Regulatory Toxicology and Pharmacology 62 (2012) 41–48

Contents lists available at SciVerse ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

# Assessment of the health risks related to the presence of drug residues in water for human consumption: Application to carbamazepine

Paul Houeto [a,*], Aude Carton [a], Michel Guerbet [b], Anne-Cécile Mauclaire [a], Chantal Gatignol [c], Philippe Lechat [a], Dominique Masset [a]

[a] Département de toxicologie, DEMEB, Agence Française de sécurité sanitaire des produits de santé (Afssaps), 143/147, Bd Anatole France, F-93285 Saint-Denis, France
[b] Laboratoire de toxicologie, ADEN EA 4311, UFR Médecine Pharmacie, Université de Rouen, 22, Boulevard Gambetta, 76183 Rouen, France
[c] Prévention des risques liés à l'environnement et Alimentation, Bureau Qualité des eaux (EA4), Direction Générale de la Santé (DGS), Ministère Travail, Emploi et Santé, 14 Avenue Duquesne, 75007 Paris, France

ARTICLE INFO

*Article history:*
Received 24 May 2011
Available online 8 December 2011

*Keywords:*
Health risk assessment methodology
Drug residues
Drinking water
Carbamazepine
10,11-Epoxycarbamazepine

ABSTRACT

Pharmaceutical residues have been detected at low (usually ng/L) concentrations in drinking water sources. The detection of drugs in water intended for human consumption (WIHC) has raised questions of safety. In the absence of regulatory or other official guidance, water utilities are faced with a problem of which pharmaceutical residues should be monitored and the toxicological limits that should be required.

In this essay, we define an approach for the assessment of health risks related to chemicals found in drinking water. We use the examples of carbamazepine and its main metabolite 10,11-epoxycarbamazepine to demonstrate our approach, which involves application of the following algorithm: (1) when there is human or animal toxicity data, a toxicity reference value (TRV) can be calculated; (2) when this is not applicable, an attempt should be made to derive the TRV using known information about the minimum therapeutic dose (MTD); and (3) when no applicable data is available, at all, a threshold of toxicological concern (TTC) should be estimated.

In the case of carbamazepine, where relevant toxicological data exists, we derived a TRV, based on the known minimum therapeutic dose (MTD). For carbamazepine's metabolite 10,11-epoxycarbamazepine, there is no toxicological data, so we applied the TTC approach. Using this approach, and combining our estimates with what is known about these chemicals' margin of exposure (MOE), suggests that there is likely to be no appreciable risk to human health exposure to carbamazepine or its major metabolite, even given the inevitable uncertainties in exposure scenarios.



## 1. Introduction

Physicochemical analysis during recent years have confirmed the presence of drug residues and their metabolites in all the different compartments of the aquatic environment: wastewater, groundwater, surface water, and drinking water (Beru et al., 2008; Cunningham et al., 2009; Mompelat et al., 2009; Schriks et al., 2010; Fent et al., 2006; Heberer, 2002). This ubiquitous contamination has been proven worldwide with over one hundred drug residues detected, all drug classes combined (Kolpin et al., 2002; Pomati et al., 2008; Ternes, 1998; Zuccato et al., 2000; Kümmerer, 2009). They are detected at low concentrations, ranging from µg to ng/L but the continuous release and fugitive emissions of these residues make micro-persistent contaminants (Daughton and Ternes, 1999; Rabiet et al., 2006). If it is accepted that the concentrations found in environmental water are too low to present a short-term risk for humans, the long-term toxicity remains to be characterized. Indeed, pharmaceutical substances are specifically designed to be chemically stable, resistant to degradation, and to have a biological effect on living organisms. In addition to the drug residues and their metabolites, transformation products resulting from degradation phenomena (biological, chemical or physical) may occur in different compartments of the aquatic environment and in sewage treatment plants (Daughton and Ternes, 1999).

For drinking water, the data available in the literature on the contamination levels of drug residues are rare. Pharmaceutical residues have been detected at low (usually ng/L) concentrations

*Abbreviations:* DWEL, drinking water equivalent level; FDA, Food and Drug Administration; IARC, International Agency for Research on Cancer; LOAEL, Lowest-Observed-Adverse-Effect-Level; MA, marketing authorization; MF, modulation factor; MTD, minimum therapeutic dose; NOAEL, No Observed Adverse Effect Level; NOEL, No Observed Effect Level; NTP, National Toxicology Program; SPE, solid phase extraction; TRV, toxicity reference value; TTC, threshold of toxicological concern; UF, uncertainty factor; WIHC, water intended for human consumption.

* Corresponding author. Fax: +33 (0) 1 55 87 35 82.
*E-mail address:* Paul.houeto@afssaps.sante.fr (P. Houeto).

0273-2300/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.yrtph.2011.11.012

in drinking water sources (Bull et al., 2011; Schriks et al., 2010; Schulman et al., 2002; Mompelat et al., 2009). These findings have been reported with little perspective on whether the small amounts that might be consumed pose a health hazard. This remains a key question and a challenge to be solved. The major source of pharmaceutical residues that occur in water intended for human consumption (WIHC) is excretion of drugs and their metabolites into municipal wastewater (Ternes, 2007). These molecules are characterized by a considerable diversity in chemical structures. The concentration levels of those molecules vary depending on their chemical stability, biodegradability, physico-chemical characteristics and the effectiveness of the treatment and filtration of particulates of wastewater treatment plants. More than 3000 human-use and 300 veterinary-use drug substances are currently available in the French market. Municipal wastewaters undergo different degrees of treatment prior to their release to surface. However, European and French water quality regulations do not take into account drug residue in the different aquatic compartments. In the absence of regulatory or other official guidance, water utilities are faced with a problem of which pharmaceutical residues should be monitored and the toxicological limits that should be required (Bound and Voulvoulis, 2004; Christensen, 1998).

Taking into account that the occurrence of pharmaceutical residues in WIHC is a common concern in the served populations, we aimed to develop a general strategy in order to determine more precisely their safety concern through three possible ways to approach this question, for individual chemicals. We propose the following algorithm: (1) when there is human or animal toxicity data, a toxicity reference value (TRV) can be calculated; (2) when this is not applicable, an attempt should be made to derive the TRV using known information about the minimum therapeutic dose (MTD); and (3) when no applicable data is available, at all, a threshold of toxicological concern (TTC) should be estimated.

In order to apply and compare the different approaches and in addition whether there is a safety concern, we will use two compounds, carbamazepine and its major metabolite 10,11-epoxycarbamazepine resulting in a list of chemicals from a national sampling survey to model such an approach. The results from the national survey have been published elsewhere, which we will briefly describe later. Through the examples of carbamazepine, where toxicological data exist, while its main metabolite there is no data, we discuss the advantages and the limitations of each approach that could be applied to the individual compounds. These different approaches would permit to anticipate monitoring actions of pharmaceutical residues in WIHC.

## 2. National sampling survey

The French General Directorate for Health (DGS) asked to the Anses (formerly French Agency for Food Safety or Afssa) to perform a national survey on drug residues in WIHC to evaluate their presence. Based on a list of 76 priority molecules established using discriminating criteria (tonnage, solubility and activity) and appropriated for the objective of the study, 45 molecules from different chemical families and therapeutic classes were tested for using a multi-residue method (LC–MSMS). In collaboration with the regional health agencies, 238 sites were retained for the national territory in order to ensure a representativity of approximately 24% in terms of population served. The sampling survey was carried out from October 2009 to June 2010. Out of the 45 molecules tested for, 19 molecules were detected at least once. Regarding the two examples that we will chose to model the different approaches, in terms of quantifiable frequency, carbamazepine is found in 4% of the sample analysed and its metabolite, 10,11 epoxycarbamazepine was found in 7.6%. This represents 11 and 21 samples of overall samples of the survey ($n$ = 280), respectively (Anses, 2011). The maximum concentration of carbamazepine found in WIHC is 33 ng/L. The maximum content of its main metabolite, 10,11 epoxycarbamazepine, is of 6 ng/L (Table 1).

## 3. Risk assessment procedure

The assessment of the health risks related to the presence of drug residues in WIHC is based on three approaches:

(1) The toxicological approach via the calculation of toxicity reference values (TRV) based on No Observed (Adverse) Effect Levels (NO(A)EL, Lowest Observed (Adverse) Effect Level (LO(A)EL) or Benchmark dose limit (BMDL) (US EPA, 2000a,b, 2002a) etc. This approach uses pharmaco-toxicological studies from the marketing authorization (MA) dossier or from the literature. However, this long and complex procedure may be hindered by the lack of data, especially in the case of metabolites and old molecules;

(2) A derivation of the TRV toxicological approach involving the use of the minimum therapeutic dose (MTD) instead of NO(A)EL or LO(A)EL or BMDL. Minimum therapeutic dose (MTD) may be used as the point of departure (POD) in assessing potential health hazards from pharmaceutical drug residues in drinking water (Bull et al., 2011). It is a fast and practical method, however it cannot be generalised to substances such as cytotoxic agents, hormones, allergens, antibiotics and metabolites. The problem of low doses arises, in particular with oestrogen compounds intended for very specific sub-populations. The toxicity of cytotoxic molecules can appear at infra-therapeutic doses. Concerning allergenic substances, the effects may appear at any dose. Otherwise, there is no posology for metabolites unless it is assumed that the latter have the same activity and strength as the original molecules (Schwab et al., 2005).

(3) The conservative approach of the threshold of toxicological concern (TTC) which is used as a risk management tool (Kroes et al., 2004). This recommended threshold is set at 0.15 μg/person/day, based on an excess of risk of $10^{-6}$ (especially for low dose substances). The TTC is defined as a human exposure dose below which the risk is believed to be sufficiently low to exempt a substance from toxicological investigations. This probabilistic approach is based on the concept of structural similarity and was built from a database of known carcinogenic substances. Even though its use is only dedicated to substances for which there is no available data, it allows to be freed from the MA dossier and cover the uncertainties related to the carcinogenic properties.

In summary, the TRV toxicological approach ($TRV_{Tox}$) should be retained as long as the MA dossier data are available. In the absence of toxicological data (NO(A)EL, LO(A)EL) or BMDL, the use of a MTD may be an alternative ($TRV_{MTD}$). However, this method cannot be generalised, especially to certain families of molecules such as cytotoxic agents, hormones, allergens, antibiotics and metabolites. The TTC approach can only be used in second line and

**Table 1**
Frequency of detection and quantification of different molecules in water intended for human consumption (Anses, 2011).

| Molecules ($n$ = 280 samples) | Frequency of quantifiable results (>LQ) | Minimum and Maximum content (ng/L) |
|---|---|---|
| Carbamazepine | 40% | 5–33 |
| Epoxycarbamazepine | 7.6% | 1–6 |

LQ, limit of quantification (1–50 ng/L); $n$, number of samples.

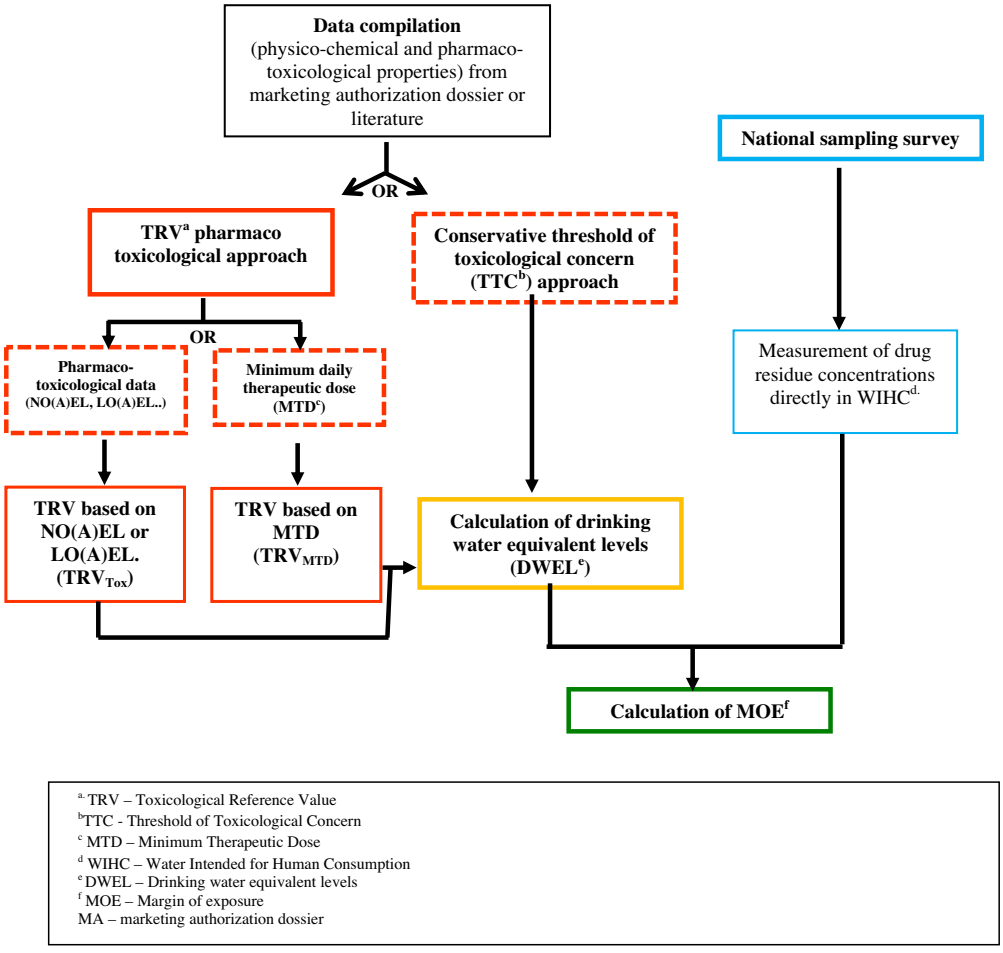


**Fig. 1.** Different methodological approaches for health risk assessment of drug residues in water intended for human consumption (Afssaps, 2010).

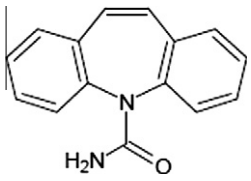


**Fig. 2.** Carbamazepine structure.

must be reserved to substances for which there is no available data, especially, metabolites.

However, it should be noted that the different approaches described are not comparable in view of the application fields and limits that have been identified, and an overall risk assessment process based on three methodologies (TRV$_{Tox}$, TRV$_{MTD}$, TTC) may be envisaged (Fig. 1). Furthermore, the questions related to multi-exposure and interactions between drug residues and other chemical origin contaminants are not considered and have not been solved to date.

## 4. Analysis of the results of the national sampling survey: carbamazepine case

### 4.1. Chemical structure

See Fig. 2: carbamazepine structure.

### 4.2. Physico-chemical properties

See Table 2: physico-chemical parameters of carbamazepine.

### 4.3. Pharmaco-toxicological data

#### 4.3.1. Pharmacology–Pharmacokinetics

Carbamazepine has anti-epileptic and psychotropic properties, and is indicated in the treatment of epilepsy (generalised tonico-clonic and partial seizures), in trigerminal nerve and glossopharyngeal neuralgias and in maniac or hypomaniac excitation conditions. Carbamazepine mainly acts on voltage-dependent sodium channels. The other mechanisms of action have been partially elucidated. The decrease in the release of glutamate and the stabilisation of neuron membranes can essentially explain the anti-epileptic effects.

The main metabolite formed by oxidation is 10,11 epoxycarbamazepine, which is pharmacologically active (Fig. 3), mainly eliminated by hydrolysis as trans-10,11-dihydroxydiolcarbamazepine (Amore et al., 1997; Miao and Metcalfe, 2003; Mockenhaupt et al., 2005) and by conjugation to form *O*-glucuronides. Anti-convulsive and analgesic properties in neuralgia have been demonstrated for 10,11 epoxycarbamazepine (Reynolds, 1996). Finally, only 1% of the 10,11 epoxycarbamazepine formed is excreted as the unaltered form. This path can also lead to the formation of minor metabolites via the aromatic hydroxylation of carbamazepine. Carbamazepine is eliminated almost exclusively as the metabolised form, 1% to 2% as the unaltered form. Excretion is mainly

**Table 2**
Physico-chemical parameters of carbamazepine (Inchem 1999; US Pharmacopoeia, 2008).

| | |
|---|---|
| CAS No. | 298-46-4 |
| ATC No. | NO3AF01 |
| Chemical formula | $C_{15}H_{12}N_2O$ |
| Molecular weight | 236.27 g $mol^{-1}$ |
| Presentation | White to yellowish-white crystalline powder |
| Solubility in water | 112.1 mg/L |
| Vapour pressure | $1.84 \times 10^{-7}$ mm Hg (25 °C) |
| Melting point | 190 to 193 °C |
| Henry's law constant | $1.08 \times 10^{-10}$ atm $m^3$ $mol^{-1}$ (25 °C) |
| pKa | 13.9 |
| Log $K_{ow}$ | 2.45 |

urinary (72%), in the form of metabolites, with a faecal excretion of 28% (Agence Française de Sécurité Sanitaire des Produits de Santé (Afssaps, 2010, 2011), proprietary medicinal product registry).

*4.3.2. Toxicology*

The toxicology data have been obtained from the MA dossiers and the literature. Several toxicity studies after repeated administration have been reported in rats (LOAEL ranging from 50 to 200 mg/kg/day), dogs (NOAEL ranging from 50 to 100 mg/kg/day and LOAEL from 100 to 300 mg/kg/day) and humans (LOAEL = 10 mg/kg/day) (FDA, 1967; Asadi-Pooya and Ghetmiri, 2006). Carbamazepine is considered to be non-mutagenic *in vitro* and *in vivo* (Glatt et al., 1975; Königstein et al., 1984; Margaretten et al., 1987; Flejter et al., 1989; Schaumann et al., 1985; Celik, 2006). A single carcinogenesis study has been found. It is a 2 years study in Sprague–Dawley rats (male and female) at doses of 25, 75 and 250 mg/kg/day. An increase in the incidence of hepatocellular tumours in females and benign testicular interstitial cell adenomas in males has been observed starting at doses of 25 mg/kg/day (United States Pharmacopeia, 2008). However, the extrapolation of these effects to humans remains unknown. Furthermore, carbamazepine is not classified as carcinogenic by any of the following organisations: NTP (National Toxicology Program), IARC (International Agency for Research on Cancer), FDA (Food and Drug Administration). Reproduction function studies have been performed in rats (LOAEL from 192 to 250 mg/kg/day) mice (NOAEL = 192 mg/kg/day), rabbits (LOAEL = 250 mg/kg/day and humans (LOAEL ranging from 3 to 11 mg/kg/day) (Samrén et al., 1997, 1999; FDA, 1967; Artama et al., 2005; Diav-citrin et al., 2001; Vorhees et al., 1990). The effects observed are weight loss of litter during lactation, decrease in the number of foetuses and fertility, increase in resorptions, bilateral twisting of ribs, increase in neural tube and urinary tract defects, cleft palate and cardiovascular disorders.

*4.4. Health risk assessment*

The assessment of the risk of carbamazepine and its metabolite, 10,11-epoxycarbamazepine was based on a lifelong consumption (70 years) of 2 L of water per day in adults (60 kg) (WHO, 2004) and 1 L of water in children (16.7 kg) (Argall et al., 2003; Kumar and Xagoraraki, 2010a,b). It was carried out according to the three approaches previously described.

The drinking water equivalent concentration (DWEL) represents the concentration of a pharmaceutical per litre of water that does not result in significant risk to the health of consumers over a lifetime of 70 years. It was estimated according to the US EPA Methodology for deriving ambient water quality criteria for the protection of human health (US EPA, 2002b) as a ratio between TRV and the adult or child drinking water ingestion rate (2 or 1 L/person-day).

Concerning the TRV toxicological approach, the study selected for the assessment of health risks was that carried out in humans (reproductive study) with a LOAEL of about 3 mg/kg/day (200 mg/day) (Samrén et al., 1997, 1999). This LOAEL corresponds to the lowest dose used in all the studies. Furthermore, it has the advantage of taking into account the pregnant women and the child sub-population. We thus observed that it is lower than the LOAEL found in the carcinogenesis study in rats over 2 years. This results in a $TRV_{Tox}$ of $3.3.10^3$ ng/kg/day with an overall uncertainty factor (UF) of 900 ($UF_1 = 3$ for the LOAEL corresponding to an effective dose on the disease, $UF_2 = 1$ for a low carry-over effect of the compound and the effect, $UF_3 = 1$ for data obtained in humans, $UF_4 = 3$ to take into account the sensitive Asian sub-population (Ferrell and McLeod, 2008), $UF_5 = 10$ for the quality of the data, $UF_M = 10$ for the uncertainty related to the oncogenicity). The DWEL calculated from the $TRV_{Tox}$ is of $99.10^3$ ng/L in adults and $55.10^3$ ng/L in children. Thus, the maximum concentration of 33 ng/L ($C_{CBZ}$) of carbamazepine found during the sampling survey is 3000 to 1667 times lower than the DWEL in adults and children, respectively. This margin of exposure (MOE) ($DWEL/C_{CBZ}$) shows that according to the toxicological approach, the presence of carbamazepine residues in drinking water does not entail any risk for human health (Table 3).

We also used the second method concerning the deriving TRV toxicological approach based on the known MTD in order to compare the two approaches. In France, based on a MTD of 10 mg/kg/day for both adults and children (from infants to 15 years old children) (Agence Française de Sécurité Sanitaire des Produits de Santé, 2011), a $TRV_{MTD}$ with an overall uncertainty factor of 810 ($UF_1 = 3$ for the LOAEL corresponding to an effective dose on the disease, $UF_2 = 3$ for a long-term study, $UF_3 = 1$ for data obtained in humans, $UF_4 = 3$ to take into account the sensitive Asian sub-population (Ferrell and McLeod, 2008), $UF_5 = 3$ for the quality of the data, $UF_M = 10$ for the uncertainty related to the oncogenicity) has been calculated. The $TRV_{MTD}$ is of $12.3.10^3$ ng/kg/day and its DWEL is estimated at $369.10^3$ ng/L in adults and $206.10^3$ ng/L in children. Thus, the $C_{CBZ}$ of 33 ng/L found during the sampling survey is 11,182 to 6242 times lower than the DWEL in adults and children, respectively. This MOE ($DWEL/C_{CBZ}$) shows that according to the approach performed via the MTD, the presence of carbamazepine residues in WIHC does not entail any risk for human health (Table 3). These approaches apply "uncertainty factors" defined previously for human health risk assessment of pharmaceuticals in waters (Schwab et al., 2005). These factors depend of quality and quantity of toxicological data and are more conservative than those proposed by some authors (Hasegawa et al., 2010; Konietzka et al., 2008).

Although data are available to calculate $TRV_{Tox}$ and $TRV_{MTD}$ we also tested the TTC-approach for carbamazepine. This conservative approach may be justified by the mechanism of action of carbamazepine on the central nervous system (CNS), and thus its neurotoxic potential long-term effect on target populations of adults and children drinking water. For a threshold of 0.0025 μg/kg/day (which corresponds to 0.15 μg/person/day divided by 60 kg) with an excess risk set at $10^{-6}$, the corresponding DWEL are 75 ng/L in adults and 41.7 ng/L in children, respectively. The maximum concentration of 33 ng/L ($C_{CBZ}$) of carbamazepine found during the sampling survey is 2.3 to 1.3 times lower than this DWEL in adults and children, respectively. The resulting MOE ($DWEL/C_{CBZ}$) is higher than one (MOE > 1) and suggests that there is likely no appreciable risk to human exposure (Table 3). However, the small MOE observed in this case clearly demonstrates that the TTC-approach is very conservative.

For carbamazepine's metabolite, 10,11 epoxycarbamazepine, there is no toxicological data and posology. So the only way to assess the health risk related to the presence of carbamazepine's

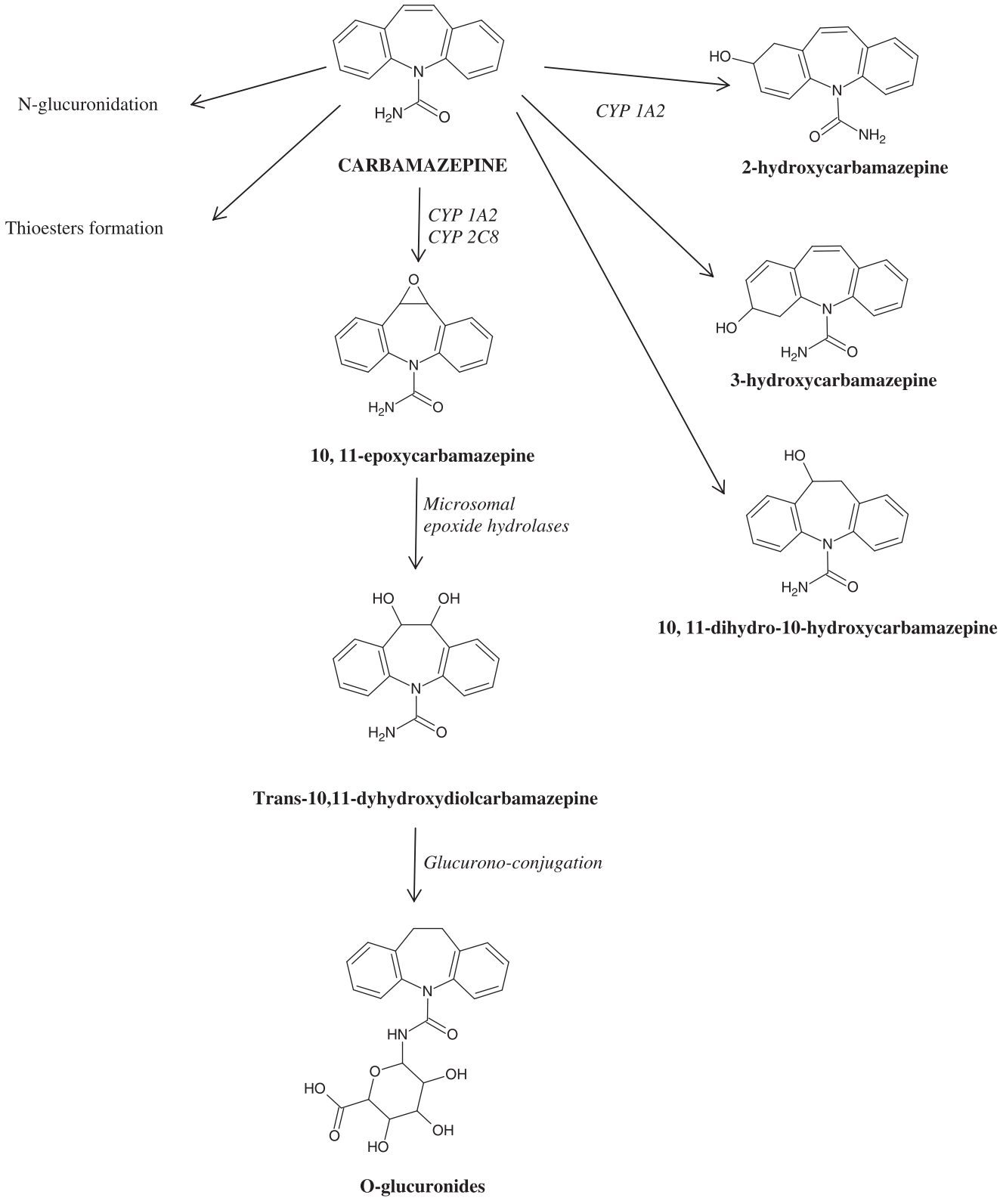


**Fig. 3.** Carbamazepine metabolism (Amore et al., 1997; Miao and Metcalfe, 2003; Mockenhaupt et al., 2005).

metabolite in WIHC is the conservative approach (TTC) with the same values as preceding. For a threshold of 0.0025 μg/kg/day (which corresponds to 0.15 μg/person/day divided by 60 kg) with an excess risk set at $10^{-6}$, the corresponding DWEL are 75 ng/L in adults and 41.7 ng/L in children, respectively. The maximum concentration of 6 ng/L ($C_{EP\text{-}CBZ}$) of 10,11 epoxycarbamazepine found during the sampling survey is 12.5 to 7 times lower than this TTC threshold. The resulting MOE ($DWEL/C_{EP\text{-}CBZ}$) is higher than one (MOE > 1) and suggests that there is likely no appreciable risk to human exposure (Table 4).

**Table 3**
MOE calculated from DWEL and carbamazepine concentration found in WIHC following the TRV toxicological approach, derivation of the TRV toxicological approach using the MTD and TTC approach.

| | Methods | DWEL (ng/L) | | Margins of exposure (MOE) | |
|---|---|---|---|---|---|
| | | Adults | Children | Adults | Children |
| Measured concentration of carbamazepine in WIHC = 33 ng/L | Toxicity reference value based on toxicological data ($TRV_{Tox}$) | 99,000 | 55,000 | 3000 | 1667 |
| | Toxicity reference value based on minimum therapeutic dose ($TRV_{MTD}$) | 369,000 | 206,000 | 11,182 | 6243 |
| | Threshold of toxicological concern (TTC) | 75 | 41.7 | 2.3 | 1.3 |

**Table 4**
MOE calculated from DWEL and 10,11-epoxycarbamazepine concentration found in WIHC following the TTC approach.

| | Methods | DWEL (ng/L) | | Margins of exposure (MOE) | |
|---|---|---|---|---|---|
| | | Adults | Children | Adults | Children |
| Measured concentration of 10,11-epoxycarbamazepine in WIHC = 6 ng/L | Threshold of toxicological concern (TTC) | 75 | 41.7 | 12.5 | 7 |

## 5. Discussion and conclusions

Pharmaceutical residues have been usually detected at low concentrations in WIHC (ng/L). (Anses, 2011; Bull et al., 2011). The present work has identified an efficient approach for an overall health risks assessment based on three methods; TRV based on toxicological data ($TRV_{Tox}$), a deriving TRV toxicological approach based on minimum therapeutic dose (MTD) ($TRV_{MTD}$) or a method using TTC concept. The $TRV_{Tox}$ approach should be retained as long as the MA dossier data are available. A derivation of the $TRV_{Tox}$ approach involving the use of MTD may be used as a point of departure (POD) if the toxicological data are missing. However, it cannot be generalised to substances such cytotoxic agents, allergens, antibiotics, hormones and metabolites. The TTC approach can only be used in last line and must be reserved to substances for which there is no available data e.g. for certain metabolites. Concerning this conservative approach, a TTC is set at 0.15 μg/person/day with an excess risk of $10^{-6}$ (Kroes et al., 2004). Although debatable, we advise that this excess risk was chosen although it is more conservative than that adopted ($10^{-5}$) in the European guideline on the limits of genotoxic impurities for human use (EMA, 2006).

The first step is to identify the $TRV_{Tox}$ $TRV_{MTD,}$ or TTC. The second step is to calculate the corresponding DWEL and compare that to concentrations encountered in WIHC for establishing MOEs for pharmaceutical residues. The variation in the frequency and the severity of effects and the mechanism of action involved must be taken into account in that process.

As, carbamazepine is one of pharmaceutical active ingredients (APIs) used in relatively high volumes (Cunningham et al., 2010), frequently detected in the aquatic environment and finally more resistant to removal from the wastewater treatment, we used this molecule and its major metabolite 10,11 epoxycarbamazepine to test the three approaches. From a methodological point of view, it seems that the application to carbamazepine and its metabolite does not allow the definition of one methodology on its own, mainly due to the types of pharmaco-toxicological studies and data available. It would therefore be appropriate to adopt an overall health assessment process combining the three methodologies ($TRV_{Tox}$, $TRV_{MTD}$, TTC). For all approaches used, the MOE indicate that there is no appreciable risk to human health exposure to carbamazepine and its major metabolite. This confirms the previous results regarding the observed risk estimates. In fact, several approaches have been developed a comprehensive ranking system of prioritizing pharmaceuticals and personal care products (PPCPs) and endocrine disruptors (EDCs) in stream water/source water and finished drinking water using criteria as occurrence, treatment, ecological effects and human effects. The EOC (Emerging Organic Contaminants) ranking system could also be used to aid water utilities in developing human health effects-based short lists of priority EOCs for monitoring and removal purposes. Regarding the carbamazepine, there is no appreciable risk to human health, in agreement with assessment results from previous studies using predicted no effect concentrations (Cunningham et al., 2010; Schwab et al., 2005) or a ranking system (Kumar and Xagoraraki, 2010a,b). However, it is important to emphasize about the inevitable uncertainties in exposure scenarios. The uncertainties in exposure scenarios concern both the method for sampling and analysis of pharmaceutical residues in water and the approaches for assessing risk due to the other sources of contamination as fish consumption. In fact, taking into account that there is no normalized analytical method for each molecule, we used a multi-residue analysis. In consequence, maximal concentrations may be overestimated or underestimated. Concerning the other sources of contamination, this manuscript takes only into account the daily water consumption but did not considered the potential effects related to consumption of fish exposure to carbamazepine and its metabolites, which is not beyond the scope of this paper. This tends to underestimate exposure because drugs are often able to pass membranes and are relatively persistent (Sanderson et al., 2004). They also may be lipophilic, which is not really the case for carbamazepine, and therefore bio-accumulate in fish. This food exposure scenario has been assessed by some authors (Schwab et al., 2005; Cunningham et al., 2010) who propose different approaches to assess risk due to fish consumption. On the basis of fish consumption of 0.0065 kg/day for children and of 0.0175 kg/day for adults, these authors suggest that carbamazepine should have no appreciable risk to human health through environmental exposures. Kumar and Xagoraraki (2010a,b) even considered accidental ingestion of water during recreational activities (around 100 ml for 2 h of activity/day). So, the exposure scenarios should take into account the cumulative amounts of these chemicals via both WIHC and fish consumption for a more relevant health hazard evaluation.

It should be emphasized that the paucity of toxicological data from the MA, which necessitated a detailed literature review for carbamazepine. For carbamazepine, where there is toxicological data, we used the TRV toxicological approach. In order to compare the three methods, we also used for carbamazepine a derivation of the TRV toxicological approach using the known MTD and the TTC approach. Concerning its metabolite, there is no toxicological data and posology, the TTC approach can be only used. However, we advise that for metabolites, another approach should be considered unless it is assumed that the latter have the same activity and strength as the original molecules (Schwab et al., 2005). For carbamazepine and its metabolite, Bourgeois and Wad (1984) suggest that antiepileptic activity and neurological toxicity of carbamazepine are proportional to the sum of the concentrations of carbamazepine

and its metabolite, 10,11-epoxycarbamazepine. Hence, 10,11-epoxycarbamazepine seems to have the similar activity as carbamazepine. Its concentration in WIHC is of 6 ng/L, i.e. approximately six times lower than that of carbamazepine (33 ng/L). Under these conditions, we may consider that such a concentration does not pose a risk to human health. Even if we push the assessment further, i.e. taking into account the possible additiveness of the two molecules, the result of their combination does not affect the previous conclusion in favour of the absence of risk. Such investigations should be considered to improve the health risk assessment.

Finally, this approach is an important aid to making decisions in individual compounds in WIHC, however, there are substantial limitations.

Some limitations of our study should be discussed. It is clear that this overall health assessment process from the prior study has not been representative of the pharmaceutical residues that are most likely to be harmful. Our results require confirmation in larger group of molecules ideally to better compare the three methodologies for assessing the risk to human health exposure. These findings represent a preliminary work and we think that further properly designed national survey related to the presence of drug residues in WIHC, are needed to reinforce this overall health assessment process principle that could vary among their frequencies or importance of their concentrations and regions (geographical distribution). One of critical point is that carbamazepine is only found in 4% of the sample analysed and its metabolite, 10,11 epoxycarbamazepine was quantified in 7.6% of overall samples. These molecules were detected at low frequencies suggesting that this approach deserves further study in this regard.

Furthermore, we do not take into account the question related to multi-exposure and interactions between pharmaceutical residues. Humans can be unintentionally exposed to trace residues of pharmaceuticals from the environment by ingesting drinking water. While this does not preclude the possibility for subtle or unmeasurable biological effects, very conservative estimates that account for synergistic, additive or antagonistic effects of drug residues mixture do not reveal the possibility of hazard; nor do the limited epidemiological studies of populations that consume drinking water. Such considerations should be taken into account showing the importance of testing the effects of mixtures of pharmaceuticals because drug residues often occur as mixtures and not as single contaminants after entering wastewaters. This mixing of substances may be resulted in overall higher concentration of drug residues. US EPA edited in 2000 a "Supplementary Guidance for conducting health risk assessment of chemical mixtures", which recommends the use of several approaches depending on the nature and quality of the available data, the type of mixture, the type of assessment being made, the known toxic effects of the mixture or of its components, the toxicological or structural similarity of mixtures or of mixture components, and the nature of the environmental exposure. Yet studies of pharmaceutical effects on human health are lacking up to now. In addition, it is therefore desirable that there is greater interest in the effects of exposure to individual or multiple pharmaceuticals that could act in additive or synergic ways through experimental studies in WIHC with perspective on whether these small amounts that might me consumed pose a health hazard.

## Acknowledgments

The authors are especially grateful to Professor Jerome R. Hoffman (Department of Emergency Medicine UCLA School of Medicine, Los Angeles, CA, USA) for his interest in revising this text. The authors are grateful to Jean François Munoz (Nancy laboratory for hydrology, National Agency for Veterinary Medicine, Anses) for providing supporting data on measured concentrations in the framework of a national sampling survey. The authors acknowledge with appreciation the technical contributions of Laurence Delva, Pascale Panetier and Gwenn Vo Van Regnault (National Agency for Veterinary Medicine, Anses).

## References

Agence Française de Sécurité Sanitaire des Produits de Santé (Afssaps), 2010. Compte rendu de la rencontre sur Médicaments et eaux: risques et enjeux sanitaires, 28 June 2010.

Agence Française de Sécurité Sanitaire des Produits de Santé (Afssaps), 2011. Répertoire des Spécialités Pharmaceutiques. Available from: <http://afssps-prd.afssaps.fr/php/ecodex/index.php>.

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), 2011. Campagne nationale d'occurrence des résidus de médicaments dans les eaux destinées à la consommation humaine. Available from: <http://pmb.santenpdc.org/opac_css/doc_num.php?explnum_id =11122>.

Amore, B.M., Kalhorn, T.F., Skiles, G.L., Hunter, A.P., Bennett, G.D., Finnell, R.H., Nelson, S.D., Slattery, J.T., 1997. Characterization of carbamazepine metabolism in a mouse model of carbamazepine teratogenicity. Drug Metab. Dispos. 25 (8), 953–962.

Argall, J.A.W., Wright, N., Mackway-Jones, K., Jackson, R., 2003. A comparison of two commonly used methods of weight estimation. Arch. Dis. Child. 88 (9), 789–790.

Artama, M., Auvinen, A., Raudaskoski, T., Isojärvi, I., Isojärki, J., 2005. Antiepileptic drug use of women with epilepsy and congenital malformations in offspring. Neurology 64 (11), 1874–1878.

Asadi-Pooya, A.A., Ghetmiri, E., 2006. Folic acid supplementation reduces the development of some blood cell abnormalities in children receiving carbamazepine. Epilepsy Behav. 8 (1), 228–231.

Beru, J., Parke, N., Fiori, J., Meyerhoff, R., 2008. Human health risk assessments for three neuropharmaceutical compounds in surface waters. Regul. Toxicol. Pharmacol. 50 (3), 420–427.

Bound, J.P., Voulvoulis, N., 2004. Pharmaceuticals in the aquatic environment: a comparaison of risk assessment strategies. Chemosphere 56 (11), 1143–1155.

Bourgeois, B.F., Wad, N., 1984. Individual and combined antiepileptic and neurotoxic activity of carbamazepine and carbamazepine-10,11-epoxide in mice. J. Pharmacol. Exp. Ther. 231, 411–415.

Bull, R., Crook, J., Whittaker, M., Cotruvo, J.A., 2011. Therapeutic dose as the point of departure in assessing potential health hazards from pharmaceutical drug residues in drinking water and recycled municipal wasterwater. Regul. Toxicol. Pharmacol. 60, 1–19.

Celik, A., 2006. The assessment of genotoxicity of carbamazepine using cytokinesis-block (CB) micronucleus assay in culture human blood lymphocytes. Drug Chem. Toxicol. 29 (2), 227–236.

Christensen, F.M., 1998. Pharmaceuticals in the environment. A human risk? Regul. Toxicol. Pharmacol. 28 (3), 212–221.

Cunningham, V., Binks, S., Olson, M., 2009. Human health risk assessment from the presence of human pharmaceuticals in the aquatic environment. Regul. Toxicol. Pharmacol. 53 (1), 39–45.

Cunningham, V., Perino, C., D'Aco, V., Hartmann, A., Bechter, R., 2010. Human health risk assessment of carbamazepine in surface waters of north America and Europe. Regul. Toxicol. Pharmacol. 356 (3), 343–351.

Daughton, C.G., Ternes, T., 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ. Health Perspect. 107 (suppl. 6), 907–938.

Diav-citrin, O., Shechtman, S., Arnon, J., Ornoy, A., 2001. Is carbamazepine teratogenic? A prospective controlled study of 210 pregnancies. Neurology 57 (2), 321–324.

EMA, 2006. Guideline on the limits of genotoxic impurities. CPMP/SWP/5199/02. EMEA/CHMP/QWP/251344/2006. Available from: <http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002903.pdf>.

EPA, 2000a. Methodology for deriving ambient water quality criteria for the protection of human health – Technical Support Document, vol. 1. Risk Assessment. EPA-822-B-00-005. Available from: <http://water.epa.gov/scitech/swguidance/standards/criteria/health/methodology/index.cfm>.

EPA, 2000b. Benchmark dose technical guidance document. External review draft, EPA/630/R-00/001. Available from: <http://www.epa.gov/ncea/pdfs/bmds/BMD-External_10_13_2000.pdf>.

EPA, Benson, B., Foureman, G., Hofmann, L., Kimmel, C., et al., 2002a. A review of the reference dose and reference concentration processes. EPA/630/P-02/002F. Available from: <http://www.epa.gov/raf/publications/review-reference-dose.htm>.

EPA, 2002b. Methodology for deriving ambient water quality criteria for the protection of human health. EPA-822-B-00-004. Available from: <http://water.epa.gov/scitech/swguidance/standards/criteria/health/methodology/index.cfm>.

FDA, 1967. NDA 16-608. Carbamazepine. Available from: <http://www.accessdata.fda.gov/drugsatfda_docs/nda/pre96/016608_Pharm_rvw2.pdf>.

Fent, K., Weston, A., Camidana, D., 2006. Ecotoxicology of human pharmaceuticals. Aquat. Toxicol. 76 (2), 122–159.

Ferrell, P.B., McLeod, H.L., 2008. Carbamazepine, HLA-B*1502 and risk of Stevens-Johnson syndrome and toxic epidermal necrolysis: US FDA recommendations. Pharmacogenomics 9 (10), 1543–1546.

Flejter, W.L., Astemborski, J.A., Hassel, T.M., Cohen, M., 1989. Cytogenetic effects of phenytoin and/or carbamazepine on human peripheral leukocytes. Epilepsia 30 (3), 374–379.

Glatt, H.R., Oesch, A., Frigerio, A., Garattini, S., 1975. Epoxides metabolically produced from some known carcinogens and from some clinically used drugs. I. Differences in mutagenicity. Int. J. Cancer 16 (5), 787–797.

Hasegawa, R. et al., 2010. Proposal of new uncertainty factor application to derive tolerable daily intake. Reg. Toxicol. Pharmacol. 58, 237–242.

Heberer, T., 2002. Occurrence, fate and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol. Lett. 131 (1–2), 5–17.

Inchem, 1999. Carbamazepine. Monography 100. Available from: <http://www.inchem.org/documents/pims/pharm/pim100.htm>.

Kolpin, D., Furlong, E., Meyer, M., Thurman, E.M., Zaugg, S., Barber, L., Buxton, H., 2002. Pharmaceuticals, hormones and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance. Environ. Sci. Technol. 36 (6), 1202–1211.

Konietzka, R., Dieter, H.H., Schneider, K., 2008. Toxicological evaluations: extrapolation beats uncertainty. J. Toxicol. Environ. Health B 11 (7), 609–611.

Königstein, M., Larisch, M., Obe, G., 1984. Mutagenicity of antieleptics drugs. I. Carbamazepine and some of its metabolites. Mutat. Res. 139 (2), 83–86.

Kroes, R., Renwick, A.G., Cheeseman, M., Kleiner, J., Mangelsdorf, I., Piersma, A., Schilter, B., van Schothorst, F., Vos, J.G., Würten, G., 2004. Structure-based threshold of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Food Chem. Toxicol. 42 (1), 65–83.

Kumar, A., Xagoraraki, I., 2010a. Human health risk assessment of pharmaceuticals in water: an uncertainty analysis for meprobamate, carbamazepine, and phenytoin. Regul. Toxicol. Pharmacol. 57 (2–3), 146–156.

Kumar, A., Xagoraraki, I., 2010b. Pharmaceuticals, personal care products and endocrine-disrupting chemicals in US surface and finished drinking waters: a proposed ranking system. Sci. Total Environ. 408, 5972–5989.

Kümmerer, K., 2009. The presence of pharmaceuticals in the environment due to human use present knowledge and future challenges. J Environ Manag 90 (8), 2354–2366.

Margaretten, N., Hincks, J., Warren, R., Coulombe, R., 1987. Effects of phenytoin and carbamazepine on human natural killer cell activity and genotoxicity in vitro. Toxicol. Appl. Pharmacol. 87 (1), 10–17.

Miao, X.S., Metcalfe, C., 2003. Determination of carbamazepine and its metabolites in aqueous samples using liquid chromatography–electrospray tandem mass spectrometry. Anal. Chem. 75 (15), 3731–3738.

Mockenhaupt, M., Messenheimer, J., Tennis, P., Schlingmann, J., 2005. Risk of Stevens-Johnson syndrome and toxic epidermal necrolysis in new users of antiepileptics. Neurology 64 (7), 1134–1138.

Mompelat, S., Le Bot, B., Thomas, O., 2009. Occurrence and fate of pharmaceutical products and by-products, from resource to drinking water. Environ. Int. 35 (5), 803–814.

Pomati, F., Orlandi, C., Clerici, M., Luciani, F., Zuccato, E., 2008. Effects and interactions in an environmentally relevant mixture of pharmaceuticals. Toxicol. Sci. 102 (1), 129–137.

Rabiet, M., Togola, A., Brissaud, F., Seidel, J.L., Budzinski, H., Elbaz-Poulichet, F., 2006. Consequences of treated water recycling as regards pharmaceuticals and drugs in surface and ground waters of a medium-sized Mediterranean catchment. Enviro Sci Technol 40 (17), 5282–5288.

Reynolds, J.E.F., 1996. The Extra Pharmacopoeia. Martindale, 31st ed.

Samrén, E.B., Van Duijn, C., Lieve Christiaens, G., Hofman, A., Lindhout, D., 1999. Antiepileptic drug regimens and major congenital abnormalities in the offsprings. Ann. Neurol. 46 (5), 739–746.

Samrrén, E.B., Van Duijn, C.M., Koch, S., Hiilesmaa, V.K., Klepel, H., Bardy, A.H., Beck Mannagetta, G., Deichl, A.W., Gaily, E., Granström, M.L., Meinardi, H., Grobbee, D.E., Hofman, A., Janz, D., Lindhout, D., 1997. Maternal use of antiepileptic drugs and the risk of major congenital malformations: a joint European prospective study of human teratogenesis associated with maternal epilepsy. Epilepsia 38 (9), 981–990.

Sanderson, H., Johnson, D.J., Reitsma, T., Brain, R.A., Wilson, C.J., Soloman, K.R., 2004. Ranking and prioritization of environmental risks of pharmaceuticals in surface waters. Regul. Toxicol. Pharmacol. 39, 158–183.

Schaumann, B., Satish, J., Johnson, S.B., Moore, K., Cervenka, J., 1985. Effects of carbamazepine on human chromosomes. Epilepsia 26 (4), 346–352.

Schriks, M., Heringa, M., Van der Kooi, M., de Voogt, P., van Wezel, A., 2010. Toxicological relevance of emerging contaminants for drinking water quality. Water Res. 44 (2), 461–476.

Schulman, L., Sargent, E., Naumann, B., Faria, E., Dolan, D., Wargo, J., 2002. A human health risk assessment of pharmaceuticals in the aquatic environment. Hum Ecol Risk Assess 8 (4), 657–680.

Schwab, B.W., Hayes, E.P., Fiori, J.M., Mastrocco, F.J., Roden, N.M., Cragin, D., Meyerhoff, R.D., D'Aco, V.J., Anderson, P.D., 2005. Human pharmaceuticals in US surface waters: a human health risk assessment. Regul. Toxicol. Pharmacol. 42 (3), 296–312.

Ternes, T., 1998. Occurrence of drugs in German sewage treatment plants and rivers. Water Res. 32 (11), 3245–3260.

Ternes, T., 2007. The occurrence of micropollutants in the aquatic environment: a new challenge for water management. Water Sci. Technol. 55 (12), 327–332.

United States Pharmacopeia, 2008. Material Safety Data Sheet Carbamazepine. Catalog number: 1093001. Available from: <http://www.usp.org/products/MSDS/>.

Vorhees, C., Acuff, K., Weisenburger, W., Minck, D., 1990. Teratogenicity of carbamazepine in rats. Teratology 41 (3), 311–317.

WHO (World Health Organization), 2004. Guidelines for drinking water quality, vol. 1, third ed. ISBN: 92 4 154638 7.

Zuccato, E., Calamari, D., Natangelo, M., Fanelli, R., 2000. Presence of therapeutic drugs in the environment. The Lancet 355, 1789–1790.