but no evidence of endocrine-specific activities have been found. It was not considered by either IUPAC (Lintelmann et al, 2003) or in the EU endocrine prioritisation exercise (BKH, 2000)
Endocrine disrupting activities of concern:	None identified
Other significant toxic effects:	There is very limited information published on the hazard profile of this substance (OECD, undated). It shows moderate acute oral toxicity (Rat oral LD_{50} = >500 mg/kg) and it is irritant to the skin and mucous membranes. It has also been stated to have only low systemic toxicity (Ash & Ash, 2004).
Study estimate of anticipated exposure via drinking water	Intake (µg/kg bw/d) of tetrabromo-o-cresol from drinking water from advanced treatment process: Infant 0.008; Child 0.005; Adult 0.002. Intake (µg/kg bw/d) based on conventional treatment: Infant 0.150; Child 0.100; Adult 0.033
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	No evidence suggestive of endocrine disrupting activity per se has been identified for this substance and little significant information on its hazard profile, other than its recognised irritant properties, is available. Although registration was anticipated by ECHA in 2010, this substance has yet to be registered in the EU (ECHA, undated). If this occurs, additional toxicity data may becoming available.

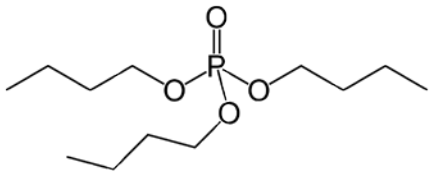
	For illustrative purposes only, if an uncertainty factor of 10,000 (100 for use of acute data only, and 10 for interspecies and 10 for inter-individual extrapolation), were to be applied to the only available toxicity data (Rat oral LD_{50} = >500 mg/kg), this would suggest that a potential study-specific value might be of the order of 5 µg/kg bw/d. The highest predicted intake of 0.150 µg/kg bw/d relates to infants given drinking water from a conventional treatment plant, and comparison of this to the study-specific illustrative estimate suggests a margin of >33-times would exist. In the absence of further hazard information, it is not possible to undertake a more meaningful risk assessment at this time. However, it is recommended that the significance of the risk posed by this substance should be reconsidered if additional toxicity data becomes available, particularly if this indicates that it may poses any properties suggestive of endocrine disrupting potential
References	Ash M & Ash I., eds (2004) *Handbook of Preservatives.* Synapse Information Resources Inc., Endicott, NY, USA. Available at Internet site http://books.google.co.uk/books?id=XZ2QB7bu5LwC&pg=PA558&lpg=PA558&dq=tetrabromocresol&source=bl&ots=6M2YtGvMu5&sig=f9TYzrzLFwMLZWftMzqndMPkFdc&hl=en&ei=vBpeToyQKcyxhAf30-H1Aw&sa=X&oi=book_result&ct=result&resnum=1&ved=0CBcQ6AEwADgK#v=onepage&q=tetrabromocresol&f=false. BKH (2000) *Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption.* BKH consulting engineers. Available at Internet site http://ec.europa.eu/environment/endocrine/strategy/substances_en.htm. Daughton CG. & Ternes TA (1999) *Pharmaceuticals and personal care products in the environment: agents of subtle change?* Environ. Health Perspect., 107, 907-938. Available at Internet site http://dx.doi.org/10.1289/ehp.99107s6907. ECHA (undated) *Information on Registered Substances.* European Chemicals Agency, Helsinki, Finland. Available at Internet site http://apps.echa.europa.eu/registered/registered-sub.aspx. Kasprzyk-Hordern B. et al. (2009) *Illicit drugs and pharmaceuticals in the environment – Forensic applications of environmental data, Part 2: Pharmaceuticals as chemical markers of faecal water contamination.* Environmental Pollution, 157, 1778-1786 . Lintelmann J. et al. (2003) *Endocrine Disruptors in the Environment* (IUPAC Technical Report). Pure Appl. Chem, 75, 631-681. OECD (undated) *The Global Portal of Information on Chemical Substances*. ChemPortal.Organisation for Economic Co-operation and Development. Available at Internet site http://www.echemportal.org/echemportal/substancesearch/page.action;jsessionid=6630EC645181D530EAF7AECB1872B0E2?pageID=9. Pharmacopoeia Site (undated) *Tetrabromocresol.* PharPhar.com-. Available at Internet site http://www.pharphar.com/Disinfectants_and_Preservatives/3259.htm

Hazard Assessment

Substance name:	**Terbuthylazine (TBA)** 
CAS No.:	5915-41-3
Synonym(s):	1,3,5-Triazine-2,4-diamine, 6-chloro-N-(1,1-dimethylethyl)-N'-ethyl-(9Cl); 2-Chloro-4-ethylamino-6-tert-butylamino-s-triazine; 2-Tert-butylamino-4-chloro-6-ethylamino-1,3,5-triazine; 2-Tert-butylamino-4-chloro-6-ethylamino-s-triazine; 2-Tert-butylamino-3-aethylamino-6-chlor-1,3,5-triazin; ChlorCaragard; Gardoprim; Primatol M; Terbutazine; Sorgoprim; Turbulethylazin; s-Triazine, 2-(tert-butylamino)-4-chloro-6-(ethylamino)-(7Cl)(8Cl)
Use and potential human exposure routes:	TBA is a chloro-triazine herbicide used on maize, sorghum, potatoes, peas, sugar cane, vines, fruit trees, citrus, coddee, oil palm, cocoa, olives, rubber and forestry in tree nurseries and new planting; it is particularly effective against annual dicotyledons. TBA application rates on most annual crops are 0.4 – 2.0 kg/ha at which no measureable residues are found among harvested produce. Residues were detected in young maize grown for silage (maximum 0.1 mg/kg) but, when given experimentally to cattle or poultry, no measureable residues occurred in meat, eggs or milk (Green, 1991). The main routes of worker exposure are via dermal contact and inhalation during preparation and use of the herbicide.
Established/Study-specific exposure limit:	An ADI of 0.004 mg/kg bw/d (4 µg/kg bw/d) was established for TBA by EFSA (2011) and a TDI of 0.0022 mg/kg bw/d (2.2 µg/kg bw/d) by WHO (2003). From the WHO (2003) TDI a recommended guideline value of 7 µg/ L was established for drinking water, based on 10% via water and a 60 kg human drinking 2 litres of water per day.
Basis for exposure limit:	The EFSA value was based on a NOAEL of 0.4 mg/kg bw/d from a dog study, described below and an uncertainty factor of 100. The WHO TDI was based on NOAEL of 0.22 mg/kg bw/d from a 2 year chronic toxicity/carcinogenicity study in rats, and an uncertainty factor of 100.
Evidence for human relevant endocrine disrupting potential:	In vitro, TBA shows weak to moderate human PXR activation;as a nuclear receptor, PXR acts as a transcription factor and, following ligand binding, functions as a heterodimer with retinoid X-receptor (RXR) in a non-permissive way, and is thus involved in the metabolism of endogenous and exogenous compounds. The EC_{50} of TBA was identified to be 3.34 E-05 (mol/L), while the maximal luciferase induction of 10 µM TBA was 32±1% (expressed as a percentage of maximal luciferase activity induced by SR12813 at 3µM) (Creutos *et al*., 2010). In an oral study on pregnant Tif/RAIfSPF rats given TBA at 0, 1, 5 or 30 mg/kg bw/day from days 6 – 15 of gestation, effects were seen on foetal

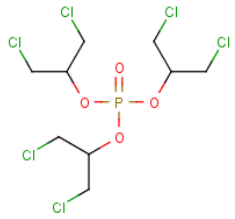
	development (delayed/absent ossification of phalanges) at 30 mg/kg bw/d, but decreased body weight gain and food consumption were also noted at the high dose; reproductive parameters were unaffected and the maternal and foetal NOAEL was 5 mg/kg bw/d EFSA, 2011). In a 2-generation dietary study in Sprague-Dawley rats, at 0, 6, 60 or 300 mg/kg diet, decreased body-weight gain and food/water consumption, and slightly higher infertile pairings and pup mortality, and retarded pup growth were noted at the high dose. At 60 mg/kg diet, decreased body weight gain and food consumption was noted in adults but reproductive parameters were unaffected (EFSA, 2011); parental NOAEL was 6 mg/kg diet (0.4 mg/kg bw/d) and reproductive NOAEL was 60 mg/kg diet (21.3 mg/kg bw/d).
Endocrine disrupting activities of concern:	Human pregnane X receptor (hPXR) agonist
Other significant toxic effects:	Acute oral toxicity of TBA is low (LD_{50} rat = 7700 mg/ kg bw). In a 1-year study in dogs administered TBA at doses of 0, 10, 50, 250 or 500 mg/kg diet, decreased body weight gain and food consumption were noted at 50 mg/kg diet and a slight reduction in red blood cell parameters occurred in females at 250 and 500 mg/kg diet; the NOAEL was 0.4 mg/kg bw/d (EFSA, 2011). In a 2 year rat chronic toxicity/carcinogenicity study, a NOAEL of 0.22 mg/kg bw/d was established based on body weight gain (WHO, 2003).
Study estimate of anticipated exposure via drinking water	No intake of TBA from drinking water is predicted following advanced water treatment. Intakes (µg/kg bw/d) based on conventional treatment were estimated at: Infant 0.005; Toddler 0.003; Adult 0.001
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	The established ADI of 4µg/kg bw/d has been established recently by an authoritative European body. Given that the 'worst case' predicted intake via drinking water (based on conservative assumptions) for infants given water from a conventional treatment processes is 0.005 µg/kg bw/d, this indicates a margin of safety of at least 800 times that of the ADI. If the more precautionary older WHO TDI values were considered, the margin of safety would still be 440-times and hence no significant cause for concern is considered to exist.
References:	Creusot, N., Kinoni, S., Balaguer, P., Tapie, N., LeMenach, K., Maillot-Maréchel, E., Pocher, J. M., Budzinski, H., Aït-Aïssa, S. (2010) Evaluation of a hPXR reporter gene assay for the detection of aquatic emerging pollutants: screening for chemicals and application to water samples. *Anal Bioanal Chem* **396**: 569 – 583. EFSA (2011) European Food Safety Authority. Conclusion on pesticide peer review: conclusion of the peer review of the pesticide risk assessment of the active substance terbuthylazine. *EFSA Journal* **9** (1): 1969. Green, D. H. (1991) Terbutylazine. Information on the active substance. September (Ciba-Geigy Document). (Cited in WHO, 2003). WHO (2003) World Health Organisation. Terbuthylazine (TBA) in drinking water: background document for development of WHO guidelines for drinking water quality. WHO/SDE/WSH/03.04/63.

Hazard Assessment

Substance name:	**Tributylphosphate (TBP)** 
CAS No.:	126-73-8
Synonym(s):	Phosphoric acid; Tributyl ester ; Butyl phosphate; Celluphos 4; TBP; Tri-N-butylphosphate; Tributilfosfato; Tributylfosfaat; Disflamoll TB
Use and potential human exposure routes:	TBP is used as a flame retardant in aircraft hydraulic fluid and as a solvent for rare earth extraction and purification. Other, minor, uses of TBP include as a defoamer additive in cement casings for oil wells, an anti-air entrainment additive for coatings and floor finishes, a solvent in nuclear fuel processing, and as a carrier for fluorescent dyes. There are no consumer products that are known to contain TBP and non-specific plant herbicides that contained TBP were reformulated in the mid-1980s and are no longer available for use (OECD, 2002). TBP has also been authorised for use as an admixture component of cements in contact with water by the UK Drinking Water Inspectorate (DWI, 2011). Human exposure is most likely to occur in workers during manufacture, formulation, processing and distribution. The general population may be potentially exposed to TBP through environmental contamination, mainly through via inhalation or oral exposure. European surface water concentrations of TBP in the range 0.1 – 3.9 µg/ L have been reported (OECD, 2002).
Established/Study-specific exposure limit:	OECD: 24.1 and 28.9 mg/kg bw/d for males and females respectively. Occupational UK Threshold Limit Values: 10 minute STEL: 5 mg/m^3. The Scottish Department of the Environment, Transport and the Regions (DETR) has set freshwater and marine environmental quality standards for TBP of 50µg/ L (annual average; AA) or 500µg/ L (maximum allowable concentration; MAC) (SEPA, 2005). US default Total Allowable Concentration (TAC) = 0.01 mg/L. Berdasco and McCready (2011) also estimated a TAC for TBP of 0.2 mg/L.
Basis for exposure limit:	In a review by the OECD, a NOAEL of 28.9 mg/ kg bw/day for females and 24.1 mg/ kg bw/d for males derived from a repeat dose toxicity study was recommended for use in risk assessment. In addition, a NOAEL for reproductive toxicity of >225 mg/kg bw/d and for developmental toxicity/ teratogenicity of >750 mg/kg bw/d were also proposed (OECD, 2002).
Evidence for human relevant endocrine disrupting potential:	This chemical was included for hazard characterisation in this study solely as a precautionary measure based on a screening QSAR analysis for protein binding carried out by the project team. Detailed subsequent examination of the available literature on the substance however confirmed that there is no reported evidence of any effects suggestible of endocrine disruptive activity
Endocrine disrupting activities of concern:	**None**

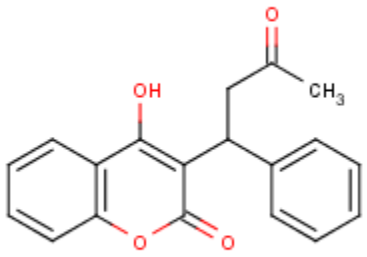
Other significant toxic effects:	A human epidemiological study has associated TBP exposure with the mucosal symptoms of sick housing syndrome (SHS) (Kanazawa *et al.*, 2009). TBP is classified as having slight to moderate oral toxicity: Rat LD_{50}= 1390 – 3350 mg/kg; Mouse LD_{50}= 400 – 1240 mg/kg; and Hen LD_{50}= 1500 – 1800 mg/kg. No evidence of embryo- or faeto-toxicity or teratogenicity has been found at doses below those associating with maternal toxicity. In Wistar rats (the most sensitive strain of rat to TBP) given 62.5, 125, 250 or 500 mg/kg bw/d, an increase in rudimentary lumbar ribs was noted at 500 mg/kg bw/d but maternal toxicity was apparent at doses of 125 mg/kg bw/d or above (Noda *et al*,. 1994). In an 18 month carcinogenicity study using CD-1 mice fed diet containing TBP at 150, 1000 or 3500 ppm, a statistically significant increase in hepatocellular adenomas was noted; NOAELs for carcinogenicity were 28.9 mg/kg bw/d for females and 24.1 mg/kg bw/d for males. The mechanism of carcinogenicity is thought to be non-genotoxic (Auletta et al., 1998).
Study estimate of anticipated exposure via drinking water	Very limited intakes via drinking water are predicted to occur following conventional or advanced treatment processes in all age groups. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.030; Toddler 0.020; Adult 0.007. Intakes (ug/kg bw/d) based on advanced treatment: Infant 0.005; Toddler 0.003; Adult 0.001
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	The OECD recommended values for risk assessment of TBP are 24.1 and 28.9 mg/kg bw/d for males and females respectively. Given that the 'worst case' predicted intake associated with consumption of drinking water generated by a conventional treatment processes (using conservative assumptions) is only 0.030 µg/kg bw/d for infants, this would suggest a margin of safety exists of > 10,000-fold for all groups considered.
References*:*	Auletta, C. S., Weiner, M. L., Richter, W. R. (1998) A dietary oncogenicity study of tributyl phosphate in the rat. *Toxicol.* **128:** 125 – 134. Berdasco, N. A. M., McCready, D. (2011) Risk assessment and class-based evaluation of three phosphate esters. *Human and Ecological Risk Assessment: An International Journal* **17** (2): 367 – 380. Bio/dynamics Inc. Bio (1991) Bio/dynamics Report A rangefinding study to evaluate the toxicity of TBP in the pregnant rabbit. Project No. 89-3536. Test conducted at the request of the Synthetic Organic Chemical Manufacturers Association, Inc (cited in OECD, 2002). DWI (2011) Defra. Drinking Water Inspectorate; Guardians of Drinking Water; list of approved products for use in pubic water supply in the United Kingdom. July 2011. Available at http://www.dwi.gov.uk/drinking-water-products/approvedproducts/soslistcurrent.pdf. Noda, T., Yamano, T., Shimizu, M., Mortia, S. (1994) Effects of TBP on pregnancy in rats. *Food Chem Toxicol*. **32:** 1031 – 1036. OECD (2002) SIDS Initial Assessment Report for 12th SIAM (Paris, France, June 2001): Tributyl Phosphate. UNEP Publications. SEPA (2005) SEPA Technical Guidance Note: Hydrological Risk Assessment of Landfills and the Derivation of Control and Trigger Levels. Version 2.12.

Hazard Assessment

Substance name:	**Tris (1,3-dichloro-2-propyl) phosphate (TDCP)** 
CAS No.:	13674-87-8
Synonym(s):	1,3-Dichloro-2-propanol phosphate; 2-Propanol, 1,3-dichloro-, phosphate; Fyrol FR 2; Tri(beta, beta'-dichloroisopropyl)phosphate; Tris(1,3-dichloro-2-propyl) phosphate; Tris (1,3-dichloroisopropyl)phosphate; Tris (1-chloromethyl-2-chloroethyl)phosphate; Tris(2-chloro-1-(chloromethyl)ethyl)phosphate
Use and potential human exposure routes:	TDCP is an additive flame retardant, i.e. it is physically incorporated with the material being treated rather than chemically-combined. Most TDCP is used in the production of flexible polyurethane (PUR) foam, which is used in automotive and furniture industries, but less than 10,000 tonnes of TDCP were consumed in the EU in 2000. Exposure of humans may be via oral, inhalation and dermal pathways. An extensive risk assessment by the EU suggested a daily human environmental intake of 1.52×10^{-5} mg/kg bw/d. This compares to a highest estimated occupational daily intake of 6.99×10^{-4} mg/kg bw/d (EU, 2008).
Established/Study-specific exposure limit:	A study-specific exposure limit of 0.05 mg/kg bw/d has been derived from a NOAEL of 5 mg/kg bw/d for hyperplasia of the convoluted tubule epithelium in male rats (Stauffer Chemical Company, 1981).
Basis for exposure limit:	In a 24-month carcinogenicity study on groups of 60 males and 60 female Sprague-Dawley rats fed TDCP in the diet at levels resulting in 0, 5, 20 or 80 mg/kg bw/day, hyperplasia of the convoluted tubule epithelium (judged a pre-neoplastic lesion) was noted at 24 months in males at >5 mg/kg bw/d. The NOAEL was thus 5 mg/kg bw/d (Stauffer Chemical Company, 1981). An uncertainty factor of 100 was applied to allow for inter-species differences.
Evidence for human relevant endocrine disrupting potential:	In the 24-month carcinogenicity study discussed above, thyroid weight was significantly increased (17%) in females at 80 mg/kg bw/d TDCP. Hyperplasia of the parathyroid glands was also increased at the high doses (Stauffer Chemical Company, 1981). Gross changes of the male reproductive tract were noted at the mid- and high-dose including discolouration, masses/nodules, enlargement and flaccidity of the testes and small seminal vesicles, in animals killed at 12 or 24 months. Testes weights were, however, unaffected (Stauffer Chemical Company, 1981). Although changes suggestive of a potential thyroid/parathyroid involvement were noted in the available carcinogenicity study, the affected animals showed evidence of significant hepatic enlargement which suggests that the underlying mechanism may not be endocrine-mediated in nature and hence should not be considered as a primary endocrine concern. Similarly the origins of the reproductive changes are unclear.

	Therefore, in the absence of any available information indicative of an endocrine-mediated mechanism it is not possible to identify a particular endocrine disruption concern at this time.
Endocrine disrupting activities of concern:	None specifically attributable to a endocrine-mediated mechanism
Other significant toxic effects:	The acute oral is low; LD_{50}rabbit = 6800 mg/kg; LD50 rat = 3160 mg/kg; LD50mice = 2670 mg/kg (EU, 2008). Data obtained from the 24-month carcinogenicity study showed increased liver weight in male and female rats; there was a LOAEL of 80 mg/kg bw/d for liver toxicity (Stauffer Chemical Company, 1981). In this study, erythroid/myloid hyperplasia of the rib-marrow and erthyroid/myloid metaplasia of the spleen were also noted at the high dose. Absolute and relative kidney weights were also statistically significantly ($p<0.05$) increased at 12 and 24 months at 80 mg/ kg bw/d while in males at terminal sacrifice, absolute kidney weight was increased by 46% and 53% at 20 and 80 mg/kg bw/d respectively.
Study estimate of anticipated exposure via drinking water	Intakes via drinking water are predicted to occur from drinking waters obtained by conventional and advanced treatment processes, for all age groups considered. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.773; Toddler 0.515; Adult 0.172. Intakes (µg/kg bw/d) based on advanced treatment: Infant 0.386; Toddler 0.258; Adult 0.086
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	The study-specific exposure limit for TDCP is 0.05 mg/kg bw/d was established on the basis of a NOAEL of 5 mg/kg bw/d for reproductive endpoints and so would be considered potentially protective of any endocrine-mechanisms of action although there is no evidence to suggest that such an endocrine-mediated mechanism exists. Given that the 'worst case' predicted intake via drinking water from a conventional treatment processes (using conservative assumptions) is 0.773 µg/kg bw/d for infants, this would suggest a minimum margin of safety of round 65 times, which is considered of low concern.
References:	EU (2008) European Union Risk Assessment: Tris(2-chloro-1-(chloromethyl)ethyl) phosphate (TDCP). Repporteur Ireland / UK. Freudenthal, R. I., Henrich, R. T. (2000) Chronic toxicity and carcinogenic potential of Tris-(1,3-dichloro-2-propyl) phosphate in Sprague-Dawley rat. *International Journal of Toxicology* **19**: 119. Stauffer Chemical Company (1981) A two year oral toxicity/carcinogenicity study of Fyrol FR-2 in rats. Unpublished report. (As cited in EU, 2008). US EPA (2011) http://www.epa.gov/dfe/pubs/flameret/altrep-v2/altrept-v2-section3a.pdf [Accessed 30.04.2011]

Hazard Assessment

Substance name:	**Warfarin** 
CAS No.:	81-81-2
Synonym(s):	Coumadin; Coumafene; Zoocoumarin
Use and potential human exposure routes:	Warfarin is the original and most frequently prescribed oral anticoagulant. Anticoagulants are used in the treatment of patients with recent deep vein thrombosis or thrombophlebitis to prevent extension and embolisation of the thrombus and to reduce the risk of pulmonary embolism or recurrent thrombus formation. In acute pulmonary embolism or venous thrombosis, anticoagulants are indicated following initial thrombolytic and/or heparin therapy to decrease the risk of extension, recurrence, or death (US EPA, 2001). Warfarin is also used as a long-term treatment in individuals at risk of developing thrombosis or embolisms. Occupational exposure to warfarin may occur through inhalation of dust and dermal contact with this compound at workplaces where warfarin is produced or used. NIOSH (NOES Survey 1981-1983) has statistically estimated that 6,132 workers (1,873 female) were potentially exposed to warfarin by such routes in the US (NIOSH, 1983). The general population may be exposed to warfarin via medical administration of this compound for the treatment of certain blood conditions, or indirectly through release into the environment during its use as a rodenticide. Warfarin and its sodium salt are also registered for use in controlling rodents (rats and mice) in and around homes, animal and agricultural premises, and commercial and industrial sites. Commercial baits typically contain 0.02% warfarin (Tomlin, 1994). When used as bait, warfarin is a potential for direct exposure of the general public; however, its therapeutic use results in significantly higher exposure of the individual. Production of warfarin for either pharmaceutical or rodenticide purposes may result in releases to the environment through various waste streams. Its use as a rodenticide may also result in releases to the environment. If released to air, warfarin will exist in both the vapour and particulate phases in the ambient atmosphere with a half-life of 2.4 hours in the vapour phase. If released to soil**,** warfarin is expected to have low soil mobility and is considered "not readily biodegradable" with only a 13% degradation (COD reduction) over a 28-day incubation period using activated sludge. If released into water, warfarin is expected to adsorb to suspended solids and

	sediment, with low potential for bioconcentration in aquatic organisms. Literature reporting warfarin levels in water bodies is sparse. Langford and Thomas (2009) report warfarin in the influent and effluent of a wastewater treatment works in Oslo, Norway at up to 5 ng/L. No U.K. data was identified.
Established/Study-specific exposure limit:	**Therapeutic doses by age range:** < 1month : 100-200 µg/kg/d 1 month to 12 years: 200 µg/kg/d (min dose) 12 to18 years: 200 µg/kg/ d (min dose) > 18 years: 0.5 – 10 mg/kg d **Occupational exposure**: Threshold Limit Values: 8 hr Time Weighted Avg (TWA) = 0.1 mg/m^3 (ACGIH, 2008). A TDI OF 0.0003 mg/kg/d has been established for warfarin by the US EPA (1991) for the general population.
Basis for exposure limit:	The US EPA TDI was based on increased prothrombin time in humans with a LOAEL of 2 mg/day, equivalent to 0.029 mg/kg/d based on a 70 kg adult (0.034 mg/kg/d for a 60kg adult). An uncertainty factor of 100 was applied: 10 to account for the use of a LOAEL and 10 to protect for sensitive individuals in the population.
Evidence for human relevant endocrine disrupting potential:	This substance was identified as of possible concern as QSAR modelling by the Study Team indicated that it may have protein binding properties (indicating the possibility of androgenic effects). However, no evidence suggestive of endocrine disrupting activity per se was identified for warfarin in subsequent literature review.
Other significant toxic effects:	Warfarin has been classified in Annex 1 of Directive 67/548/EEC (EC, 2005) as: Repr. Cat 1; R61 – may cause harm to the unborn child T: R48/25 - toxic N: R52, R53 Dangerous for the environment The US EPA has categorised warfarin as Category 1 – danger highly toxic, and WHO as Category 1b – highly toxic. Absorption of warfarin is effectively 100% from the GI-tract with maximum plasma concentrations being achieved within 2-12 hours; >99% is bound systemically to plasma protein. Warfarin is metabolised to the hydroxyl metabolites and alcohol. Excretion is bi-phasic, with a half life of between 37 – 42 hours though accumulation has been noted with repeated administration (EC, 2005). Warfarin has moderate mammalian acute toxicity in males (oral LD_{50} rat = 112 ± 15.9 mg/kg) and is highly toxicity in females (10.4 ± 1.1 mg/kg). It is not considered to be irritant to eye or skin or to be a skin sensitiser. In pregnant mice exposed to doses of 1 – 4 mg/kg bw i.p., foetal death, haemorrhaged placentaes, malformations and prolonged prothrombin time were seen (EC, 2005). Chronic exposure of humans to a therapeutic maintenance dose of between 1.0 – 13 mg/day (corresponding to 0.015-0.2 mg/kg bw/day) has been shown to prolong prothrombin time; isolated cases of bleeding episodes, skin necrosis and hepatotoxicity have also been reported following misdosing. Although a NOAEL or NOEL has not been established, a LOAEL of 0.03 mg/kg bw/day has been reported (EC, 2005). Warfarin is not considered carcinogenic or genotoxic following chronic

	exposure (EC, 2005). In a comprehensive retrospective study of pregnancies (women therapeutically treated with warfarin), doses generally ranging from 2.5-12.5 mg/day (0.03 – 0.2 mg/kg bw/day) have been found to be teratogenic (Hall et al., 1980). A critical exposure period of 6- 9 weeks of gestation has been reported for chondrodysplasia punctata, the most consistently reported malformation. Hall et al. (1980) also described CNS effects, eye disorders and developmental retardation which appeared independently of the above critical exposure window. Lowest relevant developmental adverse dose (LOAEL) in humans has been reported as 0.036 mg/kg bw/day (EC, 2005). Developmental effects have also been shown in rat studies following repeated dosing at 0.04 – 8 mg/kg bw/d; effects include haemorrhagic syndrome in foetuses, structural malformations of hind limbs, internal hydrocephalus, metabolic damage of foetal liver. At doses >100 mg/kg bw s.c. maxillonasal hypoplasia, calcium deposits in the cartilage of nasal septum and epiphyseal cartilage of vertebrae and long bones are also seen. Similar effects have been observed in humans. A NOAEL or NOEL has not been established for reproductive effects. However, a LOAEL of 0.04 mg/kg bw/day for developmental effects has been established in rats.
Primary mode of endocrine activity	None indentified
Study estimate of anticipated exposure via drinking water	Limited intake via drinking water is predicted to occur following both conventional and advanced treatment processes in all age groups. Intakes (µg/kg bw/d) based on conventional treatment: Infant 0.022; Toddler 0.015; Adult 0.005. Intakes (ug/kg bw/d) based on advanced treatment: Infant 0.003; Toddler 0.002; Adult 0.001
Assessment of endocrine disruption potential at modelled drinking water concentration(s)	No evidence suggestive of endocrine disrupting activity per se has been identified for this substance. Although, warfarin has moderate to high acute mammalian toxicity and is a recognised developmental toxicant, the daily intakes estimated in this project are well below the TDI in infants, children and adults. Given that the 'worst case' predicted intake via drinking water based on conventional treatment processes and conservative assumptions is 0.022 µg/kg bw/d for infants, this would suggest a margin of safety of 14-times against the established overall TDI. These figures were based on modelled levels in drinking water calculated with usage figures obtained from UK prescription data, undergoing conventional water treatment. It should be noted that margins of safety following advanced water treatment were significantly increased to a minimum of 100 times (for infants).
References:	EC (2005) European Commission Health and Consumer Protection Directorate-General. Review report for the active substance warfarin finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 23 September 2005 in view of the inclusion of warfarin in Annex I of Directive 91/414/EEC. Available at:http://ec.europa.eu/food/plant/protection/evaluation/exist**active/warfarin**_en.pdf Hall, J.G. et al. (1980). Maternal and fetal segualae of anticoagulation during pregnancy. Am. J. Med., 68, 122-140. Huff, B.B (Ed) 1985. Physicians Desk Reference, 39th ed. Medical Economics Co., Inc., Oradell, NJ.

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