RICARDO-AEA

Toxicological evaluation for pharmaceuticals in drinking water

Objective 6: Final report

Report for Drinking Water Inspectorate

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Executive Summary

Pharmaceuticals can be introduced into water sources through the discharge of treated sewage effluent from individuals and patients who have used these and from agricultural runoff containing livestock manure. The risks associated with the presence of specific pharmaceuticals in a water source will vary depending upon factors such as the extent of discharge into water bodies, the pharmaceuticals prescribed, used or manufactured in the area, water treatment and the size of the population in the catchment.

In 2007, wca Environment conducted a desk-based review for the Drinking Water Inspectorate (DWI) and concluded that there was unlikely to be a significant risk from pharmaceuticals discharged into drinking water sources (Watts. C *et al.*, 2007), but that a small-scale survey would be useful given the paucity of measured data in drinking water at that time. Such a study was carried out by Boxall *et al.* (2011) who detected benzoylecogonine, carbamazepine (CBZ), carbamazepine-10,11-epoxide (carbamazepine epoxide; CBZ-EP), ibuprofen and naproxen in treated water. Authors concluded that the levels of pharmaceuticals in drinking water in England do not pose a appreciable risk to human health, as they were orders of magnitude lower than therapeutic doses.

The objective of this project was to carry out a risk assessment of the pharmaceuticals measured in the previous study, by comparing potential intake values from drinking water with toxicologically-derived health-based guidance values.

Following the risk assessment paradigm, a critical assessment of the literature was carried out to identify the hazards of each pharmaceutical. Pharmaceutical companies were also approached for additional toxicological data.

Following hazard identification, hazard characterisation was carried out, by first identifying a point of departure (PoD) in the form of a no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) from the retrieved experimental animal or human epidemiology data. For some of the pharmaceuticals, i.e. for metabolites benzoylecogonine and carbamazepine epoxide, no data were retrieved. In such cases, alternative approaches were considered, including the use of Quantitative Structural Relationship Analysis (QSAR) modelling or the Threshold of Toxicological Concern (TTC) approach. Once the PoD was determined, uncertainty factors (UFs) were assessed on a case by case basis and used to calculate an acceptable daily intake (ADI).

The exposure assessment for pharmaceuticals via ingestion of drinking water was calculated based on the maximum and median concentrations of pharmaceuticals reported by Boxall *et al*., (2011) and default assumptions of body weight and daily water intake for adults, children and infants.

During risk characterisation, the intake of pharmaceuticals in drinking water was compared against the ADI and the hazard quotient (HQ) calculated. The margin of exposure (MOE) was also calculated as part of the risk communication phase.

NO(A)ELs were determined for all five pharmaceuticals, either from experimental data (carbamazepine, ibuprofen and naproxen) or via QSAR modelling (benzoylecogonine and carbamazepine epoxide). Chemical-specific UFs for all pharmaceuticals were derived, as in most instances, the default UFs of 100 were deemed inappropriate based on the data from which the NO(A)EL was derived.

The intakes of all pharmaceuticals for all receptors (adults, children and infants) were all below the calculated ADIs and the HQ were all <1. Moreover, the MOEs for all chemicals indicated it would be unlikely that they would be of concern.

Overall, when carrying out a human health risk assessment based on the levels of specific pharmaceuticals in drinking water samples reported by Boxall *et al.* (2011), using default exposure parameters for adults, children and infants, and when using toxicological endpoints as the PoD, then the levels of these pharmaceuticals measured in drinking water are not anticipated to pose an appreciable risk to public health.

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1 Introduction

1.1 Project background

Pharmaceuticals can be introduced into water sources through sewage, which carries the excreta of individuals and patients who have used these chemicals, from uncontrolled drug disposal (e.g. discarding drugs into toilets) and from agricultural runoff containing livestock manure. The risks associated with the presence of specific pharmaceuticals in a water source will vary depending upon factors such as the extent of discharge into water bodies, the pharmaceuticals prescribed, used or manufactured in the area, water treatment and monitoring, and the size of the population in the catchment.

In 2007, wca Environment conducted a desk-based review for the Drinking Water Inspectorate (DWI) and concluded that there was unlikely to be a significant risk from pharmaceuticals discharged into drinking water sources (Watts. C *et al.*, 2007) but that a small-scale survey would be useful given the paucity of measured data in drinking water at that time. Such a study was carried out by Boxall *et al.* (2011) who measured a range of pharmaceuticals in source and treated waters in England over a 12 month period. Concentrations of measured concentrations in drinking water were compared with therapeutic doses used in patients, rather than toxicological parameters. Authors concluded that the low or non-detectable levels of pharmaceuticals do not pose a risk to human health.

The objective of this current project was to carry out a risk assessment of several pharmaceuticals by comparing potential intake values from drinking water with toxicologically-derived health-based guidance values.

This report determines acceptable daily intakes (ADI) by using points of departures (PoD) identified from the data obtained from the literature search, and then reports the human health risk assessment following exposure to pharmaceuticals from drinking water, by comparing the calculated intake with such ADIs.

1.2 Selection of pharmaceuticals

Following advances in the sensitivity of analytical methods for the measurement of chemicals at very low concentrations, trace concentrations of pharmaceuticals in wastewater, various water sources and some drinking waters have been cited. In the follow-up survey commissioned by the DWI six pharmaceuticals were detected at concentrations above the limit of detection (LOD), namely caffeine, benzoylecogonine, carbamazepine (CBZ), carbamazepine-10,11-epoxide (carbamazepine epoxide; CBZ-EP), ibuprofen and naproxen, whereas the other 11 substances were not detected above their respective LODs (Boxall *et al.*, 2012).

The present report provides a summary of the current knowledge of the toxicology of benzoylecogonine, carbamazepine, carbamazepine epoxide, ibuprofen and naproxen, following a literature search of toxicological databases. Caffeine is not included in this report as it was largely monitored as a marker substance so was deemed unnecessary to include. In addition, non-pharmaceutical exposure to caffeine, i.e. the ingestion of caffeinated drinks, is most likely going to be the predominant exposure pathway for the majority of people. Caffeine was reviewed by the Committee on Toxicity in 2008, who concluded that caffeine intake during pregnancy was associated with an increased risk of fetal growth restriction and miscarriage, although there were uncertainties surrounding the latter due to recall bias and residual confounding factors (Committee on toxicity of chemicals in food, 2008).

Ongoing discussion and liaison has been held with pharmaceutical companies to identify any relevant and appropriate data held by the industry (Section 2.3 – 2.5).

1.3 Description of pharmaceuticals

Benzoylecogonine is the metabolite of cocaine. Entropin Inc, a fomer pharmaceutical research and development company manufacturered Esterom solution as a topical application. Esterom solution was derived by the esterification of benzoylmethylecgonine (cocaine), the hydroxypropyl esters of benzoylecogonine being the active molecules the Esterom solution. Under a US Food and Drug Administration (FDA) Investigational New Drug (IND) application, this Investigational Medicinal Product (IMP) underwent Phase I, II and III clinical trials, although was never licenced (Entropin Inc., 2006).

Carbamazepine is a tricyclic anticonvulsant and has been used in the treatment of epilepsy, grand mal seizures, trigeminal neuralgia and bipolar disorder since 1962. It is available by prescription only. It is a white to off-white powder that is practically insoluble in water (DrugSafetySite.com, 2013). Carbamazepine epoxide is the main metabolite of carbamazepine.

Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) used for pain relief, fever reduction and for reducing swelling (U.S. Food and Drug Administration, 1993b). It is available both by prescription but also as an over-the-counter medicine.

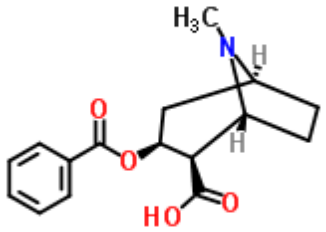
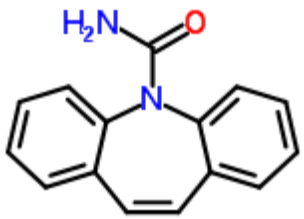
Naproxen is also a NSAID, widely used in the treatment of pain and inflammation. It is available both by prescription but also as an over-the-counter medicine. It is an odourless, white to off-white crystalline substance that is practically insoluble in water (Roche, 2006, U.S. Food and Drug Administration, 1993a) (Table 1).

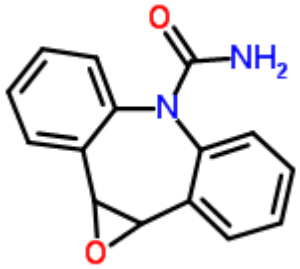
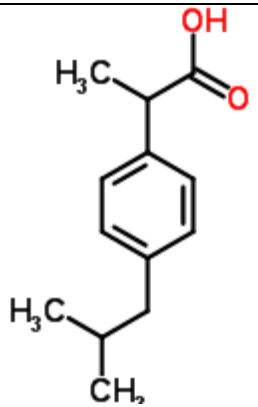
1.4 This report

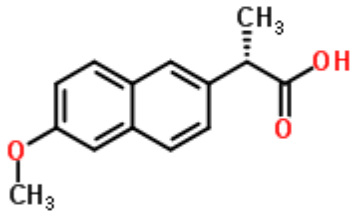
This report is structured as follows:

- Chapter 2 sets out the literature search methodology to gather information regarding the toxicity of the named pharmaceuticals.
- Chapter 3 presents a toxicological summary of the data obtained from the literature search and from the pharmaceutical companies.
- Chapter 4 identifies the PoD for each pharmaceutical, either from experimental data or via alternative approaches such as Quantitative Structural Relationship Analysis (QSAR) modelling or the Threshold of Toxicological Concern (TTC) approach.
- Chapter 5 presents the risk assessment for the identified pharmaceuticals, by comparing intake from drinking water with ADI calculated from the PoD previously determined.

Table 1. Pharmaceutical characteristics

Compound	Structure	Brand name	Class and use	Maximum conc. in treated water (ng/L)	Highest median conc. in treated water (ng/L)
Benzoylecgononine Cas No. 519-09-5			Cocaine metabolite	3.51 (site 4)	1.98 (site 4)
Carbamazepine Cas No. 298-46-4		Biston, Calepsin, Convulsine, Epitol, Finlepsin, Hermolepsin, Karbamazepine, Lexin, Mazepine, Neuritol, Neurotol, Neurotop, Nordotol, Servimazepine, Sirtal, Stazepine, Tegretal, Tegretol, Telesmin, Temporol, Teril,Timonil, Trimonil Retard	Anti-convulsant and mood stabilising drug used for epilepsy, bipolar disorder and trigeminal neuralgia	148 (site 2)	11.8 (site 1)

Compound	Structure	Brand name	Class and use	Maximum conc. in treated water (ng/L)	Highest median conc. in treated water (ng/L)
Carbamazepine epoxide Cas No. 36507-30-9			Carbamazepine metabolite	16.6 (site 2)	6.24 (site 4)
Ibuprofen Cas No. 22204-53-1		Anadin Ibuprofen, Anadine Joint Pain, Artofen, Brufen, Brufen Retard, Calprofen, Ebufac, Fenpaed, Galprofen, Ibugel, Ibuleve, Nurofen, Orbifen, Rimafen	Non-steroidal anti-inflammatory drug used for pain and inflammation	3.07	<2

Compound	Structure	Brand name	Class and use	Maximum conc. in treated water (ng/L)	Highest median conc. in treated water (ng/L)
Naproxen Cas No. 15687-27-1		Aleve, Anaprox, Antalgin, Apranax, Feminax Ultra, Flanax, Inza, Midol Extended Relief, Nalgesin, Naposin, Naprelan, Naprogesic, Naprosyn, Narocin, Proxen, Soproxen, Synflex and Xenobid	Non-steroidal anti-inflammatory drug used for pain and inflammation	2.72	<1

2 Literature search and data collection

The objective of milestone 1 was to conduct a literature search and summarise current knowledge of the toxicology of the five selected pharmaceuticals.

2.1 Selection of information sources and search terms

The project team identified a range of information sources for interrogation and access was made to a number of scientific and bibliographic databases as well as publicly available websites (Appendix 1). In general, opinions from toxicology databases were primarily considered, including hazardous substances databank (HSDB), or authoritative bodies such as Medicines and Healthcare products Regulatory Agency (MHRA), US FDA, Agency for Toxic Substances and Disease Registry (ATSDR) etc, but in cases where data were scarce, the primary literature such as scientific publications was searched. This occurred predominantly for benzoylecogonine and carbamazepine epoxide, as there were few data availbale and such data that had not been assessed by authoritative bodies.

Search terms were developed to retrieve published literature relating to the named pharmaceuticals and associated toxicity and/or terms relating to the derivation of health-based guidance values (HBGVs) (Appendix 2). The search terms were adapted according to the information source being interrogated and depending on the quality and quantity of literature found. Synonyms were used where appropriate as well as the CAS number. The latter were particularly useful for the metabolites, to distinguish them from the source compound.

Following preliminary searches by the information scientist, the team screened the titles and abstracts of the published data obtained to ascertain whether sufficient relevant data had been retrieved. The Klimisch criteria were not applicable at this stage because of the nature of the data obtained for the toxicology summary, but were more relevant when identifying and selecting relevant points of departure (Milestone 3).

If the data were considered to be sufficient, no further searches were conducted. If few data were obtained, searched terms were amended and additional search tools were used.

2.2 Data extraction

All relevant literature was systematically reviewed and data were extracted by team members. Because of the nature of the data obtained i.e. in the form of narrative reviews, safety data sheets, toxicity excerpts etc, data abstraction forms outlining the study characteristics, such as number of animals used, route of administration, controls, statistical methods etc. were inappropriate to use at this stage of the project. However, they played a critical role in the derivation of the HBGVs as data such as study type, species, exposure duration, PoD (e.g. no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL)) and effect from each study were tabulated (Milestone 3).

2.3 Identification of pharmaceutical companies

The objective of milestone 2 was to communicate with the pharmaceutical companies to obtain any relevant toxicity data for use in the toxicology review and in deriving HBGVs.

During the initial stages of the project the pharmaceutical companies that held the original licence for the compounds in question were identified (Table 2).

Table 2. Pharmaceutical manufacturers

Generic name	Brand name	Pharmaceutical company
Benzoylecogonine	Not applicable	Entropin, Inc. No longer trading
Carbazepine	Biston, Calepsin, Convulsine, Epitol, Finlepsin, Hermolepsin, Karbamazepine, Lexin, Mazepine, Neuritol, Neurotol, Neurotop, Nordotol, Servimazepine, Sirtal, Stazepine, Tegretal, Tegretol, Telesmin, Temporol, Teril,Timonil, Trimonil Retard	Novartis
Carbamazepine epoxide	Not applicable	Not applicable – metabolite of carbamazepine
Naproxen	Aleve, Anaprox, Antalgin, Apranax, Feminax Ultra, Flanax, Inza, Midol Extended Relief, Nalgesin, Naposin, Naprelan, Naprogesic, Naprosyn, Narocin, Proxen, Soproxen, Synflex and Xenobid	Roche
Ibruprofen	Anadin Ibuprofen, Anadine Joint Pain, Artofen, Brufen, Brufen Retard, Calprofen, Ebufac, Fenpaed, Galprofen, Ibugel, Ibuleve, Nurofen, Orbifen, Rimafen	Abbott

2.4 Communication with pharmaceutical companies

Communication with the relevant pharmaceutical companies was initially made via telephone to identify the most appropriate contact person, after which letters were sent to all concerned to more fully outline the project, along with a pro forma that they were asked to complete for their pharmaceutical. All companies acknowledged the request for toxicity data and identified, internally, the contact person who was responsible for retrieving the data. All communications are outlined in Appendix 3.

A log of all correspondence is given in the Appendix 3, as well as copies of the letters sent to the company (Appendix 4) and the pro forma (Appendix 5).

Entropin Inc, the company that manufactured Esterom solution (benzoylecogonine), ceased trading in 2005. However, prior to this, initial registrations for Esterom solution were made to the FDA. In late 1987, Entropin Inc. filed an IND application with the FDA seeking approval of Esterom solution as a topical application. Subsequently, based on review of four preclinical animal studies, the FDA approved the application permitting the company to proceed with human testing. However, due to the lack of statistically significant efficacy levels required by the FDA, no approval was gained and the company subsequently ceased business. As part of the project, we carried out a Freedom of Information Act request for the non-clinical safety assessment data from acute studies all the way through the safety assessment programme to carcinogenicity studies. We also requested data from any study involving exposure via oral administration although stated that studies involving other routes of exposure would also be of interest, particularly if they included toxicokinetic information.

2.5 Response from pharmaceutical companies

2.5.1 Novartis

Novartis were unable to provide any information regarding the toxicological data on carbamazepine and/or carbamazepine epoxide.

2.5.2 Roche

Roche provided a poster entitled 'Proposal for an Environmental Quality Standard according to the EU Water Framework Directive for the anti-hyperlipidaemic pharmaceutical Bezafibrate' and a Safety Data Sheet for naproxen.

2.5.3 Abbott

Abbott provided a scientific publication entitled 'Absorption, distribution and toxicity of ibuprofen' by Adams *et al.* (1969).

2.5.4 Food and Drug Administration

The Food and Drug Administration responded stating that the '*type of information requested is complex and would require a detailed disclosure review prior to release because it could contain confidential commercial information or trade secret information as well as personal privacy information. This type of request would be at least 24 months before it came up in the queue and likely to be approximately 1-2 thousand dollars'*. Therefore we did not proceed with the application.

3 Toxicological summary

3.1 Benzoylecgonine

3.1.1 Pharmacokinetics

Chemically, benzoylecogonine is ecgonine benzoate and is the primary metabolite of benzoylmethylecgonine, otherwise known as cocaine. It is formed in the liver by the metabolism of cocaine, catalysed by carboxylesterases and is subsequently excreted in the urine (Hamilton *et al.*, 1977). Approximately 30-40% of a dose of cocaine is hydrolysed spontaneously to form benzoylecogonine and another 30-40% metabolised by liver enzymes to form ecgonine methyl ester. Both products are water soluble and appear in the urine as benzoylecogonine with a half life ($T_{1/2}$) of 7.5 hours.

3.1.2 Mechanism of action

In their paper examining dermal penetration, McDonald and Lunte (2003) describe work they conducted on the IMP Esterom solution. Entropin Inc, the manufacturers of this topical solution, Esterom, described the conduct of Phase I, II and II clinical trials with the product in their annual report of 2006. Attempts to define a mechanism of action were undertaken at Harvard Medical School and University of Arizona Medical Centre. It was reported that Esterom works by blocking nerve impulse conduction resulting in anaesthetic and potentially long-lasting analgesic properties. However, the product was a mixture of hydroxypropyl esters of benzoylecogonine and the effects could not be attributed to benzoylecogonine alone (McDonald and Lunte, 2003).

3.1.3 Therapeutic dose

Benzoylecgonine was used as the main pharmaceutical ingredient in the IMP, Esterom, a topical solution used for the relief of muscle pain, but did not gain regulatory approval. As this product was a mixture of related esters of benzoylecogonine, a dose concentration of benzoylecogonine alone was not established and there remains some debate as to whether this specific metabolite of cocaine is itself biologically active (McDonald and Lunte, 2003).

It has been established that benzoylecogonine might be responsible for some of the delayed cerebrovascular events previously attributed to cocaine. For instance Brogan *et al.* (1992) associated the recurrent vasoconstriction of human coronary arterial (after 90 minutes of cocaine administration) to a temporal correspondence with an increasing blood concentration of cocaine's main metabolites, including benzoylecogonine . Madden *et al.* (1995) found that cat's middle cerebral arteries contracted more vigorously to benzoylecogonine than to cocaine itself. Moreover, they showed that cocaine and benzoylecogonine exert vasoconstrictor effects through different mechanisms: cocaine appears to act primarily through adrenergic nerves and receptors coupled with activation of intracellular calcium stores; benzoylecogonine's action appears to depend extensively on the influx of extracellular calcium. Nikolettos *et al.* (2012) showed also that benzoylecogonine increases myometrium contractility in the rat through an adrenergic mechanism.

Despite this knowledge of benzoylecogonine's pharmacological effects in humans and vertebrates, no data appears to be available on the acute or chronic effects on non-target organs.

3.1.4 Toxicity

3.1.4.1 Acute toxicity

Acute oral lethal dose (LD_{50}) levels are 300 mg/kg in mice, 980 mg/kg in rats and 3200 mg/kg in rabbits (Clearsynth Labs Pvt. Ltd.).

3.1.4.2 Chronic toxicity

There were no data retrieved on irritation / corrosivity, sensitisation, chronic toxicity, mutagenicity / genotoxicity, carcinogenicity, developmental or reproductive toxicity.

3.2 Carbamazepine

3.2.1 Pharmacokinetics

Carbamazepine is almost completely absorbed but the rate of absorption is slow and may vary between patients. The concentration of unchanged substance in the cerebrospinal fluid is the unbound portion in plasma i.e. 20-30% of the total plasma concentration. It is metabolised in the liver by oxidative pathways to pharmacologically active metabolite, carbamazepine epoxide. This may constitute up to 30% of the circulating active material as carbamazepine. Less than 1 % of carbamazepine is excreted in the urine in the unchanged form, as most is converted to the metabolite (Medicines and Healthcare products Regulatory Agency, 2013).

3.2.2 Mechanism of action

Carbamazepine appears to exert its anticonvulsant properties by reducing polysynaptic responses and blocking the post-tetanic potentiation. Carbamazepine greatly reduces or abolishes pain induced by stimulation of the infraorbital nerves and depresses thalamic potential and bulbar and polysynaptic reflexes. It is chemically unrelated to other anticonvulsants or other drugs used to control the pain of trigeminal neuralgia although the mechanism of action is unknown (Novartis Pharmaceuticals Corporation, 2013, U.S. Food and Drug Administration).

Current experimental evidence shows that carbamazepine reduces the ability of neurons to fire at high frequency by enhancing sodium channel inactivation (Cunningham *et al.*, 2010).

Carbamazepine is thought to be carcinogenic, but believed that the mode of action for hepatoma formation is mostly due to high liver metabolic activity and possible liver enzyme induction rather than a genotoxic mechanism (Cunningham *et al.*, 2010).

The principal metabolite of carbamazepine, carbamazepine epoxide, also has anticonvulsant activity as demonstrated in several in vivo animal models of seizures (Daily Med, 2012a, b, Novartis Pharmaceuticals Corporation, 2013, U.S. Food and Drug Administration, 1968). It has proved to be effective in limiting high frequency repetitive firing at concentrations comparable to those of carbamazepine. Though clinical activity for carbamazepine epoxide has been postulated, the significance of its activity with respect to the safety and efficacy of CBZ has not been established (Cunningham *et al.*, 2010).

Signs of toxicity have been reported above the upper limit of the therapeutic level and are due to effects on the central nervous system, gastrointestinal irritation, arrhythmogenic properties as well as its anti-diuretic properties (World Health Organisation, 1988).

3.2.3 Therapeutic dose

The lowest therapeutic dose for carbamazepine is 200 mg/day and 100 mg/day for adults and children respectively (2.86 mg/kg bw/day for adults or 3.33 mg/kg bw/day, based on a 70 kg adult and 30 kg child) (Cunningham *et al.*, 2010). The maximum human daily dose (MHDD) is 1600 mg/day, used in rare instances, the norm being 1200 mg/day (17.14 mg/kg

bw/day) for adults or 1000 mg/day for children (33.3 mg/kg bw/day) (Novartis Pharmaceuticals Corporation, 2013).

The maximum recommended therapeutic dose (MRTD) for carbamazepine was 26.7 mg/kg bw/day (U.S. Food and Drug Administration, 2009).

3.2.4 Toxicity

3.2.4.1 Acute toxicity

Acute oral LD_{50} levels are 529-3750 mg/kg in mice, 1957-4025 mg/kg in rats and 3850-4025 in rabbits (Novartis Pharmaceuticals Corporation, 2013, NSF International, 2012, Sigma-Aldrich, 2013, World Health Organisation, 1988).

3.2.4.2 Irritation / corrosivity and sensitisation

There were no data retrieved on irritation / corrosivity or sensitisation.

3.2.4.3 Chronic toxicity

Head, Eyes, Ears, Nose and Throat

Mydriasis and nystagmus are common. Hearing loss has also been reported (TOXNET, 2013a).

Carbamazepine has mild anticholinergic activity so may cause an increase intraocular pressure in patients already with elevated pressures (FDA Drug Information Online, 2013, Novartis Pharmaceuticals Corporation, 2013).

Cardiovascular toxicity

Sinus tachycardia is common following exposure and cardiac conduction defects (prolonged PR, QRS and QTc intervals) have been noted. Hypotension and myocardial depression may occur in severe overdose (TOXNET, 2013a).

Respiratory toxicity

Respiratory depression and apnea may occur and acute interstitial pneumonia and ARDS have occasionally been reported after severe overdose (TOXNET, 2013a).

Neurotoxicity

Ataxia, slurred speech, nystagmus, dystonias, and varying degrees of CNS depression are common. Myoclonic activity, seizures and abnormal tendon reflexes have also been reported (TOXNET, 2013a).

Gastrointestinal toxicity

Delayed gastric emptying may occur as an anticholinergic side effect (TOXNET, 2013a).

Hepatotoxicity

Hepatitis has been reported after chronic therapy (TOXNET, 2013a).

Haematotoxicity

Hematopoietic toxicity (neutropenia, thrombocytopenia, agranulocytosis and aplastic anemia) has been reported following therapeutic doses over a longer period of time, but not after acute overdose (TOXNET, 2013a).

Aplastic anemia and agranulocytosis have also been reported. Data from a population-based case control study demonstrate that the risk of developing these reactions is 5 to 8 times greater in patients receiving carbamazepine than in the general population.

Although reports of transient or persistent decreased platelet or white blood cell counts are not uncommon following the use of carbamazepine, data are not available to estimate accurately their incidence or outcome. However, the vast majority of the cases of leukopenia have not progressed to the more serious conditions of aplastic anemia or agranulocytosis

(Daily Med, 2012b, FDA Drug Information Online, 2013, Novartis Pharmaceuticals Corporation, 2013).

Genitourinary system toxicity

Following administration of carbamazepine to rats for 4 to 52 weeks at dose levels of 50 to 400 mg/kg body weight (bw)/day, testicular atrophy was reported. In addition, rats receiving carbamazepine in the diet for 2 years at dose levels of 25, 75, and 250 mg/kg bw/day had a dose-related incidence of testicular atrophy and aspermatogenesis, with a LOAEL being 250 mg/kg bw/day (FDA Drug Information Online, 2013, Novartis Pharmaceuticals Corporation, 2013)

Neurotoxicity

Reversible neurotoxicity can occur in 5-14% of patients during the first few weeks of therapeutic doses and persons with prior brain injury and elderly may be more sensitive. Typical neurotoxicity symptoms include diplopia, drowsiness, blurred vision, disturbed equilibrium and paresthesae. Long-term or irreversible neurotoxic effects are not known to occur with carbamazepine therapy (Minnesota Department of Health, 2013).

Endocrine disruption

Thyroid effects in animal studies were noted at human equivalent doses over 10 times higher than the human LOAEL (3.8 mg/kg bw/day based on the minimum therapeutic dose for children). The human equivalent doses for thyroid effects in animals are over 3,000 times higher than the reference dose (RfD) (0.013 mg/kg bw/day) and 400 times higher than the RfD for reproductive effects (Minnesota Department of Health, 2013).

Endocrine effects, including decreased thyroid hormones, in the absence of clinical hypothyroidism, have occurred in multiple human studies and in only a few animal studies (Minnesota Department of Health, 2013).

3.2.4.4 Mutagenicity/genotoxicity

There was no evidence that carbamazepine was mutagenic as bacterial and mammalian mutagenicity studies were negative (Cunningham *et al.*, 2010, Minnesota Department of Health, 2011, 2013, World Health Organisation, 1988)

3.2.4.5 Carcinogenicity

Following administration of carbamazepine to Sprague-Dawley rats for two years in the diet at doses of 25, 75, and 250 mg/kg bw/day, a dose-related increase in the incidence of hepatocellular tumors in females and of benign interstitial cell adenomas in the testes of males was reported (FDA Drug Information Online, 2013). The significance of these findings to humans was unknown (FDA Drug Information Online, 2013, World Health Organisation, 1988), although MHRA stated that there is no evidence that this observation is of importance for the therapeutic use of carbamazepine in humans (Medicines and Healthcare products Regulatory Agency).

The mutagenicity data suggest that carbamazepine is non-genotoxic hence a threshold of toxicity is likely to occur with respect to the hepatomas. The LOAEL for hepatomas in females was 25 mg/kg bw/day (Cunningham *et al.*, 2010).

Carabamazepine has not been identified by International Agency for the Research on Cancer (IARC) as a probable, possible or confirmed human carcinogen (Sigma-Aldrich, 2013).

3.2.4.6 Reproductive toxicity

Developmental toxicity

There is a large body of evidence in both experimental animal species and humans to suggest that there is an association between carbamazepine taken during pregnancy and adverse developmental effects.

Experimental animal data

In rats, carbamazepine has been shown to have adverse effects in reproduction studies in rats when given orally in dosages 10 to 25 times the MHDD of 1,200 mg on a mg/kg basis or 1.5-4 times the MHDD on a mg/m^2 basis. In rat teratology studies, 2 of 135 offspring showed kinked ribs at 250 mg/kg and 4 of 119 offspring at 650 mg/kg showed other anomalies (cleft palate, 1; talipes, 1; anophthalmos, 2). In reproduction studies in rats, nursing offspring demonstrated a lack of weight gain and an unkempt appearance at a maternal dosage level of 200 mg/kg (FDA Drug Information Online, 2013, MICROMEDEX, 2010, Novartis Pharmaceuticals Corporation, 2013).

Oral administration of carbamazepine to mice, rats and rabbits during organogenesis led to increased embryonic mortality at daily doses that caused maternal toxicity (>200 mg/kg bw/day) i.e. equating to 20 times the usual human dose**.** In the rat there was also some evidence of abortion at 300 mg/kg bw/day. Maternally toxic doses also cause growth retardation in near term rat foetuses. No evidence of a teratogenic effect was observed in the mice, rats or rabbits. One study reported defects in 4.7% of exposed foetuses compared with 1.3% in controls following oral administration of 40 to 240 mg/kg bw/day carbamazepine (Medicines and Healthcare products Regulatory Agency, 2008, Novartis Pharmaceuticals Corporation, 2013).

Most developmental effects in animal studies have occurred at doses near or above 200 mg/kg bw/day, with a human equivalent dose >44 mg/kg bw/day, which is over 8 times higher than the human LOAEL (3.8 mg/kg bw/day) and over 2,000 times higher than the RfD (0.013 mg/kg bw/day) . Slight effects on skeletal and brain development and slight foetal and pup growth retardation were reported in a small number of animals at the human equivalent doses at or near the human LOAEL (human equivalent dose ranging from 4.4 to 9.75 mg/kg bw/day) but which are over 200 times higher than the RfD (0.013 mg/kg bw/day). Study limitations prevented use of the animal studies for quantitative evaluation. The Minnesota Department of Health (MDH) based the RfD, in part, on developmental effects observed in humans at therapeutic dose levels (Minnesota Department of Health, 2013).

Human data

Offspring of epileptic mothers are known to be more prone to developmental disorders, including malformations. The possibility that carbamazepine, like all major antiepileptic drugs, increases this risk has been reported. Developmental disorders and malformations, including spina bifida and also other congenital anomalies, e.g. craniofacial defects, cardiovascular malformations, hypospadias and anomalies involving various body systems, have been reported in association with Tegretol (FDA Drug Information Online, 2013, Novartis Pharmaceuticals Corporation, 2013).

A number of studies have been identified that report an association between carbamazepine (doses of approximately 600-1,600 mg/day) and congenital anomalies, including intrauterine growth retardation, poor neonatal performance, postnatal growth deficiency , developmental delay, microcephaly, upslanting palpebral fissures, short nose with long philtrum, hypoplastic nails, cardiac defect and spina bifida (DrugSafetySite.com, 2013). Other authors concluded, based on the combined results from the retrospective and prospective studies that carbamazepine exposure was associated with a pattern of congenital malformations whose principal features consisted of minor craniofacial defects, fingernail hypoplasia, and developmental delay (DrugSafetySite.com, 2013).

Human developmental effects have also been reported at therapeutic doses in many prospective studies of epileptic women who have taken carbamazepine during pregnancy.

Overall, carbamazepine use in pregnancy is associated with an increased incidence of major and minor malformations, including an estimated 1% risk of spina bifida. A foetal carbamazepine syndrome has been proposed consisting of minor craniofacial defects,

fingernail hypoplasia, and developmental delay, although the latter abnormality is controversial as not all studies reported mild mental retardation (DrugSafetySite.com, 2013, Novartis Pharmaceuticals Corporation, 2013, U.S. Food and Drug Administration).

Carbamazepine is classified as FDA pregnancy category D: There is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience or studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks (U.S. Food and Drug Administration). Craniofacial defects, fingernail hypoplasia and developmental delays were found in a series of 35 patients whose mothers took only carbamazepine. Human and animal studies indicate that carbamazepine and its epoxide metabolite are found and excreted in breast milk and may affect the nursing child (TOXNET, 2013a).

Female reproductive toxicity

Experimental animal data

A small number of studies in animals have reported associations between carbamazepine exposure and parameters such as pre- and post-implantation mortality, litter size and live birth index. Carbamazepine has produced decreased fertility in animal studies at human equivalent doses of 52 mg/kg bw/day or more (over 10 times higher than the human LOAEL and over 2500 times higher than the RfD) (Medicines and Healthcare products Regulatory Agency, 2013)

No data on human female reproductive toxicity associated with carbamazepine were identified (U.S. Food and Drug Administration).

Male reproductive toxicity

Experimental animal data

A study in rats exposed by injection for 3 months post weaning revealed lowered epididymal sperm count but no effect on fertility.

Reported effects on testes and spermatogenesis in animals occurred at human equivalent doses from 7.3 to 23 mg/kg bw/day (corresponding to the human therapeutic maintenance dose range) and decreased fertility was reported in animals at human equivalent doses of over 8 times higher than the human LOAEL and the maximum dose level of approximately 17 mg/kg bw/day (1200 mg/day) for human adults (Minnesota Department of Health, 2013).

3.3 Carbamazepine epoxide

3.3.1 Pharmacokinetics

Carbamazepine is metabolised into carbamazepine epoxide, which is pharmacologically active and potentially toxic. The epoxide is the main active metabolite of carbamazepine, which, in turn, is inactivated to the transdiol derivative (Mayo Medical Laboratories, Medicines and Healthcare products Regulatory Agency).

In rat studies, plasma carbamazepine levels in plasma were significantly lower than levels of carbamazepine epoxide, with epoxide levels being consistently three to five times higher than the parent compound (Finnell *et al.*, 1986).

3.3.2 Mechanism of action

In humans, it has been suggested that carbamazepine epoxide may be responsible for the congenital abnormalities that are often associated with carbamazepine used during pregnancy. However, the reproductive studies in animals do not support this suggestion. (see Section 3.3.4.2 below). Toxicity may arise in patients whose serum concentrations of carbamazepine are within therapeutic ranges, but who are producing significant levels of the active epoxide metabolite. Potentially toxic levels of the epoxide may occur following co-

administration of drugs that induce hepatic oxidizing enzymes (hence more epoxide metabolite is formed), or inhibits the epoxide metabolism to the inactive transdiol metabolite (Mayo Medical Laboratories).

3.3.3 Therapeutic dose

The therapeutic concentration of carbamazepine epoxide is 0.4-4 μg/mL (4.0-12.0 μg/mL for carbamazepine) (Mayo Medical Laboratories).

3.3.4 Toxicity

3.3.4.1 Acute and chronic toxicity

There were no data retrieved on acute toxicity, irritation / corrosivity, sensitisation, chronic toxicity, mutagenicity / genotoxicity, or carcinogenicity, or reproductive toxicity.

3.3.4.2 Reproductive toxicity

Developmental toxicity

Contrary to the human epidemiology data described above, there was no correlation between adverse pregnancy outcome and carbamazepine or the epoxide metabolite in rats. Despite the increasing concentrations of carbamazepine and the epoxide in plasma, there was no parallel increase in the rate of foetal abnormalities (Finnell *et al.*, 1986).

Similarly, carbamazepine epoxide was not shown to be embryotoxic to either mice or rat embryos, suggesting that the parent carbamazepine is responsible for the embryotoxicity, rather than the metabolite (Hansen *et al.*, 1996).

Later studies in rats indicated that carbamazepine epoxide potentially increased the risk of teratogenesis following administration of carbamazepine in combination with other anticonvulsants, although other authors suggested that it has not yet been established whether the presence of carbamazepine epoxide in carbamazepine monotherapy was associated with adverse effects on the foetus (California Environmental Protection Agency, 1996).

3.4 Ibuprofen

3.4.1 Pharmacokinetics

Ibuprofen is rapidly absorbed after oral administration with C_{max} values being reached 1.6-3.1 hours after oral administration. Repeated administration does not appear to result in any accumulation of ibuprofen. Ibuprofen is extensively bound to plasma proteins (99%) (European Medicines Agency, 2010). Ibuprofen is present as a racemate and following absorption, it undergoes interconversion in the plasma from the R-isomer to the S-isomer. Both the R- and S- isomers are metabolized to two primary metabolites: (+)-2-4'-(2-hydroxy-2-methyl-propyl) phenyl propionic acid and (+)-2-4'-(2-carboxypropyl) phenyl propionic acid, both of which circulate in the plasma at low levels relative to the parent.

Ibuprofen is eliminated from the systemic circulation with $T_{1/2}$ values ranging from 1.8 to 2.6 hours after single dose administration of Combunox. Urinary excretion of unchanged ibuprofen is minimal (less than 0.2% of administered ibuprofen dose) (Davies, 1998).

3.4.2 Mechanism of action

Nonsteroidal anti-inflammatory drugs such as ibuprofen work by inhibiting the enzyme cyclooxygenase (COX), which converts arachidonic acid to prostaglandin H_2 (PGH_2). PGH_2, in turn, is converted by other enzymes to several other prostaglandins (which are mediators of pain, inflammation, and fever) and to thromboxane A_2 (which stimulates platelet aggregation, leading to the formation of blood clots).

Ibuprofen is a nonselective COX inhibitor, in that it inhibits two isoforms of COX, COX-1 and COX-2. The analgesic, antipyretic, and anti-inflammatory activity of NSAIDs appears to operate mainly through inhibition of COX-2, whereas inhibition of COX-1 would be responsible for unwanted effects on the gastrointestinal tract (Rao and Knaus, 2008). However, the role of the individual COX isoforms in the analgesic, anti-inflammatory, and gastric damage effects of NSAIDs is uncertain and different compounds cause different degrees of analgesia and gastric damage (Kakuta *et al.*, 2008).

3.4.3 Therapeutic dose

Ibuprofen has a dose-dependent duration of action of around four to eight hours, which is longer than suggested by its short half-life. The recommended dose varies with body mass and indication. A dose of 400 mg per dose and 1200 mg per day is considered the maximum amount for over-the-counter use in the United States (U.S. Food and Drug Administration).

The MRTD for ibuprofen was 40.0 mg/kg bw/day (U.S. Food and Drug Administration, 2009).

3.4.4 Toxicity

3.4.4.1 Acute Toxicity

Acute oral LD_{50} values in the range of 400-1600 mg/kg are quoted for oral administration to rats and 800-897 mg/kg for mice (Adams *et al.*, 1970, Adams *et al.*, 1969, European Chemicals Bureau, 2000, European Medicines Agency, 2010).

3.4.4.2 Irritation / corrosivity

Along with other NSAIDs, ibuprofen has been associated with the onset of pemphigoid-like blistering (Chan, 2011) and also reported to be a weak photosensitising agent (Bergner and Przybilla, 1992). NSAIDs, including Combunox, can cause serious skin adverse events such as exfoliative dermatitis, Stevens-Johnson Syndrome and toxic epidermal necrolysis, which can be fatal (Forest Pharmaceuticals Inc, 2007)).

3.4.4.3 Sensitisation

In an OECD 406 Guinea pig maximisation test performed in 1981 a 10% ibuprofen gel was found to be non-sensitising (European Chemicals Bureau, 2000).

3.4.4.4 Chronic toxicity

Cardiovascular toxicity

Chronic ibuprofen use at high doses has been found to correlate with a risk of hypertension (Forman *et al.*, 2005) and myocardial infarction (Hippisley-Cox and Coupland, 2005) and doses of 1800 mg per day were found to significantly increase systolic blood pressure (Gurwitz *et al.*, 1996). Similar cardiovascular events are cited in Forest Pharmaceuticals Medical Data Sheet (Forest Pharmaceuticals Inc, 2007).

Respiratory toxicity

Pulmonary lesions were recorded in rats treated with 120 mg/kg bw/day in the diet for 2 years (European Chemicals Bureau, 2000).

Gastrointestinal toxicity

Experimental animal data

In a 13 week oral toxicity study in rats, gastrointestinal tract irritation (ulcers, diverticuli, mucosal necrosis of the jejunum and erosions of the stomach mucosa) was reported at a dose level including 100 mg/kg bw/day. A no observed effect level (NOEL) was established at 25 mg/kg bw /day (European Medicines Agency, 2010). Gastrointestinal damage was also induced at dose levels of 180 mg/kg bw/day and above in a 13 and 26 week oral study in rats, as well as following 120 mg/kg bw/day in the diet for 2 years (European Chemicals Bureau, 2000). A similar effect was reported in mice treated at 300 mg/kg bw/day in the diet

for 13 weeks (European Chemicals Bureau, 2000). No effects such as these were detected in dogs up to an oral dose level of 16 mg/kg bw/day over 14 days. However, over treatment periods of 30 days and up to 26 weeks, daily doses to dogs of 8 mg/kg bw/day and above and to baboons of 100 mg/kg bw/day were frequently associated with gastric ulcers or erosions and intestinal inflammation (European Chemicals Bureau, 2000).

At 180 mg/kg bw/day, one treatment-related death occurred in rats, due to intestinal lesions. Histological differences were also observed in rats treated with 180 mg/kg bw/day, which caused intestinal ulcers. A dose level of 540 mg/kg bw/day caused intestinal ulceration with peritonitis after 4 days of dosing (Adams *et al.*, 1969).

Dogs treated with 8 and 16 mg/kg bw/day ibuprofen for 30 days showed gastric ulcers and erosions, post-morten, as well as intestinal inflammation. No effects were seen in dogs treated with 4 mg/kg bw/day. Administration of 16 mg/kg bw/day ibuprofen to female dogs for 26 weeks caused gross gastrointestinal disturbances, such as vomiting, diarrhoea with fresh blood, anorexia and loss of weight. Male dogs showed no adverse effects. On post mortem, organ weights were normal and pathologic changes were confined to ulcerative lesions in the gastrointestinal tract (Adams *et al.*, 1969).

Rabbits treated with 7.5, 20 or 60 mg/kg bw/day all showed some gastric ulcers or lesions, the severity of which was dose-related (Adams *et al.*, 1969).

Human data

Ibuprofen can cause serious gastrointestinal adverse events including inflammation, bleeding, ulceration, and perforation of the stomach, small intestine, or large intestine, which can be fatal (TOXNET, 2013b)

Hepatotoxicity

Liver lesions were recorded in rats treated with 120 mg/kg bw/day ibuprofen in the diet for 2 years (European Chemicals Bureau, 2000). Increased liver weights were also recorded in male and female rats treated with 180 mg/kg bw/day ibuprofen for 26 weeks, although no histological abnormalities were observed. Enlarged livers also occurred in females following 13 weeks administration (Adams *et al.*, 1969).

Nephrotoxicity

Experimental animal data

In a 13 week oral toxicity study in rats, renal papillary necrosis was reported at 100 mg/kg bw/day ibuprofen and a NOEL was established at 25 mg/kg bw/day (European Medicines Agency, 2010). Renal papillary changes were recorded in rats treated with 60 or 120 mg/kg bw/day in the diet for 2 years (European Chemicals Bureau, 2000). No effects such as these were detected in dogs up to an oral dose level of 16 mg/kg bw/day over 14 days and up to 26 weeks. However, in a 52 week oral administration study, delivery of ibuprofen in gelatine capsules to baboons caused cortical pitting and scarring in the kidneys as well as minor focal papillary changes at a dose level of 100 mg/kg bw/day (European Chemicals Bureau, 2000). An increase in kidney weights were also recorded in male (180 mg/kg bw/day) and female (20 mg/kg bw/day) rats treated with ibuprofen for 13 weeks. 540 mg/kg bw/day caused slight renal tubular dilatation after 4 days of dosing (Adams *et al.*, 1969).

Human data

Long-term administration of NSAIDs has resulted in renal papillary necrosis and other renal injury (Forest Pharmaceuticals Inc, 2007, Rocca *et al.*, 2005).

Haematoxicity

Experimental animal data

In a 26 week rat study by oral administration all animals treated at 180 mg/kg bw/day were anaemic (low erythrocyte count and haemoglobin levels) (Adams *et al.*, 1969).

Human data

Anemia is sometimes seen in patients receiving NSAIDs, including ibuprofen as found in Combunox. This may be due to fluid retention, occult or gross GI blood loss, or an incompletely described effect upon erythropoiesis. NSAIDs inhibit platelet aggregation and have been shown to prolong bleeding time in some patients (Forest Pharmaceuticals Inc, 2007, Rocca *et al.*, 2005).

3.4.4.5 Mutagenicity/genotoxicity

Ibuprofen has been proven to be negative in an Ames test including Salmonella typhimurium strains TA98, TA100, TA102, TA1535 and TA1537 up to 5000 µg/plate (with and without metabolic activation) in 1986 and also showed a weak positive result in a sister chromatid exchange test in the bone marrow cells of mice (1997) (Australian Government, 2013). However, a sister chromatid exchange assay using human lymphocytes and a cytogenetic assay in human lymphocytes both showed negative responses in both tests (European Chemicals Bureau, 2000).

3.4.4.6 Carcinogenicity

Experimental animal data

Ibuprofen was not carcinogenic to mice after administration of 100 mg/kg bw/day for 80 weeks, or to rats after administration of 60 mg/kg bw/day for 104 weeks. These (1970) studies were not GLP-compliant and did not incorporate toxicokinetic measurements (Australian Government, 2013). A long-term carcinogenicity study in F344 male rats was reported to have a no effect level of 16 mg/kg bw/day (Brambilla *et al.*, 2012). Another 2-year rat study via dietary administration showed no carcinogenic potential at doses up to 120 mg/kg bw/day (European Chemicals Bureau, 2000).

Human data

A 51% increase in the rate of renal cell carcinoma, the most common form of kidney cancer, has been reported in nurses who regularly took ibuprofen and other NSAIDs (Cortez, 2011).

3.4.4.7 Reproductive toxicity

Developmental toxicity

Experimental animal data

Pregnant rats were treated with combination doses of oxycodone:ibuprofen (0.25:20, 0.5:40, 1.0:80, or 2.0:160 mg/kg bw/day) by oral gavage on days 7-16 of gestation. There was no evidence for developmental toxicity or teratogenicity at any dose, although maternal toxicity was noted at doses of 0.5:40 mg/kg bw/day and above. The highest dose tested in the rat (2.00:160 mg/kg bw/day) is equivalent to the MRTD (20:1600 mg/day) based on a body surface area (mg/m^2) basis. This dose was associated with maternal toxicity (death, clinical signs, decreased body weight) (Forest Pharmaceuticals Inc, 2007).

Similar effects were seen in a study with an ibuprofen and diphenhydramine hydrochloride combination in which rats were treated during days 6-15 of gestation, producing maternal toxicity at a dose of 60 mg/kg bw/day, without effects on foetal morphological observations (European Medicines Agency, 2010).

Pregnant rabbits were also treated by oral gavage with combination doses of oxycodone/ibuprofen (0.38:30, 0.75:60, 1.50:120 or 3.00:240 mg/kg bw/day) on gestation days 7-19. Oxycodone/ibuprofen treatment was not teratogenic under the conditions of the assay. Maternal toxicity was noted at doses of 1.5:120 (reduced body weight and food consumption) and 3:240 mg/kg bw/day (mortality). The NOAEL for maternal toxicity, 0.75:60 mg/kg bw/day, is 0.75 fold the proposed MHDD based upon the body surface area. Developmental toxicity, as evidenced by delayed ossification and reduced fetal body weights, was noted at the highest dose, which is approximately 3 times the MHDD on a mg/m^2 basis,

and is likely due to maternal toxicity. The fetal NOAEL of 1.50:120 mg/kg bw/day is approximately 1.5 times the MHDD on a mg/m^2 basis (Forest Pharmaceuticals Inc, 2007).

Similar effects were seen in a study with an ibuprofen and diphenhydramine hydrochloride combination in which rabbits treated during days 6-18 of gestation showed signs of maternal toxicity at a dose of 60 mg/kg bw/day without effects on foetal morphological observations (European Medicines Agency, 2010). In a separate study in rabbits, a maternally toxic dose of 60 mg/kg bw/day ibuprofen was also found not to be teratogenic (European Medicines Agency, 2010).

Various congenital abnormalities were observed in rabbits, although no consistent pattern was observed and no dose-response relationship. Overall, it was concluded that ibuprofen is not teratogenic (Adams *et al.*, 1969). In embryofoetal studies, rats, mice and rabbits, treated with ibuprofen alone from 6-19 or 6-29 days of pregnancy showed no signs of developmental toxicity at doses up to 100 mg/kg bw/day or 50 mg/kg bw/day, respectively (European Chemicals Bureau, 2000).

Potential effects of NSAIDs (including ibuprofen) during pregnancy were also addressed by Cappon *et al.* (2003) who demonstrated that the compounds, which are specific COX-1 or nonspecific COX inhibitors, show a greater potency to induce malformation during the sensitive periods for heart development and midline closure in rats and rabbits. Therefore, the selective COX-2 inhibitors pose minor risk of inducing heart anomalies even at the greater exposures.

Female reproductive toxicity

Experimental animal data

Fertility and pre- and post-natal studies in rats via oral administration showed no effects on the reproductive endpoints at a dose level of 20 mg/kg bw/day. At the higher dose level of 120 mg/kg bw/day, excessive haemorrhage, weakness, prolonged and incomplete delivery were reported at parturition. Litters of surviving dams were also less than normal size (European Chemicals Bureau, 2000).

An increase in ovary weight was observed in female rats following administration to 60 and 180 mg/kg bw/day for 13 weeks, although no histological abnormalities were reported. Ibuprofen altered the organ to body weight ratio of gonads and secondary sex organs at 180 kg/kg bw/day (Adams *et al.*, 1969).

Rabbits receiving 60 mg/kg bw/day gave birth prematurely to normal pups. The number of live foetuses per litter was decreased compared to controls, but there was no difference in the number of dead or resorbed foetuses. There was a reduction in the ratio of implants to corpora lutea, suggesting the decrease in live litter size is due to interruptions in early pregnancy. Overall, it was concluded that ibuprofen may reduce fertility by affecting early pregnancy (Adams *et al.*, 1969).

Human data

Based on literature data, an increased risk of miscarriage with the use of NSAIDs has been found. However, several other studies did not find an association.

Nielsen *et al*. (2001) compared pregnancies where mothers had used NSAIDs (n = 1462) with those who did not use (n = 17259). The main outcome measures were incidences of congenital abnormality, low birth weight, preterm birth, and miscarriage. It was concluded that the use of NSAIDs during pregnancy does not seem to increase the risk of congenital abnormality, low birth weight, or preterm birth. However, a significant association with miscarriage in the first trimester was demonstrated (Nielsen *et al.*, 2011).

Male reproductive toxicity

Experimental animal data

Fertility and pre- and post-natal studies in rats via oral administration showed no effects on the male libido endpoint at dose levels up to 120 mg/kg bw/day (European Chemicals Bureau, 2000). Ibuprofen altered the organ to body weight ratio of gonads and secondary sex organs at 180 kg/kg bw/day (Adams *et al.*, 1969).

Human data

A study has linked long-term (over three months) use of NSAIDs, including ibuprofen, with a 140% higher risk of erectile dysfunction. The study reported, "regular nonsteroidal anti-inflammatory drug use is associated with erectile dysfunction beyond what would be expected due to age and other conditions" (Shiri *et al.*, 2006).

3.5 Naproxen

3.5.1 Pharmacokinetics

Naproxen itself is rapidly and completely absorbed from the gastrointestinal tract with an in vivo bioavailability of 95%. Even with the observed differences in pattern of absorption between the different formulations, the elimination $T_{1/2}$ of naproxen is unchanged across products ranging from 12 to 17 hours. Peak plasma concentrations are obtained within 1 to 4 hours, depending on the formulation. At therapeutic levels, naproxen is more than 99% bound to albumin. It is metabolised extensively to inactive metabolites, and approximately 95% of the dose is excreted in the urine, as naproxen (<1%), or metabolites (Roche, 2006, U.S. Food and Drug Administration, 1993a).

3.5.2 Mechanism of action

Naproxen has pharmacologic actions similar to those of other NSAIDs and exhibits anti-inflammatory, analgesic and antipyretic activity. As with ibuprofen, it is thought that naproxen inhibits prostaglandin synthesis via the inhibition of COX. The anti-inflammatory, analgesic, and antipyretic activity is largely due to the inhibition of the COX-2 isoenzyme, whereas inhibition of COX-1 is largely responsible for the undesirable side effects such as irritation of the GI mucosa and platelet aggregation (Rao and Knaus, 2008).

3.5.3 Therapeutic dose

The lowest therapeutic dose for naproxen in adults is 1000 mg/day and 300 mg/day for adults and children respectively (14.29 mg/kg bw/day for adults or 10.00 mg/kg bw/day). The MHDD is 1650 mg/day for a limited period, the norm being 1100 mg/day (15.7 mg/kg bw/day) (Medicines and Healthcare products Regulatory Agency, 2008, Roche, 2006).

3.5.4 Toxicity

3.5.4.1 Acute toxicity

The acute oral LD_{50} in rats is 248 - 534 mg/kg, 360 mg/kg in mice, 665 mg/kg in guinea pigs and >1000 mg/kg in dogs (British Pharmacopoeia, 2013, Cayment Chemical Company, 2005, Pfizer, 2007, Roche, 2006).

3.5.4.2 Irritation / corrosivity

Naproxen has little or no irritating effect on the skin or eyes (British Pharmacopoeia, 2013, Roche, 2006).

3.5.4.3 Sensitisation

Naproxen has no reported sensitizing effects (British Pharmacopoeia, 2013).

3.5.4.4 Chronic toxicity

Cardiovascular toxicity

Clinical trials of up to three years duration have shown an increased risk of serious cardiovascular thrombotic events, myocardial infarction and stroke. Patients with known cardiovascular disease or risk factors for cardiovascular disease may be at greater risk (Drugs Information Online, 2013). Meta-analyses of observational studies reported an increased risk of cardiovascular outcomes, such as myocardial infarction, stroke and cardiovascular disease. In contrast, other studies reported that naproxen was not associated with an increased risk of cardiovascular outcomes (myocardial infarction, stroke or cardiovascular death) as estimated rate ratios from randomised trials were not significantly different following naproxen administration compared to the placebo (European Medicines Agency, 2012).

Gastrointestinal toxicity

NSAIDs cause an increased risk of serious gastrointestinal adverse events including inflammation, bleeding, ulceration, and perforation of the stomach, small intestine, or large intestine, which can be fatal. Elderly patients are at greater risk for serious gastrointestinal events (Drugs Information Online, 2013).

Hepatotoxicity

Elevations of liver enzymes, such as aspartate transaminase or alanine transaminase may occur in up to 15% of patients taking NSAIDs, although such hepatic abnormalities may be due to hypersensitivity rather than direct toxicity. In rare cases, severe hepatic reactions, including jaundice and fatal fulminant hepatitis, liver necrosis and hepatic failure have been reported (Roche, 2006, Unit Dose Services).

Nephrotoxicity

Experimental animal data

Nephropathy was seen in some mice at high dose levels of naproxen (data not given) but not in monkeys or miniature pigs (Hallesy *et al.*, 1973).

Human data

Long-term administration of NSAIDs in patients has resulted in renal necrosis and other renal toxicity. Patients at greatest risk are those with impaired renal function, hypovolemia, heart failure, liver dysfunction, salt depletion, those taking diuretics and angiotensin converting enzyme inhibitors or angiotensin receptor blockers, and the elderly (Drugs Information Online, 2013).

Haematotoxicity

Anemia is sometimes reported following treatment with naproxen, due to fluid retention, occult or gross GI blood loss, or an effect upon erythropoiesis. Naproxen also inhibits platelet aggregation and have been shown to prolong bleeding time in some patients (Drugs Information Online, 2013).

3.5.4.5 Mutagenicity/genotoxicity

The mutagenic effects of naproxen were inconclusive. Naproxen showed a weak but significant increase in revertant colonies in the Ames test, but no dose response was demonstrated. Similarly, a small increase in sister chromatic exchanges were reported in the bone marrow of mice (Philipose *et al.*, 1997).

In a later study, naproxen was shown to be negative in a bacterial reverse mutation assay, with or without metabolic activation indicating it was not mutagenic to the bacterial system. (Center for Drug Evaluation & Research, 1999).

In applications to the MHRA for product licensing, the Summary of Product Characteristic reports stated that mutagenicity was not seen in Salmonella typhimurium (5 cell lines),

Sachharomyces cerevisisae (1 cell line) and a mouse lymphoma test (Bristol Labatories Ltd, 2012, Medicines and Healthcare products Regulatory Agency, 2008)

3.5.4.6 Carcinogenicity

There is no evidence that naproxen is carcinogenic in rats. Following administration of 8, 16 or 24 mg/kg bw/day naproxen to Sprague-Dawley rats in food for 24 months, a NOAEL of 24 mg/kg bw/day was determined (Medicines and Healthcare products Regulatory Agency, 2008, Pfizer, 2007, Roche, 2006).

Naproxen is not listed as a carcinogen by IARC, National Toxicology Programme (NTP) or Occupational Safety and Health Administration (US OSHA) (Pfizer, 2007).

3.5.4.7 Reproductive toxicity

Developmental toxicity

Experimental animal data

Teratology studies were carried out with naproxen in Sprague-Dawley rats and New Zealand albino rabbits.

In rats administered 2, 10 or 20 mg/kg or 15 or 30 mg/kg naproxen (corresponding to 0.2, 0.9 or 1.7 times or 1.3 and 2.6 times MRTD in humans, respectively) between gestation day 6 and 15, a decrease in number of live fetuses with a concomitant increase in number of dead fetuses and resorptions were seen in females treated with the highest dose (30 mg/kg). No visceral or skeletal abnormalities were seen in pups born to these animals (Hallesy *et al.*, 1973).

Rats given daily oral doses of 2, 10, 20 and 30 mg/kg naproxen (0.2 to 2.6 times the MRTD) between days 14 of pregnancy and weaning of the offspring 21 days postpartum only showed signs of dystocia or difficulties during parturition, including excessive bleeding during parturition and/or partial or complete inability to deliver the litters. Such effects increased in a dose dependent manner (Hallesy *et al.*, 1973). Overall, naproxen was reported not to be teratogenic in rats or rabbits (Hallesy *et al.*, 1973).

In later studies, exposure of rats to 35.2 mg/kg bw/day naproxen resulted in maternal toxicity (death, reductions in body weight, body weight gain and feed consumption values, adhesions ofhte visceral and fluid in the abdominal cavity) as well a foetal toxicity (reduced foetal growth and caused delayed skeletal ossification). The duration and route of exposure was not stated. The maternal NOAEL was 17.6 mg/kg kg/day (Center for Drug Evaluation & Research, 1999).

In their material safety data sheet, Pfizer stated a NOEL for fertility and embryonic development following oral exposure to naproxen was 20 mg/kg bw/day in the rat (Pfizer, 2007).

In rabbits administered doses of 2, 10 or 20 mg/kg, or 20 or 40 mg/kg naproxen (0.2, 0.9 or 1.7 times or 1.7 or 3.4 times the MRTD) between day 6 and 18 of pregnancy, a slight increase in number of dead fetuses was seen at 20 and 40 mg/kg in the second study, although such an effect was not reported in the first study (Hallesy *et al.*, 1973).

In their material safety data sheet, Pfizer stated a NOEL for fertility and embryonic development following oral exposure to naproxen was 20 mg/kg bw/day in the rabbit (Pfizer, 2007).

The NOEL for fertility and embryonic development following oral exposure to naproxen was 170 mg/kg bw/day in the mouse, the basis of which is unknown (Pfizer, 2007).

The Summary of Product Characteristics reports stated that naproxen was not teratogenic when administered orally at doses of 20 mg/kg bw/day during organogenesis to rats and rabbits and that reproduction studies performed in rats, rabbits and mice at doses up to 6 times the human dose revealed no evidence of harm to the foetus (Bristol Labatories Ltd, 2012, Medicines and Healthcare products Regulatory Agency, 2008).

Female reproductive toxicity

Experimental animal data

Fertility, reproduction, and peri- and post-natal studies were carried out with naproxen in Sprague-Dawley rats.

Groups of 13 female rats were given 2, 10 or 20 mg/kg naproxen (0.2, 0.9 or 1.7 times the MRTD) 14 days before mating and continuing until offspring were weaned. Ten male and 20 female pups from each group were used for a second generation study. Following such treatment, the only effects observed was an inhibition of parturition seen in one female given 10 mg/kg bw/day (died on day 24 but had had 13 well-formed pups) and one given 30 mg/kg bw/day (died on day 27 but had 9 well-formed pups). Overall, naproxen did not affect fertility or reproductive performance in female rats, but had an inhibitory effect on parturition (Hallesy *et al.*, 1973).

The Summary of Product Characteristics reports stated that naproxen did not affect the fertility of rats when administered orally at doses of 30 mg/kg bw/day to males and 20 mg/kg bw/day to females, and that reproduction studies performed in rats, rabbits and mice at doses up to 6 times the human dose revealed no evidence of harm to the foetus. Oral administration of 2, 10 or 20 mg/kg bw/day naproxen to female rats during the third trimester resulted in difficult labour, which appears to be a known effect of NSAIDs (Bristol Laboratories Ltd, 2002, Medicines and Healthcare products Regulatory Agency, 2008).

Human data

Reproductive effects have been cited, 30 weeks after conception following oral exposure to 20 mg/kg naproxen. It has been classified as Repro class 1A. (known to have produced an adverse effect on the reproductive ability or capacity or on development in humans) (British Pharmacopoeia, 2013).

The use of naproxen in late pregnancy should be avoided because it may cause premature closure of the ductus arteriosus (Drugs Information Online, 2013).

Male reproductive toxicity

Experimental animal data

To assess male fertility, 20 male rats were given doses 2, 10 or 30 mg/kg naproxen (0.2, 0.9 or 2.6 times the MHTD) 60 days before mating and continuing through the mating period. The male rats were matched with untreated female rats, half of which were sacrificed on day 13 of pregnancy and the numbers of pregnant animals, corpora lutea, implantations, live fetuses or resorptions per animal were recorded. The remaining females were allowed to come to term and raise their litters to weaning. No changes in fertility or reproductive performance related to administration of naproxen were seen for male rats (Hallesy *et al.*, 1973).

4 Identification of Points of Departure

The objective of milestone 3 was to identify relevant PoDs based on the data gathered for the pharmaceuticals detected in drinking water (Appendix 6).

4.1 Data collection and extraction

4.1.1 Selection of a PoD from published data

Toxicological data for each chemical were previously identified in milestone 1 and 2, from which published PoDs such as the NOAEL or LOAEL were collated (where possible).

4.1.1.1 Test species

Data from studies in experimental animals such as rats, mice, guinea pigs, rabbits, non-human primates and humans were considered.

4.1.1.2 Route of exposure

In general, toxicity studies using an oral route of exposure are most appropriate for the risk assessment of contaminants in drinking water. Therefore, wherever possible the PoD was determined for oral toxicity studies. Other routes of exposure i.e. intravenous, intraperitoneal or subcutaneous injections were deemed inappropriate for use.

4.1.1.3 Critical toxicological effect

All adverse toxicological effects in animals were taken into account if they were appropriate to humans.

All relevant literature was systematically reviewed and relevant data, such as PoD (NOAEL/LOAEL/LD_{50}), species (mouse/rat), route of exposure (oral gavage/oral diet) and sensitive endpoint (teratogenicity/maternal toxicity) from each study were tabulated in data abstraction forms (in Excel format).

It was anticipated that articles would be evaluated by using the Klimisch ToxRTool, which helps consider reliability, relevance and adequacy of the data; adequacy being defined as 'the usefulness of data for risk assessment purpose'. However, as published PoDs were predominantly used, the data needed to complete the ToxRTool, such as purity and source of the test substance, species, strain, sex, age, body weight and number of test animals, route of administration and doses administered, study endpoints, statistics and study design were largely unavailable.

4.1.2 Alternative approaches to deriving a PoD

For those chemicals where PoDs were not published (e.g. carbamazepine epoxide and benzolecgonine), various methods were used to determine a PoD (Figure 1). For example, LD_{50} data for the metabolite and parent compound were compared (Section 4.1.3), structural alerts were identified (Section 4.1.4), the TTC approach was investigated (Section 4.1.5) and QSAR modelling (read across/trend analysis) was carried out in order to determine a NOEL or lowest observed effect level (LOEL) (Section 4.1.6). The decision on which PoD to select was carried out on a case by case basis. The typical decision stream for this process being primarily a QSAR modelling to derive a NOAEL followed by a LOAEL, the TTC approach or using a LD50.

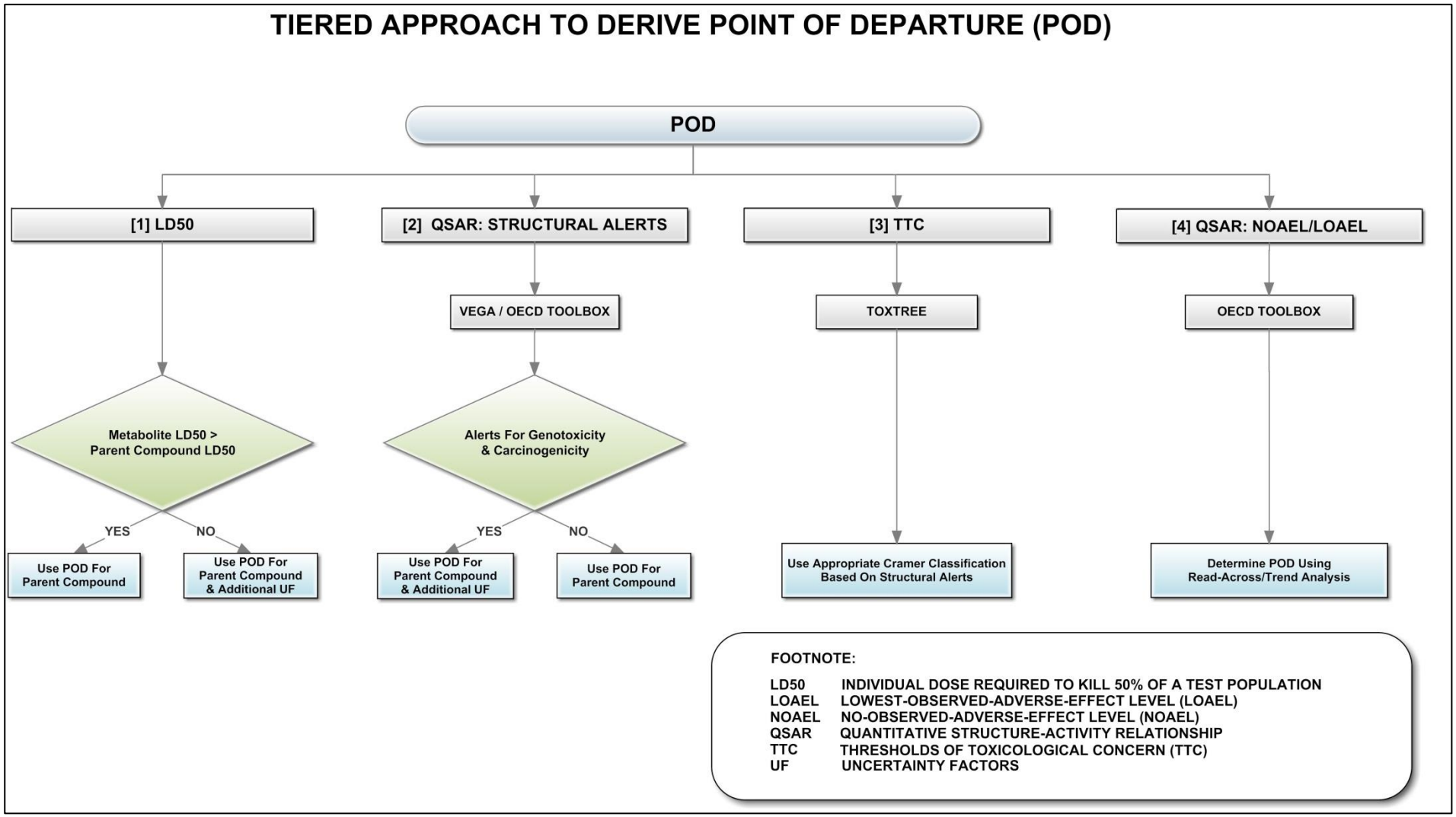


Figure 1. Tiered approach to determine a point of departure

4.1.3 Comparison of LD_{50} values

Two of the contaminants found in drinking water are metabolites. Carbamazepine epoxide is the metabolite of parent drug, carbamazepine, which is also detected in drinking water, and benzoylecogonine is the metabolite of cocaine.

To identify if the metabolite exerts similar, lower or greater (acute) toxicity, the LD_{50} values could be compared. If the LD_{50} value for the metabolite is higher, indicating it to be less toxic, then toxicity data for the parent compound could be used as a proxy for the determination of a PoD, as this would represent a conservative approach. If the LD_{50} is lower, indicative of a higher toxicity, then the parent compound may not be appropriate to use, or additional uncertainties could be accounted for, via the use of additional uncertainty factors (UFs).

4.1.4 Identification of structural alerts

In the absence of toxicological data, QSAR modelling can be used to predict the property of the chemical based on its chemical structure. Such in silico models can be used for risk assessment, hazard classification and prioritization.

Several models are now available, including ToxTree, VEGA (including Caeser and ToxTree) and the OECD Toolbox. The models can relate the target chemical to results obtained for structurally similar chemicals, by carrying out 'read-across' or 'trend analysis'.

ToxTree can be used to predict the toxic effect of the chemical by applying a decision tree approach, as described below. Toxtree and VEGA (CAESAR and ToxTree) software and the OECD toolbox can also be used identify structural alerts for mutagenicity, carcinogenicity and reproductive toxicity.

Once identified, this information could be used to inform the selection of the UFs to be used with the PoD from the parent compound.

4.1.5 Threshold of Toxicological Concern Approach

The TTC approach is intended for use as a screening tool for chemicals for which substance-specific toxicity data are not available. It is a risk assessment tool that establishes exposure threshold values for chemicals below which there is a very low probability of adverse effects to human health occurring. This probabilistic approach concept is based on the premise that a "safe" level of exposure can be identified for many chemicals based on their structure similarity and the known toxicity of chemicals that share similar structural characteristics (Kroes *et al.*, 2004). It has been used in the EU in the evaluation of pesticide metabolites in groundwater (Lapenna, 2011), pharmaceuticals and their metabolites in drinking water (Houeto *et al.*, 2012) and for determining and testing impurities in pharmaceuticals that possess the potential for genotoxicity (Muller *et al.*, 2006).

The TTC approach is exclusively designed as a substitute for substance-specific information in situations where there is limited or no information on the toxicity of the compound and information on exposure indicates that human exposure is very low.

ToxTree applies three rulebases relevant to TTC assessment, which are:

(a) Cramer decision tree,

(b) the Cramer rulebase with extensions, and

(c) the Kroes TTC decision tree (including the Beningni/Bossa rules for the identification of genotoxic carcinogens).

Practical application of the TTC approach to chemicals with no structural alerts for genotoxicity is performed by analysing the structure and using Cramer classification as an indicator of systemic toxicity.

The TTC values for non-cancer endpoints are as follows:

Cramer class 1.

- Substances with simple chemical structures and for which efficient modes of metabolism exist, suggesting a low order of oral toxicity.
- 1800 µg/person/day corresponding to 30 µg/kg bw/day for substances without genotoxicity alerts

Cramer class II.

- Substances which possess structures that are less innocuous than class I substances, but do not contain structural features suggestive of toxicity like those substances in class III.
- 540 µg/person/day corresponding to 9 µg/kg bw/day for substances without genotoxicity alerts.

Cramer Class III.

- Compounds that permit no strong initial presumption of safety, suggest significant toxicity or have reactive functional groups
- 90 µg/person/day corresponding to 1.5 µg/kg bw/day for substances without genotoxicity alerts.

For substances with genotoxicity alerts and hence possible DNA reactive carcinogens, the default TTC value of 0.15 µg/person/day corresponding to 2.5 ng/kg bw/day was derived. This value was derived based on the analysis of the dose-response data for various carcinogenic compounds and is associated with a 1 in 10^6 (1 in a million) excess lifetime cancer risk (Kroes *et al.*, 2004). Houeto *et al.* (2012) also used a TTC approach to carry out a risk assessment of drug residues in drinking water focussing on carbamazepine and its epoxide metabolite.

4.1.6 OECD toolbox modelling

The OECD toolbox can be used to group chemicals based on their mechanism of action or their structural similarity, extract data for similar chemicals and fill data gaps using read across, trend analysis or QSAR models. Depending on the data available, NO(A)EL and/or LO(A)EL data could be determined using the modelling software.

4.2 Benzoylecgonine

4.2.1 Comparison of LD_{50} values

LD_{50} values for benzoylecogonine and cocaine are 980 and 95 mg/kg, respectively. Both were determined in rats (Bedford *et al.*, 1982, Clearsynth Labs Pvt. Ltd.). The PoD for cocaine could be used as a proxy for benzoylecogonine, representing the worse-case scenario, as benzoylecogonine is less toxic than the parent cocaine. However, very little toxicity data in animals were retrieved following oral exposure. Most studies carried out reported results following intravenous (Mactutus, 1999), intraperitoneal (Frankfurt *et al.*, 2011, Xiao *et al.*, 2009) or subcutaneous injections (Henderson and McMillen, 1990), which were deemed inappropriate for use to determine a PoD.

4.2.2 Identification of structural alerts

The VEGA (CAESAR and ToxTree) QSAR model predicted benzoylecogonine to be non-mutagenic and non-carcinogenic. It was predicted to be a toxicant in the development toxicity model, although this result was deemed unreliable by the model (Table 3).

Table 3. Toxicity predictions from VEGA QSAR model

Model	Prediction	Reliability	Comments
Mutagenicity model (CAESAR)	Non-mutagen	Compound could be out of model Applicability Domain	Accuracy of prediction for similar molecules found in the training set is not optimal
Mutagenicity SarPy model	Non-mutagen	Compound could be out of model Applicability Domain	Accuracy of prediction for similar molecules found in the training set is not optimal
Benigni-Bossa mutagenicity (ToxTree) model	Non-mutagen	Compound is in model Applicability Domain	Only moderately similar compounds with known experimental value in the training set have been found
Carcinogenicity model (CAESAR)	Non-carcinogen	Compound could be out of model Applicability Domain	Accuracy of prediction for similar molecules found in the training set is not adequate
Benigni-bossa carcinogenicity (ToxTree)	Non-carcinogen	Compound is in model Applicability Domain	The result appears reliable
Developmental toxicity model (CAESAR)	Toxicant	Compound is out of model Applicability Domain	Result may be not reliable. Careful check of the information should be done, paying particular attention to the following issues: - similar molecules found in the training set have experimental values that disagree with the predicted value - accuracy of prediction for similar molecules found in the training set is not adequate

As there are no structural alerts for genotoxicity or carcinogenicity, a Cramer classification for non-genotoxic compounds could be used.

4.2.3 Threshold of Toxicological Concern approach

Using ToxTree and OECD toolbox, benzoylecogonine was designated Cramer class 3, based on it being heterocyclic (Q7). The general default value for Cramer class III compounds is 1.5 µg/kg bw/day.

4.2.4 OECD toolbox modelling

The OECD toolbox identified the aliphatic amines and esters as the structural alerts.

Using 'Data gap filling' in the OECD toolbox, read across from category members gave a NOEL of 81.9 mg/kg bw/day based on five values within the range of 0.1-1000 mg/kg bw/day from five nearest neighbours compared by prediction descriptors. The target chemical fell within the Applicability domain. Category members are single chemicals or mixtures that are selected based on the profile of the target chemical. Only chemicals with experimental data (from rats) were used in the prediction. Experimental data for the target chemicals (if any) were not used in prediction calculations.

A NOEL of 94.3 mg/kg bw/day is predicted from category members using trend analysis based on 15 values with the range of 0.1 – 1000 mg/kg bw/day from 15 category members. The target chemical fell within the Applicability domain. Only chemicals that have experimental data (in rat) were used in the category. Again, experimental data for the target chemicals (if any) were not used in prediction calculations.

4.2.5 Proposed PoDs

Based on the data obtained, the following PoDs are proposed for benzoylecogonine:

- A NOEL of 81.9 mg/kg bw/day based on read across data
- A NOEL of 94.3 mg/kg bw/day based on trend analysis
- A TTC value of 0.0015 mg/kg bw/day

The target compound fell within the Applicablility Domain of both categories and only chemicals with experimental data were used in the predictions. However, the range of NOELs derived was quite large for both models adding uncertainty to the prediction. Therefore the lowest NOEL of 81.9 mg/kg bw/day based on read across data was selected, in order to be most conservative. Due to the uncertainty of the prediction, the TTC value will also be used in risk characterisation to provide a weight of evidence approach.

4.3 Carbamazepine

4.3.1 Selection of PoD from published data

4.3.1.1 Toxicological effects – humans

Various epidemiological studies have reported an association between carbamazepine use during pregnancy (for the treatment of epilepsy) and the risk of congenital anomalies such as spina bifida, cardiovascular effects, oral clefts and urinary tract defects (Hernandez-Diaz *et al.*, 2001, Samrén *et al.*, 1997). Such effects are thought to occur at the minimum therapeutic concentration of 200 mg/day.

4.3.1.2 Toxicological effects – animals

Various toxicity studies have been carried out in experimental animals.

No effects were seen in rats and rabbits treated with 20-200 mg/kg bw/day or mice (170-300 mg/kg bw/day) (Daily Med, 2012b).

In rats, higher doses of carbamazepine caused reduced fetal body weight (192-200 mg/kg bw/day), kinked ribs (250 mg/kg bw/day), cleft palate, club foot or absence of eyes (650 mg/kg bw/day). In mice, 225 mg/kg bw/day caused increased resorptions and 400 mg/kg bw/day decreased fertility and the number of foetuses (Snyder *et al.*, 2008a).

Rats treated with carbamazepine for 2 years showed a dose-related increase in hepatocellular tumours in females and benign interstitial cell adenomas of the testes in males. However, MHRA stated that there is no evidence that this observation is of importance for the therapeutic use of carbamazepine in humans (Medicines and Healthcare products Regulatory Agency). Moreover, mutagenicity data suggest that carbamazepine is non-genotoxic hence a threshold of toxicity is likely to occur with respect to the hepatomas and carbamazepine has not been identified by IARC as a probable, possible or confirmed human carcinogen (Sigma-Aldrich, 2013).

4.3.2 Proposed PoDs

Based on the data obtained, the following PoDs are proposed for carbamazepine:

- A NOAEL of 20 mg/kg bw/day based on impaired fertility and fetotoxicity in the rat and rabbit.
- A LOAEL of 3.33 mg/kg bw/day is also proposed, based on the epidemiology data indicating a significant risk of congenital abnormalities when taking carbamazepine at the minimum therapeutic dose of 200 mg/day (Novartis Pharmaceuticals Corporation, 2013) during pregnancy. This was based on an adult weighing 60 kg.

The NOAEL of 20 mg/kg bw/day based on animal data was selected for further derivations. This value has been selected as a no effect level is considered preferable over a low effect level. Moreover, the LOAEL was based on the therapeutic dose of carbamazepine used in humans.

4.4 Carbamazepine epoxide

4.4.1 Comparison of LD_{50} values

The LD_{50} for carbamazepine is 1100-3750 mg/kg and 3850-4025 mg/kg for mice and rats, respectibely (Daily Med, 2012b). However, no LD_{50} values could be found for the epoxide metabolite.

4.4.2 Identification of structural alerts

The VEGA (CAESAR and ToxTree) QSAR model predicted carbamazepine epoxide to be mutagenic and a toxicant. It was predicted to be a carcinogenic toxicant in the CAESER carcinogenicity model and the ToxTree Benigni-Bossa carcinogenicity, although neither of these results may be reliable (see Table 4).

As there are structural alerts for genotoxicity, the PoD for carbamazepine could be used as a proxy for carbamazepine epoxide but additional uncertainties could be proposed when deriving the ADI to account for the genotoxic endpoint. Therefore, a NOAEL of 20 mg/kg bw/day could be used, based on impaired fertility and fetotoxicity of carbamazepine in the rat and rabbit plus additional UFs.

An alternative approach for non-threshold chemicals would be to estimate a regulatory virtually safe dose by dividing the maximum tolerated dose (MTD) by 740,000 (Snyder and Snyder, 2008). The MTD is the highest dose predicted to produce minimal systemic toxicity during a carcinogenicity study typically obtained from 90 day rodent studies. However, this approach was not appropriate in this instance as no data on carbamazepine were retrieved.

4.4.3 Threshold of Toxicological Concern approach

Using ToxTree, carbamazepine has been designated Cramer class 3, based on it comprising a heterocyclic ring with complex substituents (Q11).

ToxTree and the OECD toolbox also designated carbamazepine epoxide as Cramer class 3, based on its heterocyclic structure (Q7) and its 3-membered heterocycle (Q10).

The OECD toolbox and CAESAR identified the epoxide and aziridine as the structural alerts for genotoxicity and carcinogenicity. Due to these structural alerts the default Cramer classes are unsuitable, a TTC value of 2.5 ng/kg bw/day (corresponding to 0.15 µg/person/day) could be used.

4.4.4 OECD toolbox modelling

The OECD toolbox identified the epoxide and aziridine as the structural alerts.

Using ‘Endpoints’ in the toolbox that retrieves experimental results from resident databases for the chemical in question, data showed that carbamazepine epoxide was negative in the Ames test so was not genotoxic. No other data were retrieved.

Using ‘Data gap filling’ in the OECD toolbox, read across from category members gave a NOEL of 34.8 mg/kg bw/day based on two values within the range of 12.5 – 25.0 mg/kg bw/day from two nearest neighbours compared by prediction descriptors. The target chemical fell within the Applicability domain. Only chemicals with experimental data (from rats) were used in the prediction. Experimental data for the target chemicals (if any) were not used in prediction calculations.

A NOEL of 17.7 mg/kg bw/day is predicted from category members using trend analysis based on two values with the range of 12.5 – 25.0 mg/kg bw/day from two category members. The target chemical fell within the Applicability domain. Only chemicals that have experimental data (in rat) were used in the category. Again, experimental data for the target chemicals (if any) were not used in prediction calculations.

4.4.5 Proposed PoDs

Based on the data obtained, the following PoDs are proposed for carbamazepine epoxide:

- A proxy NOAEL of 20 mg/kg bw/day based on carbamazepine-induced impaired fertility and fetotoxicity in the rat and rabbit
- A NOEL of 17.7 mg/kg bw/day based on trend analysis data
- A TTC value of 0.0000025 mg/kg bw/day

There is no clear scientific rationale for selecting which NO(A)EL to use for further derivations, therefore the lowest value of 17.7 mg/kg bw/day has been chosen. However, the TTC value will also be used in risk characterisation to provide a weight of evidence approach.

Table 4. Toxicity predictions from VEGA QSAR model for carbamazepine epoxide

Model	Prediction	Reliability	Comments
Mutagenicity model (CAESAR)	Mutagen Epoxides and aziridines structural alerts	Compound is in model Applicability Domain	The result appears reliable. Some issues could be not optimal: - Only moderately similar compounds with known experimental value in the training set have been found - One or more fragments related to mutagen activity were found: Epoxides and aziridines
Mutagenicity SarPy model	Mutagen	Compound is in model Applicability Domain	Result appears reliable. One or more fragments related to mutagen activity were found: SA 97
Benigni-Bossa mutagenicity (Toxtree) model	Mutagen	Compound is out of model Applicability Domain	Result may be not reliable. - Only moderately similar compounds with known experimental value in the training set have been found - Similar molecules found in the training set have experimental values that disagree with the predicted value - Accuracy of prediction for similar molecules found in the training set is not adequate - A prominent number of atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments - One or more fragments related to mutagen activity were found: SA7
Carcinogenicity model (CAESAR)	Carcinogen Epoxides and aziridines structural alerts	Compound is out of model Applicability Domain	Prediction is carcinogen, but the result may be not reliable. - similar molecules found in the training set have experimental values that disagree with the predicted value - some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments - One or more fragments possibly related to carcinogenic activity were found: Epoxides and aziridines
Benigni-Bossa carcinogenicity (Toxtree)	Carcinogen	Compound is out of model Applicability Domain	Prediction is carcinogenic, but the result may be not reliable. - Only moderately similar compounds with known experimental value in the training set have been found - Accuracy of prediction for similar molecules found in the training set is not adequate - Some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments - One or more fragments related to mutagen activity were found: SA7
Developmental toxicity model (CAESAR)	Toxicant	Compound is in model Applicability Domain	The result appears reliable.

4.5 Ibuprofen

4.5.1 Selection of PoD from published data

4.5.1.1 Toxicological effects – animals

Various toxicity studies have been carried out in experimental animals.

No effects were seen in rats (7.5-180 mg/kg bw/day) and rabbits (7.5-60 mg/kg bw/day) for maternal toxicity and teratogenicity, respectively. Primates (16 mg/kg bw/day) and dogs (4-16 mg/kg bw/day) also showed no adverse effects (Australian Government, 2013, Centre for Drug Evaluation and Research, European Medicines Agency, 2010, Norwegian Food Safety Authority, 2012).

In rats, 50 mg/kg bw/day caused decreased body weight and food intake, 60 mg/kg bw/day caused increased kidney and thyroid weights and maternal toxicity, and 100 mg/kg bw/day caused gastrointestinal irritation (ulcers, diverticuli, necrosis and erosions of the stomach mucosa) as well as renal papillary necrosis (Centre for Drug Evaluation and Research, European Medicines Agency, 2010).

Rats and mice treated with 60 or 100 mg/kg bw/day, respectively, showed no evidence of tumours compared with controls (Australian Government, 2013, Brambilla *et al.*, 2012).

4.5.2 Proposed PoDs

Based on the data obtained, the following PoD is proposed for ibuprofen:

- A NOAEL of 7.5 mg/kg bw/day based on maternal toxicity in rats and rabbits.

The occurrence of gastro-intestinal ulceration and renal papillary changes are common changes following chronic oral exposure to this class of compound. The renal changes reported were, as expected, seen in animals generally over longer-term exposure at higher dose ranges (i.e. NOELs for this change were 25 mg/kg/day in rat; 16 mg/kg/day in dogs; <100 mg/kg/day in monkeys). The more prevalent effect of gasto-intestinal irritation is generally seen in laboratory animals at the lower dose exposure ranges (i.e. NOELs for this change were >100 mg/kg/day in rats; 4 mg/kg/day in dogs; 100 mg/kg/day in monkeys; <7.5 mg/kg/day in rabbits). However, other studies in rabbits had shown 7.5 mg/kg/day to be a NOAEL when administered orally over the period of gestation in female animals. Therefore, as a result of this variation at this particular low dosage and the absence of significant changes at daily doses of 16 mg/kg/day (rats) and 100 mg/kg/day (mice) over lifespan studies, the PoD was designated 7.5 mg/kg/day.

4.6 Naproxen

4.6.1 Selection of PoD from published data

4.6.1.1 Toxicological effects – animals

Various toxicity studies have been carried out in experimental animals.

There was no evidence of impaired fertility or foetal effects in rats or rabbits treated with 10-30 mg/kg bw/day naproxen during pregnancy, or mice treated with treated with 170 mg/kg bw/day (Hallesy *et al.*, 1973, Roche, 2006). 10 mg/kg bw/day caused no gastrointestinal effects in rats following a six week exposure period. However, gastrointestinal lesions were in 'a few rats' from all treatment levels (2, 10 and 30 mg/kg bw/day) for 22 weeks. Dystocia (obstructive labour) also reported in rats treated with 2-30 mg/kg bw/day naproxen during the third trimester of pregnancy (Bristol Labatories Ltd, 2012, Hallesy *et al.*, 1973).

Rats treated with naproxen for 2 years (8-24 mg/kg bw/day) showed no evidence of tumours compared with controls (Roche, 2006).

4.6.2 Proposed PoDs

Based on the data obtained, the following PoD is proposed for naproxen:

- A NOAEL of 10 mg/kg bw/day based on gastrointestinal tract effects in rats and teratogenic effects in the rabbit.

An extensive study of naproxen in a range of reproductive toxicology studies in rats, rabbits and mice has been conducted by oral exposure with a dose range of 2 to 40 mg/kg/day. These doses were administered either during the period of gestation of these animals for assessment of developmental changes or for longer periods in the assessment of fertility. The weight of evidence showed a potential distinction between an effect and no effect level in the region of 10 mg/kg/day. The specific effect of problems at parturition only affected one female dosed at 10 mg/kg/day and also one female dose at 30 mg/kg/day. Therefore, in the absence of a dose-dependency for this low incidence change and, in the absence of other effects, the dose of 10 mg/kg/day was designated as the PoD.

4.7 Summary

In summary, a PoD for all pharmaceuticals has been determined using variety of methods, depending on the data available. NO(A)ELs/LO(A)ELS based on experimental animal data or therapeutic doses were used, or if data were not available, specifically for the drug metabolites benzoylecogonine and carbamazepine epoxide, then the approaches outlined in Figure 1 were applied.

Table 5 gives a summary of all PoDs proposed.

Table 5. Summary of PoD for all pharmaceuticals

Pharmaceutical	PoD mg/kg bw/day			
	NO(A)EL	LO(A)EL	LD50	TTC
Benzoylecgonine	**81.9**[a]	-	980	0.0015
Carbamazepine	**20**[b]	3.33[c]	-	-
Carbamazepine epoxide	**17.7**[a]-20[b]	-	-	0.0000025
Ibuprofen	**7.5**[b]	-	-	-
Naproxen	**10.0**[b]	-	-	-

[a]QSAR modelling; [b] Animal data; [c] Therapeutic doses

Figures in bold represent the selected PoD.

5 Risk assessment for study compounds

The objective of milestone 4 was to perform a risk assessment for the five pharmaceuticals detected in drinking water, namely benzoylecogonine, carbamazepine, carbamazepine epoxide, ibuprofen and naproxen.

In carrying out the risk assessment, we followed the risk assessment paradigm, namely

- Hazard identification
- Hazard characterisation
- Exposure assessment
- Risk characterisation

In doing so, we built on data presented above in order to determine an ADI and carry out a risk assessment of the named pharmaceuticals in drinking water.

5.1 Hazard identification

During the hazard identification phase, the type and nature of potential adverse effects are identified by using human epidemiology data, animal toxicity data, in vitro data or QSAR modelling (see objective 1).

5.2 Hazard characterisation

Hazard characterisation encompasses a qualitative or quantitative description of inherent properties of the pharmaceutical agent having the potential to cause adverse health effects. HBGVs such as ADI or tolerable daily intake (TDI) are used to provide an estimate of the amount of chemical that can be ingested over a lifetime without appreciable risk to health.

5.2.1 Proposed PoDs

The PoD, in the form of a NOAEL or LOAEL are identified from the literature search or determined from data retrieved from the search (see objective 3). The NOAEL is the highest dose at which no adverse effects are seen in the toxicity study, and the LOAEL is the lowest dose at which some adverse effects are seen.

If a NOAEL cannot be determined from the data, due to effects being seen at even the lowest dose tested, a LOAEL is selected. If there is more than one good quality study, the lowest NOAEL is selected to represent the most sensitive endpoint of toxicity.

For those chemicals where there are little data, then alternative approaches may be considered when deriving a PoD, such as QSAR modelling or the TTC approach.

5.2.2 Selection of proposed UFs

In general, a default UF of 100 is typically used, consisting of a factor of 10 for interspecies variability (4 for toxicokinetics and 2.5 for toxicodynamics) and 10 to account for intraspecies differences (3.2 for toxicokinetics and 3.2 for toxicodynamics) (European Food Saftey Authority, 2012). However, in some cases such default factors may not be applicable. The UFs presented in Table 6 are proposed in such cases.

Table 6. Consideration for the selection of UFs

	Possible range	Comment
Interspecies differences	1-10	UF used to account for differences in sensitivity between species • 10 is proposed if animals data are used • 1 is used if human data are used
Intraspecies differences	1-10	UF used to account for differences in sensitivity between individuals • 3 is proposed if human data are used, depending on the sub-population • 3 is proposed if using a therapeutic dose
Conversion of LOAEL to NOAEL	3-10	UF used to account for the test dose causing some adverse effect • 10 is proposed when NOAEL is not available • 10 is proposed if the LOAEL is based on a therapeutic dose
Use of subchronic exposure data	1-10	UF used to account for the test study being less than chronic exposure. • 10 is proposed when no chronic data are available • 1 is proposed when chronic data are used • 3 is proposed for epidemiology studies or developmental studies
Inadequate databases	1-10	UF used to account for less than adequate datasets. • 3 is proposed if QSARs were used • 3 is proposed if the NOAEL is the highest dose tested

5.2.3 Derivation of proposed ADI

The ADI is calculated by using equation:

$$ADI = \frac{PoD}{UF}$$

5.3 Exposure assessment

During the exposure assessment phase, evaluation of the concentration or amount of a particular agent that reaches a target population is carried out, taking into account the frequency, duration and route of exposure.

Exposure to pharmaceuticals was assumed to be long term, although actual levels may vary. Individuals are likely to be exposed to the study compounds from water predominantly through the ingestion of drinking water and water used for cooking, and through dermal exposure via showering and bathing, although dermal exposure is considered to be a minor exposure pathway.

The measured concentration of the pharmaceutical is converted to an intake value. This is achieved by using default assumptions on body weight and volume of water ingested to allow the intake to be expressed on a body weight basis (World Health Organisation, 2011). The following assumptions are used:

- 60 kg adult drinking 2 L per day
- 10 kg child drinking 1 L per day

- 5 kg infant drinking 0.75 L per day

Once the intake has been estimated for the appropriate receptor, it is then compared against the HBGV during the risk characterisation phase.

5.4 Risk characterisation

During risk characterisation, the estimated intake of the pharmaceutical is compared with the HBGV and a qualitative statement about the estimated exposure relative to the HBGV is provided.

The hazard quotient (HQ) can be calculated, which is the ratio of the estimated intake with the level at which no adverse effects is expected (the HBGV). If the HQ is <1, then no adverse effects are expected following exposure. If HQ is >1, then the exposure is greater than the no effect level, and there is a possibility that adverse effects could occur.

5.5 Risk communication

To aid in risk communication, the margin of exposure (MOE) approach is commonly used. The MOE is defined as the ratio of the NOAEL or benchmark dose for the critical effect to the estimated exposure. The magnitude of the MOE gives an indication of the level of concern, i.e. the larger the MOE, the smaller the potential risk posed by exposure to the compound under consideration (Benford *et al.*, 2010).

For non-carcinogenic compounds, EPA stated that an acceptable MOE based on a NOAEL and LOAEL is 100 and 1000, respectively (US Environmental Protection Agency, 2012). Others compare the MOE with UFs and if the MOE is larger, then the exposure is considered to pose little risk (National Institute of Technology and Evaluation, 2011).

EFSA has also endorsed the use of the MOE approach and stated that 'a MOE of 10,000 or higher, when used with the 95% lower confidence limit of a benchmark dose (BMDL10) would be of low concern from a public health point of view with respect to carcinogenic effects, whereas a MOE of less than 10,000 indicates that exposure may be of concern' (European Food Saftey Authority, 2005, 2012). Previous work has been carried out by AWWARF where the TDI was used in the MOE calculation as a replacement for the NOAEL (Snyder *et al.*, 2008b).

For the purpose of this project we have calculated the MOE based on the NOAEL, although for completeness we also have presented MOEs calculated from a ADI.

5.6 Benzoylecgonine

5.6.1 Hazard Identification

There was little toxicological information retrieved on benzoylecogonine. The LD_{50} is 980 mg/kg, classifying it as category 4 under classification, labelling and packaging (CLP) regulations. QSAR modelling indicated it is not likely to be a mutagen or carcinogen.

5.6.2 Hazard characterisation

There are no HBGVs determined for benzoylecogonine.

5.6.2.1 Proposed PoDs

Based on the data obtained, the following PoD is proposed for benzoylecogonine (see objective 3).

- A NOEL of 81.90 mg/kg bw/day based on read across data
  (A TTC value of 0.0015 mg/kg bw/day)

5.6.2.2 Selection of proposed UFs

The proposed UFs for use with the PoDs selected are presented in Table 7.

Table 7. Proposed UFs for benzoylecogonine

	UF	Comments
PoD (mg/kg bw/day)	81.90	
Interspecies differences	10	Based on animal data from read across
Intraspecies differences	10	To account for human variability
Conversion of LOAEL to NOAEL	1	NOAEL used as PoD
Use of subchronic exposure data	1	Based on read across from chronic data
Inadequate databases	3	Precautionary value due to using QSARs
Total UFs	300	

5.6.2.3 Derivation of proposed ADI

The proposed ADI is:

- 273000 ng/kg bw/day (273 µg/kg bw/day) using a NOAEL as the PoD

5.6.3 Exposure assessment

The maximum concentration of benzoylecogonine measured in drinking water was 3.51 ng/L and the highest median was 1.98 ng/L (<1-3.51 ng/L)[1].

Based on default factors,

- For an adult, the daily intake would be 0.12 and 0.07 ng/kg bw/day (0.00012 and 0.00007 µg/kg bw/day) based on maximum and median concentrations, respectively.
- For a child, the daily intake would be 0.35 and 0.20 ng/kg bw/day (0.00035 and 0.002 µg/kg bw/day) based on maximum and median concentrations, respectively.
- For an infant, the daily intake would be 0.53 and 0.30 ng/kg bw/day (0.00053 and 0.0003 µg/kg bw/day) based on maximum and median concentrations, respectively.

5.6.4 Risk characterisation

The maximum intake of benzoylecogonine via drinking water by adults, children and infants (0.12-0.53 ng/kg bw/day) is less than the proposed ADI (273,000 ng/kg bw/day) and the HQ is <1. The estimated intakes are also lower than the TTC value (1500 ng/kg bw/day). Therefore it is not anticipated that any adverse public health effects will occur following exposure to benzoylecogonine via drinking water.

[1] The levels measured in the three other sites were <1 ng/L. Benzoylecgonine was only measured at site 4, where the range measured was <1-3.51 ng/L.

5.6.5 Risk communication

The MOEs for benzoylecogonine, based on the NOAEL and the maximum intake, are 700,000,000 for adults, 230,000,000 for children and 160,000,000 for infants.

For comparison, the MOEs based on the ADI (derived from the NOAEL), are 2,300,000 for adults, 780,000 for children and 520,000 for infants. Estimated intakes are also below the TTC value.

The MOEs and the TTC approach indicate exposures are of low concern in terms of risk to public health.

5.7 Carbamazepine

5.7.1 Hazard Identification

The principal effects of carbamazepine appear to be reproductive and developmental toxicity, haematotoxicity and neurotoxicity in both animals and humans.

Carbamazepine did induce hepatocellular tumours in female rats and benign interstitial cell adenomas of the testes in males although MHRA stated that there is no evidence that this observation is of importance for the therapeutic use of carbamazepine in humans (Medicines and Healthcare products Regulatory Agency).

5.7.2 Hazard characterisation

A number of ADIs have been published for carbamazepine.

Kumar and Xagoraraki (2010) derived an ADI of 42,000 and 95,000 ng/kg bw/day (42 and 95 µg/kg bw/day) for adults and children based on the minimum therapeutic dose.

Snyder *et al.* (2008a) derived an ADI of 10,000 ng/kg bw/day (10 µg/kg bw/day) based on development abnormalities from epidemiology studies and 340 ng/kg bw/day (0.34 µg/kg bw/day) based on the maximum tolerated dose.

Minnesota Department of Health (2013) derived ADI of 13,000 and 6,000 ng/kg bw/day (13 and 6 µg/kg bw/day) based on the minimum therapeutic dose in children and adults, respectively.

de Jongh *et al.* (2011) derived an ADI of 16,000 ng/kg bw/day (16 µg/kg bw/day) based on the lowest therapeutic dose.

Environment Protection and Heritage Council (2008) derived an ADI of 2,800 (2.8 µg/kg bw/day) based on the lowest therapeutic doses for adults.

5.7.2.1 Proposed PoDs

Based on the data obtained, the following PoD is proposed for carbamazepine (see objective 3).

- A NOAEL of 20 mg/kg bw/day based on impaired fertility and fetotoxicity in the rat and rabbit

5.7.2.2 Selection of proposed UFs

The proposed UFs for use with the PoD selected are presented in Table 8.

Table 8. Proposed UFs for carbamazepine

	UF	Comments
PoD (mg/kg bw/day)	20	
Interspecies differences	10	Based on animal data
Intraspecies differences	10	To account for human variability
Conversion of LOAEL to NOAEL	1	NOAEL used as PoD
Use of subchronic exposure data	3	Based on embryofoetal tox (subchronic) dataset
Inadequate databases	3	Quoted figure-no data available
Total UFs	900	

5.7.2.3 Derivation of proposed ADI

The proposed ADI is:

- 22,000 ng/kg bw/day (22 µg/kg bw/day) using a NOAEL as the PoD

5.7.3 Exposure assessment

The maximum concentration of carbamazepine measured in drinking water was 148 ng/L and the highest median was 11.8 ng/L (8.37-17.3 ng/L).

Based on default factors,

- For an adult, the daily intake would be 4.93 and 0.39 ng/kg bw/day (0.0049 and 0.00039 µg/kg bw/day) based on maximum and median concentrations, respectively
- For a child, the daily intake would be 14.8 and 1.18 ng/kg bw/day (0.0148 and 0.00118 µg/kg bw/day) based on maximum and median concentrations, respectively
- For an infant, the daily intake would be 22.2 and 1.77 ng/kg bw/day (0.022 and 0.0017 µg/kg bw/day) based on maximum and median concentrations, respectively

5.7.4 Risk characterisation

The maximum intake of carbamazepine via drinking water by adults, children and infants (4.93-22.2 ng/kg bw/day) is less than the proposed ADI (22,000 ng/kg bw/day) and the HQ is <1. Therefore it is not anticipated that any adverse public health effects will occur following exposure to carbamazepine via drinking water.

5.7.5 Risk communication

The MOEs for carbamazepine, based on the NOAEL and the maximum intake, are 4,100,000 for adults, 1,400,000 for children and 900,000 for infants.

For comparison, the MOEs based on the ADI (derived from the NOAEL), are 4,500 for adults, 1,500 for children and 1,000 for infants.

The MOEs indicate exposures are of low concern in terms of risk to public health.

5.8 Carbamazepine epoxide

5.8.1 Hazard Identification

There was little toxicological information retrieved on carbamazepine epoxide. QSAR modelling indicated structural alerts for developmental toxicity, genotoxicity and carcinogenicity.

5.8.2 Hazard characterisation

There are no health based guidance values determined for carbamazepine epoxide.

5.8.2.1 Proposed PoDs

Based on the data obtained, the following PoD is proposed for carbamazepine epoxide (see objective 3).

- A NOEL of 17.7 mg/kg bw/day based on trend analysis data
  (A TTC value of 0.0000025 mg/kg bw/day)

5.8.2.2 Selection of proposed UFs

The proposed UFs for use with the PoDs selected are presented in Table 9.

Table 9. Proposed UFs for carbamazepine epoxide

	UF	Comments
PoD (mg/kg bw/day)	17.7	
Interspecies differences	10	Based on animal data from read across
Intraspecies differences	10	To account for human variability
Conversion of LOAEL to NOAEL	1	NOAEL used as PoD
Use of subchronic exposure data	1	Based on read across from chronic data
Inadequate databases	3	Precautionary value due to using QSARs
Total UFs	300	

5.8.2.3 Derivation of proposed ADI

The proposed ADI is:

- 59,000 ng/kg bw/day (59.00 µg/kg bw/day) using a modelled NOAEL as the PoD

5.8.3 Exposure assessment

The maximum concentration of carbamazepine epoxide measured in drinking water was 16.6 ng/L and the highest median was 6.24 ng/L (1.93-10.7 ng/L).

Based on default factors,

- For an adult, the daily intake would be 0.55 and 0.21 ng/kg bw/day (0.00055 and 0.00021 µg/kg bw/day) based on maximum and median concentrations, respectively
- For a child, the daily intake would be 1.66 and 0.62 ng/kg bw/day (0.0016 and 0.00062 µg/kg bw/day) based on maximum and median concentrations, respectively
- For an infant, the daily intake would be 2.49 and 0.94 ng/kg bw/day (0.00025 and 0.00094 µg/kg bw/day) based on maximum and median concentrations, respectively

5.8.4 Risk characterisation

The maximum intake of carbamazepine epoxide via drinking water by adults, children and infants (0.55-2.49 ng/kg bw/day) is less than the proposed ADIs (59,000 ng/kg bw/day) and the HQ is <1. The estimated intakes are also lower than the TTC value (2.5 ng/kg bw/day). Therefore it is not anticipated that any adverse public health effects will occur following exposure to carbamazepine epoxide via drinking water.

5.8.5 Risk communication

The MOEs for carbamazepine epoxide, based on the trend analysis NOEL as the PoD and the maximum intake are 32,000,000 for adults, 11,000,000 for children and 7,100,000 for infants.

The MOEs based on the ADI (derived from the modelled NOEL), are 106,627 for adults, 35,542 for children and 23,695 for infants. Estimated intakes are also below the TTC value.

The MOEs based on the NOAELs and the TTC approach indicate exposures are of low concern in terms of risk to public health.

5.9 Ibuprofen

5.9.1 Hazard Identification

The principal effects of ibuprofen appear to be gastrointestinal toxicity, hepatotoxicity, cardiovascular toxicity, nephrotoxicity, haematotoxicity and developmental toxicity.

5.9.2 Hazard characterisation

Schwab *et al.* (2005) derived an ADI of 110,000 ng/kg bw/day (110 µg/kg bw/day) based on the lowest single therapeutic dose.

Environment Protection and Heritage Council (2008) derived an ADI of 114,000 ng/kg bw/day (114 µg/kg bw/day) based on the lowest therapeutic doses for adults.

Brooks and Huggett (2012) derived an ADI of 110,000 ng/kg bw/day (110 µg/kg bw/day) and an ADI of 1,200,000,000 ng/kg bw/day (1,200,000 µg/kg bw/day).

5.9.2.1 Proposed PoDs

Based on the data obtained, the following PoD is proposed for ibuprofen (see objective 3).

- A NOAEL of 7.5 mg/kg bw/day based on maternal toxicity in rats and rabbits

5.9.2.2 Selection of proposed UFs

The proposed UFs for use with the PoD selected are presented in Table 10.

Table 10. Proposed UFs for ibuprofen

	UF	Comments
PoD (mg/kg bw/day)	7.5	
Interspecies differences	10	Based on animal data
Intraspecies differences	10	To account for human variability
Conversion of LOAEL to NOAEL	1	NOAEL used as PoD
Use of subchronic exposure data	3	Based on data from developmental studies
Inadequate databases	1	Adequate data
Total UFs	300	

5.9.2.3 Derivation of proposed ADI

The proposed ADI is:

- 25,000 ng/kg bw/day (25 µg/kg bw/day) using a NOAEL as the PoD

5.9.3 Exposure assessment

The maximum concentration of ibuprofen measured in drinking water was 3.07 ng/L and the highest median was <2 ng/L[2].

Based on default factors,

- For an adult, the daily intake would be 0.10 and 0.07 ng/kg bw/day (0.0001 and 0.00007 µg/kg bw/day) based on maximum and median concentrations, respectively
- For a child, the daily intake would be 0.31 and 0.20 ng/kg bw/day (0.00031 and 0.0002 µg/kg bw/day) based on maximum and median concentrations, respectively
- For an infant, the daily intake would be 0.46 and 0.30 ng/kg bw/day (0.00046 and 0.0003 µg/kg bw/day) based on maximum and median concentrations, respectively

5.9.4 Risk characterisation

The maximum intake of ibuprofen via drinking water by adults, children and infants (0.10-0.46 ng/kg bw/day) is less than the proposed ADI (25,000 ng/kg bw/day) and the HQ is <1. Therefore it is not anticipated that any adverse public health effects will occur following exposure to ibuprofen via drinking water.

[2] The median levels measured at all sites was <2 ng/L. Ibuprofen was only detected at site 1, where the range measured was <2-3.07 ng/L.

5.9.5 Risk communication

The MOEs for ibuprofen, based on the NOAEL and the maximum intake, are 73,000,000 for adults, 24,000,000 for children and 16,000,000 for infants.

The MOEs based on the ADI (derived from the NOAEL) are 244,000 for adults, 81,000 for children and 54,000 for infants.

The MOEs indicate exposures are of low concern in terms of risk to public health.

5.10 Naproxen

5.10.1 Hazard Identification

The principal effects of ibuprofen appear to be gastrointestinal toxicity, hepatotoxicity, cardiovascular toxicity, nephrotoxicity, haematotoxicity and developmental toxicity.

5.10.2 Hazard characterisation

Snyder and Snyder (2008) derived an ADI of 570,000 ng/kg bw/day (570 µg/kg bw/day) based on developmental effects in the mouse.

Environment Protection and Heritage Council (2008) derived an ADI of 6300 ng/kg bw/day (6.3 µg/kg bw/day) based on the lowest therapeutic doses for adults.

5.10.2.1 Proposed PoDs

Based on the data obtained, the following PoD is proposed for naproxen (see objective 3).

- A NOAEL of 10 mg/kg bw/day based on gastrointestinal tract effects in rats and teratogenic effects in the rabbit

5.10.2.2 Selection of proposed UFs

The proposed UFs for use with the PoDs selected are presented in Tabe 11.

5.10.2.3 Derivation of proposed ADI

The proposed ADI is:

- 33,000 ng/kg bw/day (33 µg/kg bw/day) using a NOAEL as the PoD

5.10.3 Exposure assessment

The maximum concentration of naproxen measured in drinking water was 2.72 ng/L and the highest median was <1 ng/L[3].

Based on default factors,

- For an adult, the daily intake would be 0.09 and 0.03 ng/kg bw/day (0.00009 and 0.00003 µg/kg bw/day) based on maximum and median concentrations, respectively
- For a child, the daily intake would be 0.27 and 0.10 ng/kg bw/day (0.00027 and 0.00010 µg/kg bw/day) based on maximum and median concentrations, respectively
- For an infant, the daily intake would be 0.41 and 0.15 ng/kg bw/day (0.00041 and 0.00015 µg/kg bw/day) based on maximum and median concentrations, respectively

[3] The median levels measured at all sites was <1 ng/L. Naproxen was only detected at site 1, where the range measured was <1-2.72 ng/L.

Table 11. Proposed UFs for naproxen

	UF	Comments
PoD (mg/kg bw/day)	10	
Interspecies differences	10	Based on animal data
Intraspecies differences	10	To account for human variability
Conversion of LOAEL to NOAEL	1	NOAEL used as PoD
Use of subchronic exposure data	3	Based on subchronic exposure in reproductive studies
Inadequate databases	1	Adequate data
Total UFs	300	

5.10.4 Risk characterisation

The maximum intake of naproxen via drinking water by adults, children and infants (0.09-0.41 ng/kg bw/day) is less than the proposed ADI (33,000 ng/kg bw/day) and the HQ is <1. Therefore it is not anticipated that any adverse public health effects will occur following exposure to naproxen via drinking water.

5.10.5 Risk communication

The MOEs for naproxen, based on the NOAEL and the maximum intake, are 110,000,000 for adults, 170,000,000 for children and 290,000,000 for infants.

The MOEs based on the ADI (derived from the NOAEL), are 370,000 for adults, 120,000 for children and 82,000 for infants.

The MOEs indicate exposures are of low concern in terms of risk to public health.

6 Summary and conclusions

A summary of the data for all pharmaceuticals is presented in Table 12.

Table 12. Summary of PoD, ADI and MOE for all pharmaceuticals

Pharmaceutical	Max conc in DW	PoD	ADI	Max intake by most sensitive receptor	MOE (adult)	MOE (child)	MOE (infant)
	ng/L	mg/kg bw/day	µg/kg bw/day				
Benzoylecgonine	3.51	81.9	273	0.00053	700,000,000	230,000,000	160,000,000
Carbamazepine	148	20.0	22	0.022	4,100,000	1,400,000	900,000
Carbamazepine epoxide	16.6	17.7	59	0.00025	32,000,000	11,000,000	7,100,000
Ibuprofen	3.07	7.5	25	0.00046	73,000,000	24,000,000	16,000,000
Naproxen	2.72	10.0	33	0.00041	110,000,000	170,000,000	290,000,000

During the hazard characterisation phase of the risk assessment, the NO(A)EL was selected as the most robust PoD for all five substances. For benzoylecogonine and carbamazepine epoxide, a TTC approach was also considered as it has been used previously by Houeto *et al.* (2012) for the risk assessment of carbamazepine epoxide in drinking water. Authors stated that the small MOE seen in the study clearly demonstrated that the TTC approach is very conservative. In light of this and the fact that a NOEL could be modelled, the use of the NOELs in the risk assessment was considered more robust, although the TTC was still used to provide additional support as part of a weight of evidence approach.

During the exposure assessment phase, the levels of pharmaceuticals in drinking water were based on data reported by Boxall *et al.* (2012), who measured levels in treated water at four sites over a one year period. Authors identified the four sites as being of high risk status due to their geographical location being downstream from highly populated areas. Although there were detectable levels of all five substances in many of the samples over the measurement period, several pharmaceuticals showed seasonal and site variability. For example, highest concentrations occurred predominantly in the latter part of the year, and site four had higher levels of many of the pharmaceuticals in comparison to the other three sites. Moreover, potential outliers in the data were observed i.e. higher levels of carbamazepine were measured in November at site 1, and ibuprofen was measured above the LOD only at site 1 in January. For the purposes of this risk assessment the maximum concentration of each pharmaceutical was used, although the intake by all receptors was also calculated based on the median value.

During the risk characterisation phase, the intake of the five pharmaceuticals by adults, children and infants was calculated based on the maximum and median concentrations in drinking water, as described above. Such intakes were compared against the ADI, indicative of the concentration of the substance that may be ingested over a lifetime without appreciable risk to health. The intake of pharmaceuticals by all receptors was below the ADI and the HQ for all substances was <1.

The MOEs calculated for each of the pharmaceuticals was between 900,000 (infant exposure to carbamazepine) to 700,000,000 (adult exposure to benzoylecogonine). For non-carcinogenic compounds, the US EPA stated that an acceptable MOE based on a NOAEL and LOAEL is 100 and 1000, respectively (US Environmental Protection Agency, 2012).

Overall, this human health risk assessment was based on the levels of specific pharmaceuticals in drinking water samples reported by Boxall *et al.* (2011), and used default exposure parameters for adults, children and infants. When using toxicological endpoints as the PoD rather than therapeutic values, then the levels of these pharmaceuticals measured in drinking water are not anticipated to pose an appreciable risk to public health.

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8 Abbreviations

ADI	Acceptable daily intake
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	Benchmark dose
CBZ	Carbamazepine
CBZ-EP	Carbamazepine-10,11-epoxide
CLP	Classification, labelling and packaging
COX	Cyclooxygenase
DWI	Drinking Water Inspectorate
FDA	Food and Drug Administration
HBGV	Health based guidance value
HQ	Hazard quotient
HSDB	Hazardous Hazardous Substances Databank
IARC	International Agency for the Research on Cancer
LD_{50}	Lethal dose
IMP	Investigational Medicinal Product
IND	Investigational New Drug
LOAEL	Lowest observed adverse effect level
LOEL	Lowest observed effect level
LOD	Limit of detection
MDH	Minnesota Department of Health
MHDD	Mmaximum human daily dose
MHRA	Medicines and Healthcare products Regulatory Agency
MHTD	Maximum human therapeutic dose
MOE	Margin of exposure
MRTD	Maximum recommended therapeutic dose
MTD	Maximum tolerated dose
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NSAID	Nonsteroidal Nonsteroidal anti-inflammatory drug
NTP	National Toxicology Programme
PoD	Point of departure
QSAR	Quantitative Structure Activity Relationship modelling

RfD	Rreference dose
TDI	Tolerable daily intake
TTC	Threshold of Toxicological Concern
UF	Uncertainty factor

Appendix 1 – Information sources

- ScienceDirect
- PubMed
- Medicines and Healthcare products Regulatory Agency
- Hazardous Substances Data Bank (HSDB)
- Commission on Human Medicines
- TOXNET
- European Medicines Agency
- FDA Adverse Event Reporting System (FAERS)
- MedEffect Canada
- British Pharmacopoeia
- Google Scholar
- Worldwide Science.org,
- Canada Vigilance Adverse Reaction Online database
- European database of suspected adverse drug reaction reports
- Toxline
- Environmental Protection Agency (EPA) databases ChemAbs
- National Institute for Health and Care Excellence
- NICE Evidence Services
- Health Canada Vigilance Adverse Reaction Online
- British Pharmacopoeia Commission
- British National Formulary
- Computational Toxicology Resaerch Programme (ACToR)
- US Food and Drug Administration

Appendix 2 – Literature review search terms

Benzoylecogonine, benzoylecgonine, Esterom, 3-Benzoyloxy-8-methyl-8-azabicyclo[3.2.1]octane-4-carboxylic acid, 519-09-5

Carbamazepine, Biston, Calepsin, Convulsine, Epitol, Finlepsin, Hermolepsin, Karbamazepine, Lexin, Mazepine, Neuritol, Neurotol, Neurotop, Nordotol, Servimazepine, Sirtal, Stazepine, Tegretal, Tegretol, Telesmin, Temporol, Teril,Timonil, Trimonil Retard, 5*H*-dibenzo[*b*,*f*] azepine-5-carboxamide, 298-46-4

Carbamazepine epoxide, Carbamazepine-10,11-epoxide,36507-30-9

Ibuprofen, Anadin Ibuprofen, Anadine Joint Pain, Artofen, Brufen, Brufen Retard, Calprofen, Ebufac, Fenpaed, Galprofen, Ibugel, Ibuleve, Nurofen, Orbifen, Rimafen, *RS*)-2-(4-(2-methylpropyl)phenyl)propanoic acid, 15687-27-1

Naproxen, Aleve, Anaprox, Antalgin, Apranax, “Feminax Ultra”, Flanax, Inza, “Midol Extended Relief”, Nalgesin, Naposin, Naprelan, Naprogesic, Naprosyn, Narocin, Proxen, Soproxen, Synflex, Xenobid, (+)-(*S*)-2-(6-methoxynaphthalen-2-yl)propanoic acid, 22204-53-1

AND

Toxicity, carcinogenicity, mutagenicity, immunotoxicity, developmental, reprotoxicity, immunotoxicity, neurotoxicity, “in vitro”, “side effect”

OR

Health-based guidance value, acceptable daily intake, tolerable daily intake, no observed adverse effect level, lowest observed adverse effect level, benchmark dose, lethal dose, therapeutic dose, margin of exposure, margin of safety, safety factor, uncertainty factor, maximum recommended daily dose, summary of product characteristics, SmPC, clinically effective dose

Appendix 3 – Communications log

The following log outlines communication with the pharmaceutical companies.

Date	Activity	Response	Person involved
14-Oct-2013	Finalised generic background & request letter		Jo Carter
17-Oct-2013	Called Novartis to discuss project and requirements		Jo Carter
18-Oct-2013	Sent follow-up email to Novartis with letter attached		Jo Carter
23-Oct-2013	Called Abbott to discuss project and requirements		Jo Carter
24-Oct-2013	Sent follow-up email to Abbott with letter attached	Automated reply received	Jo Carter
25-Oct-2013	Called Roche to discuss project and requirements	Enquiry tracking reference number provided	Jo Carter
28-Oct-2013	Sent follow-up email to Roche with letter attached	Automated reply received	Jo Carter
29-Oct-2013	Data extraction form drafted		Jo Carter
07-Nov-2013	Carried out Novartis follow up call.	Novartis could not track original enquiry. Will follow up and respond by 11-12 Nov	Jo Carter
07-Nov-2013	Carried out Roche and Abbott follow up call		Jo Carter
08-Nov-2013	Sent Roche and Abbott follow up email forwarding initial correspondence and data abstraction form		Jo Carter
14-Nov-2013	Resent Abbott follow up email		Jo Carter
14-Nov-2013	Called Roche to confirm enquiry was currently with product specialist	Roche confirmed they are seeking relevant data	Jo Carter
14-Nov-2013	Called Abbott to confirm enquiry was currently with product specialist	Abbott confirmed they are seeking relevant data	Jo Carter
15-Nov-2013	Received telephone call from Abbott (Julia Beegan)	Abbott confirmed they are looking into the enquiry and will provide data as soon as possible	Sarah Bull
15-Nov-2013	Received confirmation email from Abbott (Julia Beegan)	Abbott confirmed they are looking into the enquiry and will	Jo Carter

		provide data as soon as possible	
15-Nov-2013	Received confirmation email from Roche Medinfo	Roche are currently working on a response	Jo Carter
15-Nov-2013	Called Novartis and resent initial correspondence and data abstraction form.	Novartis confirmed the enquiry had been passed on to their regulatory colleagues for follow up.	Jo Carter
15-Nov-2013	Researched FDA FOI requests		Jo Carter
22-Nov-2013	Called FDA FOI office	Received only voicemail message	Jo Carter
25-Nov-2013	Sent reminder email to Novartis		Sarah Bull
25-Nov-2013	Sent reminder email to Abbott		Sarah Bull
25-Nov-2013	Received automatic e mail from Abbott		Sarah Bull
25-Nov-2013	Received call from Abbott asking for deadline.	Abbott confirmed that the enquiry was being discussed with global colleagues	Jo Carter
27-Nov-2013	Called FDA FOI office	Submitted on line request. Payment on retrieval of data if found in archives	Jo Carter
5-Dec-2013	Received telephone call from Abbott (Julie Burns)	Abbott confirmed that the enquiry was being discussed with global colleagues and will provide data as soon as possible	Sarah Bull
5-Dec-2013	Online request completed, ref number FDA 1308339		Jo Carter
9-Dec-2013	Received receipt of online request for information		Sarah Bull
11-Dec-2013	Received e-mail response from Novartis	Novartis were unable to provide any information	Sarah Bull
12-Dec-2013	Received e-mail response from Roche	Roche provided a poster publication and a Safety Data Sheet	Sarah Bull
7-Jan-2014	Received e-mail response from FDA	FDA were unable to provide any data	Sarah Bull
8-Jan-2014	Received e-mail response from Abbott	Abbott provided a publication on ibuprofen	Sarah Bull

Appendix 4 – Letter to pharmaceutical companies

Copy of the explanatory letter sent to pharmaceutical companies.

Ref: A refined toxicological evaluation for pharmaceuticals detected in drinking water WT1289

Dear Sirs,

The purpose of this letter is to seek your company's assistance with a Defra research project. The project aims to understand better any risks that may arise from the trace quantities of pharmaceuticals that have been found in drinking water. The assistance we are seeking is the provision of toxicological information on certain compounds.

Pharmaceuticals can be introduced into water sources through excretion after normal use or through improper disposal (e.g. discarding drugs into toilets). Reports of trace concentrations of pharmaceuticals in the water cycle have raised concerns over potential human health risks from exposure to very low levels of pharmaceuticals in drinking water. The World Health Organisation (WHO) considers that appreciable adverse impacts on human health are very unlikely at current levels of exposure in drinking water and is maintaining a watching brief on this subject without seeing a need at this time for any specific guidelines (WHO 2012), whereas the US Environmental Protection Agency (EPA) has published a list of chemicals, including a number of pharmaceuticals, to be considered for government regulation.

In the UK, a desk based study previously commissioned by the Drinking Water Inspectorate concluded that 'there is no significant risk from pharmaceuticals discharged to drinking water sources, even in worse case situations', but recommended that a small scale study be carried out to measure concentrations of pharmaceuticals in UK drinking waters. In this survey, six pharmaceuticals were detected at concentrations above the LOD, namely *caffeine, benzoylecogonine, carbamazepine, carbamazepine epoxide, ibuprofen and naproxen,* whereas another 11 substances were not detected above their respective LODs. The authors used the therapeutic dose of the pharmaceuticals as the point of departure to assess the risk associated with exposure via drinking water and concluded that the low or non-detectable levels of pharmaceuticals present in water do not pose an appreciable risk to human health.

In order to better define the risk to human health, the Drinking Water Inspectorate have recently commissioned a study to carry out a risk assessment of these pharmaceuticals from exposure via drinking water. This project will support the Drinking Water Inspectorate's strategic objective maintaining public confidence in the water supply through of a better understanding of the risk.

Ricardo-AEA has been contracted to carry out the above mentioned study, namely 'A refined toxicological evaluation for pharmaceuticals detected in drinking water WT1289', where the aim is to compare the concentrations of pharmaceuticals measured in drinking water with toxicological end points to provide a human health risk assessment. Due to the sensitivity surrounding public perception of water quality, we need to ensure the risk assessment of pharmaceuticals is based on reliable, relevant and robust toxicological data to give as accurate a prediction of risk to public health as possible. In order to do so, we would like to base the risk assessment on points of departure such as no observed (adverse) effect levels

or lowest observed (adverse) effect levels from toxicological studies. The type of data we are looking for would include, but not necessarily be limited to non-clinical safety assessment data from acute studies all the way through the safety assessment programme to carcinogenicity studies. Any study involving exposure via oral administration would be useful although studies involving other routes of exposure would also be of interest, particularly if they include toxicokinetic information. In addition, any data from human studies involving exposure to the substances would be helpful.

As the Marketing Authorisation holder of Naproxen we are approaching you to request the provision of study reports outlining such points of departure if available, or alternatively, any toxicological information you may have on Naproxen from which we could derive such values. The data provided will be used as a comparator to the levels of pharmaceuticals measured in drinking water as part of the risk assessment and may be cited in a final report to Drinking Water Inspectorate, which will be publically available.

We appreciate you may have concern about releasing such data and would be happy to sign any necessary confidentiality/disclosure agreements and to discuss with you how any data provided would be used/reported.

I would greatly appreciate if you could spare some time to discuss this matter further and thank you in advance for your support. Please contact me on 07584 430300 or carterjojc@gmail.com

Yours sincerely,

Jo Carter
Independant Consultant
(on behalf of Ricardo-AEA)

Dr Sarah Bull
Toxicology consultant, Ricardo-AEA

Sign off Ricardo AEA

Appendix 5 – Toxicology pro forma

ABSTRACT/STUDY	
Reference	
Study Type	
Study Population (species, strain, sample size, sex, age)	
Pharmaceutical under investigation	Ibuprofen
Doses, route of administration	Oral
Sampling times, end-points	
Relevant PoD: NOAEL, LOAEL, LD_{50}, Bench Mark Dose (BMD)	
Results	
Details of study quality	
Comments	

PoD; point of departure

NOAEL; no observed adverse effect level

LOAEL; low observed adverse effect level

LD_{50}; lethal dose 50

BMD; benchmark dose

Appendix 5 – ADI derivations

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