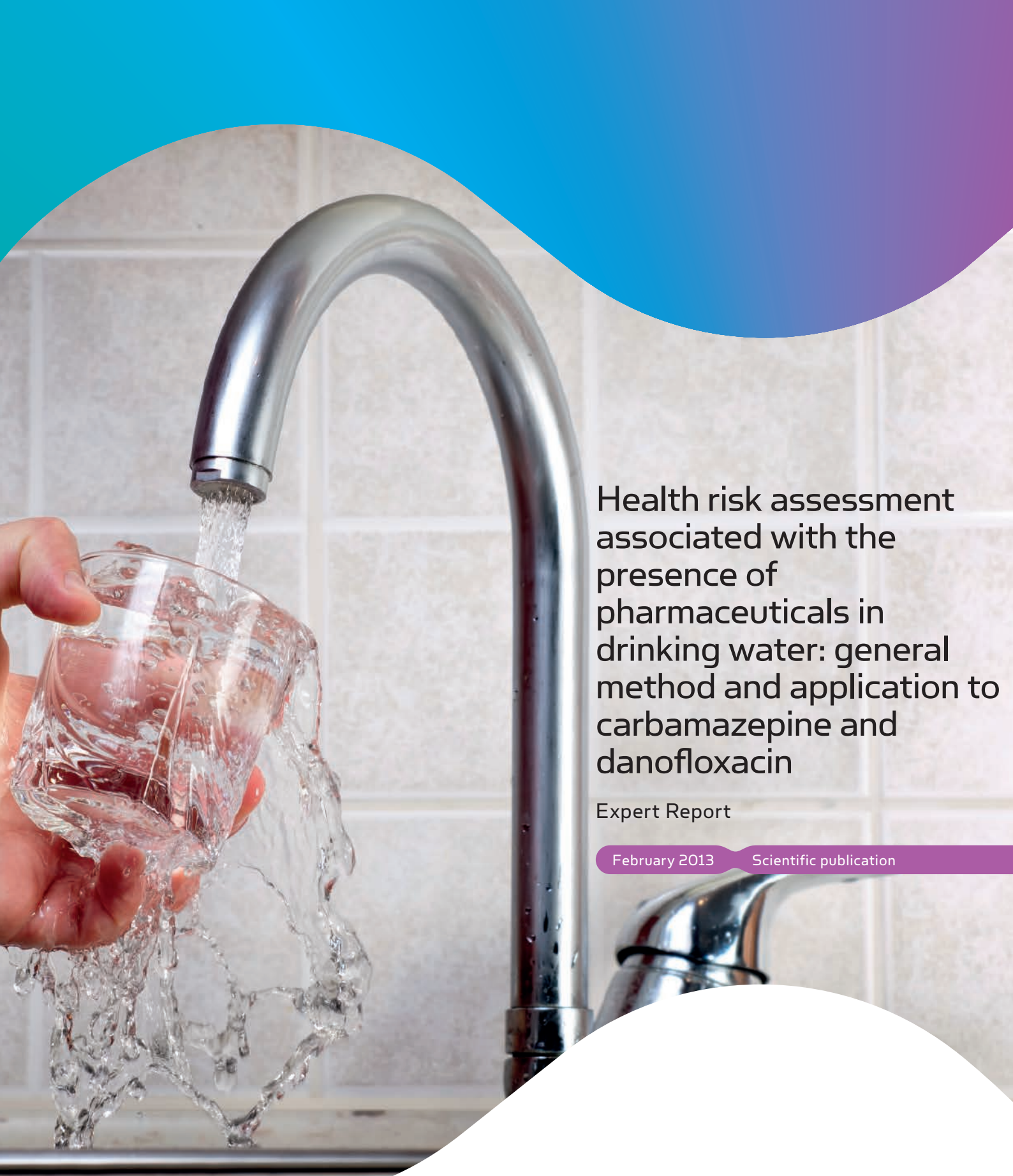

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Expert Report

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Request No 2009-SA-0210 – Pharmaceuticals and drinking water

Collective expert

REPORT

Expert Committee on Water

Working Group on Pharmaceuticals in drinking water: general health risk assessment methodology applied to the example of carbamazepine - 2

February 2013

French Agency for Food, Environmental and Occupational Health & Safety,
27-31 av. du Général Leclerc, 94701 Maisons-Alfort Cedex
Telephone: + 33 (0)1 49 77 13 50 - Fax: + 33 (0)1 49 77 26 26 - www.anses.fr

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Presentation of participants

Preamble: The external experts, members of the expert committees or working groups, or designated rapporteurs, have all been appointed in a personal capacity, *intuitu personae,* and do not represent their respective parent organisations.

WORKING GROUP (WG)

Chair

Mrs Claude CASELLAS - Professor, University of Montpellier I, Faculty of Pharmacy - Micropollutants in water

Members

Mr Jean CARRÉ - Professor, EHESP - Water flows and pollutant transfer, Hydrogeology

Mr Serge CHIRON - Research Director, Hydrosciences Montpellier - Environmental chemical reactivity

Mr Michel JOYEUX - Director of Research, Development and Water Quality, Eau de Paris - Environmental toxicology, HRA

Mr Yves LÉVI - Professor, Paris Sud University, Faculty of Pharmacy - Drinking water, Public health, Micropollutants

Mr Patrick MAZELLIER - Professor, University of Bordeaux, Périgueux University Technology Institute (IUT) - Drinking water treatment

Mr Antoine MONTIEL - Retired - Drinking water treatment

Mr Christophe ROSIN - Deputy Head of Unit, LHN, ANSES - Analytical methods

EXPERT COMMITTEE (CES)

The work covered by this report was monitored and adopted by the following CES:

- CES on WATER

Chair

Mr Yves LÉVI - Professor, Paris Sud University, Faculty of Pharmacy - Drinking water, Public health, Micropollutants

Members

Mr Yves ANDRÈS - Professor, École des Mines de Nantes - Reuse of greywater, Water microbiology, Process engineering

Mr Jean-Luc BOUDENNE - Lecturer, University of Provence - Water chemistry and metrology

Mrs Corinne CABASSUD - Professor, INSA Toulouse - Water treatment methods, including membranes

Mr Jean CARRÉ - Professor, EHESP - Water flows and pollutant transfer, Hydrogeology

Ms Catherine CHUBILLEAU - Hospital Doctor, Niort Hospital Centre - Epidemiology, Reuse of treated wastewater, HRA

Mr Olivier CORREC - Study and Research Engineer, CSTB - Water chemistry, Modelling of internal systems, Corrosion

Mr Christophe DAGOT - Professor, University of Limoges, ENSIL - Process engineering, Water reuse, Water microbiology and chemistry

Ms Sylvie DUBROU - Director, LHVP - Hygiene and Microbiology of water

Mr Alain HÉDUIT – Research Director, IRSTEA - Purification process engineering

Mr Jean-François HUMBERT - Research Director, INRA - Cyanobacteria

Mr Michel JOYEUX - Director of Research, Development and Water Quality, Eau de Paris - Environmental toxicology, HRA

Ms Colette LE BÂCLE - Medical Advisor in Occupational Health, INRS - Occupational medicine

Mr Pierre LE CANN - Research Professor, EHESP - Water microbiology

Mrs Laurence MATHIEU - Lecturer, École pratique des hautes études - Microbiology, Water, Aerosols, Exposure to biological contaminants

Mr Patrick MAZELLIER - Professor, University of Bordeaux, Périgueux University Technology Institute (IUT) - Drinking water treatment

Mr Jacques MUDRY - Professor, University of Franche-Comté - Hydrogeology

Mr Maxime PONTIÉ - Professor, University of Angers - Process engineering

Mrs Anne-Marie POURCHER - Research Director, IRSTEA - Management and treatment of livestock manure

Mr Robert TARDIF - Professor in the Department of Environmental and Occupational Health, University of Montreal - Toxicology

Mrs Michèle TREMBLAY - Medical Officer in Occupational Health and Infectious Diseases, Montréal Public Health Department - Occupational medicine, Occupational risks

Mrs Bénédicte WELTÉ - Deputy Director of Research, Development and Water Quality, Eau de Paris - Treatment of water intended for human consumption

ANSES PARTICIPANTS

Scientific coordination

Mrs Morgane BACHELOT - Water-related risk assessment unit - Risk Assessment Department - ANSES

Ms Gwenn VO VAN-REGNAULT - Water-related risk assessment unit - Risk Assessment Department - ANSES

Scientific contribution

Mr Thierry GODARD - MA Department – Chemical veterinary medicinal product assessment unit - ANMV-ANSES

Mrs Pascale PANETIER - Head of the Water-related risk assessment unit - Risk Assessment Department - ANSES

Administrative secretariat

Mrs Odile BENDER - Risk Assessment Department - ANSES

ANSM PARTICIPANTS

Scientific contribution

Mr Paul HOUETO - Assessment Department - Non-clinical and clinical toxicology unit - ANSM

Mr Dominique MASSET - Assessment Department - Head of the Non-clinical and clinical toxicology unit - ANSM

Contents

Acronyms and Abbreviations

ADI: Acceptable daily intake
AFSSA: French Food Safety Agency (until 01/07/2010)
AFSSAPS: French Health Products Safety Agency (until 30/04/2012)
ANMV: French Agency for Veterinary Medicinal Products (part of ANSES)
ANSES: French Agency for Food, Environmental and Occupational Health & Safety (resulting from the merger between AFSSA and AFSSET on 01/07/2010)
ANSM: French National Agency for Medicines and Health Products Safety (AFSSAPS until 30/04/2012)
ARS: Regional Health Agency
BMD: Benchmark dose
BRGM: French Geological Survey
CES: Expert Committee
DGS: French Directorate General for Health
EMA: European Medicines Agency (formerly EMEA)
FAO: Food and Agriculture Organization of the United Nations
FDA: Food and Drug Administration (USA)
GV: Guideline value
- GV_{tox}: Guideline value derived from toxicological studies
- $GV_{posology}$: Guideline value derived from the minimum daily posology
- GV_{TTC}: Guideline value derived from the TTC approach

HRA: Health risk assessment
IRSTEA: National Research Institute of Science and Technology for Environment and Agriculture (formerly Cemagref)
JECFA: Joint FAO/WHO Expert Committee on Food Additives
LD_{50}: Median lethal dose
LHN: Nancy Laboratory for Hydrology (ANSES)
LOAEL: Lowest observed adverse effect level
LOD: Limit of detection
LOQ: Limit of quantification
MA: Marketing Authorisation
MIC: Minimum inhibitory concentration
MRL: Maximum residue limit
NOAEL: No observed adverse effect level
NTP: National Toxicology Program (USA)
PC: Predicted concentration
PEC: Predicted environmental concentration
SF: Slope factor
SPE: Solid-phase extraction
TDI: Tolerable daily intake
TRV: Toxicity Reference Value
- TRV_{tox}: Toxicity reference value derived from toxicological studies
- $TRV_{posology}$: Toxicity reference value derived from the minimum daily posology
- TRV_{TTC}: Toxicity reference value derived from the TTC approach

TTC: Threshold of Toxicological Concern
UF: Uncertainty factor
WG: Working group
WHO: World Health Organization
WWTP: Wastewater treatment plant

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1 Context, purpose and procedure for responding to the request

1.1 Context

More than 3000 active substances for human use and 300 for veterinary use are currently available on the French market. These compounds have been selected, produced and used for their biological effects and are characterised by a great diversity of activities and chemical structures.

Residues of human and veterinary medicines are introduced into the environment from various sources. For example, drugs used in therapy or diagnosis are mainly excreted in faeces and urine, in their original form or as one or more metabolites.

Concentration levels in the receiving environments vary depending on the physico-chemical characteristics of the compounds that affect their chemical stability and biodegradability, on the routes by which they are introduced and on the types and performance of the treatment plants, mainly with regard to human medicines. The first recorded traces of pharmaceuticals in the environment date back to the 1980s. Since then, many studies have shown their presence at concentrations ranging from nanograms to micrograms per litre in surface water or groundwater. Some of these resources are used to produce drinking water and, depending on the efficacy of the treatments in place, residues have sometimes been identified in the water supply (ANSES, 2011; Stackelberg *et al.*, 2007; Ternes, 2001; Togola and Budzinski, 2008).

Current European and French regulations on water quality do not require screening for pharmaceuticals in drinking water. Nevertheless measurement campaigns have been conducted by different organisations, both international and French (the French health protection agency ANSES (formerly AFSSA), the Water Agencies, the BRGM, the Irstea, the ARSs, university laboratories, water distribution and sanitation management unions, etc.).

1.2 Purpose of the request

The French Directorate General of Health (DGS) made a formal joint request to ANSES and the French National Agency for Medicines and Health Products Safety (ANSM, formerly AFSSAPS) for an opinion on the assessment of the health risks associated with the presence of pharmaceuticals in drinking water. This required defining a general methodological approach for the health risks assessment (HRA) associated with the presence of pharmaceuticals in drinking water and testing its application on several compounds including carbamazepine. Indeed, existing studies show that this compound is the most frequently encountered, especially in drinking water. Apart from carbamazepine, which is a human medicine, it was agreed to extend the risk assessment to a veterinary medicine also quantified in drinking water: danofloxacin.

ANSES initially responded with a report published in June 2010 that addressed exposure *via* drinking water to human and veterinary drugs (AFSSA, 2010). This present report describes a general method for assessing the health risks associated with the presence of pharmaceuticals in drinking water based on the report published in 2010 and updated by the working group. The general HRA approach was then applied to carbamazepine and danofloxacin, which had both been quantified during the national analysis campaign conducted by ANSES's Nancy Laboratory for Hydrology (LHN) (ANSES, 2011).

1.3 Procedure for responding: measures deployed and organisation

The expert appraisal was carried out in accordance with French standard NF X 50-110 “Quality in Expertise – General requirements of Competence for Expertise activities (May 2003)”.

ANSES entrusted the examination of this request to the Working Group (WG) “pharmaceuticals in drinking water: general health risk assessment methodology applied to the example of carbamazepine – 2”, which reported to the Expert Committee (CES) on Water. A representative of ANSES’s French Agency for Veterinary Medicinal Products (ANMV) and a representative of the ANSM also took part in the WG’s work.

The methodological and scientific aspects of the work were regularly submitted by the WG to the CES. The report produced by the WG takes account of observations and additional information supplied by the members of the CES.

This work was therefore conducted by a group of experts with complementary skills.

2 General method for assessing the health risks associated with the presence of pharmaceuticals in drinking water

The general method for assessing the health risks associated with the presence of pharmaceuticals in drinking water adopted by the WG and the CES involves calculating guideline values (GVs) that are compared to concentrations measured in drinking water. This method is based on the one proposed by the World Health Organization (WHO, 2011) and on the approach for health, risk assessment of non-compliance with water intended for human consumption parametric values, which was published by the Agency in 2007 (AFSSA, 2007). Applying these methods to pharmaceuticals in drinking water is limited by the difficulty in obtaining data from Marketing Authorisation (MA) dossiers and by the absence of chronic toxicity studies published in the scientific literature concerning applicable toxicity reference values (TRVs).

The main steps of this method are as follows:

Module A: Characteristics of the compound

Module B: Identification of relevant metabolites formed in humans or animals

Module C: Identification of relevant transformation products formed in the environment and in water purification systems

Module D: Assessment of human exposure *via* drinking water

Module E: Biological effects

Module F: Determination of toxicity reference values (TRVs)

Module G: Determination of a guideline value

Module H: Risk assessment

Modules A to D of the method, initially published in the report *"Drug residues in water intended for human consumption: Part on General Methodology for assessing human exposure to pharmaceuticals via drinking water"* (AFSSA, 2010), are included in this report with some updates.

The conventional approach varies depending on whether or not there is a threshold of appearance of a biological effect induced by the compound considered.

- The effect is said to be "deterministic" if it is possible to define a threshold dose below which no biological effect is observed. Above this threshold, the intensity of the effect increases as a function of the dose administered.
- If it is not possible to define a no-effect threshold, the effect is then called "probabilistic or stochastic", which is the case with genotoxic carcinogens.

For most pharmaceuticals, the predominant route of exposure to be considered is ingestion. In specific cases where toxicity results from exposure by another route (dermal, inhalation), this should be mentioned and included in the HRA approach.

2.1 Module A: Characteristics of the compound

The characteristics of the compound must be known precisely before the HRA can even be undertaken, specifically by gathering the following informations:

- The compound's International Nonproprietary Name (INN) and CAS number.
- Its use(s) in France in human and/or veterinary medicine, so as to identify the source of information to be given priority: ANSM and/or ANMV (Annex 2).
- Possible sources other than medical uses that might explain its presence in drinking water:
  - Are there any natural source (e.g. hormones)?
  - Is the compound also used for other purposes (e.g. biocide)?
  - Could the compound be the metabolite or transformation product of another drug (e.g. oxazepam is used as a medicine but is also the metabolite of other benzodiazepines)?

  This information is especially important for determining the share of exposure resulting from medical use. Measuring the concentration of exposure via drinking water makes it possible to estimate the exposure of the subjects involved, whether or not the source is related to medical uses. Calculating a predictable concentration from amounts used in medicine does not take these other sources into account and in this case, predictive exposure of humans *via* drinking water may therefore be underestimated.
- The other routes of human exposure: food (excluding drinking water) may lead to exposure to low concentrations of drug and pesticide residues. This is the case for example with veterinary drugs intended for animal species used to produce food intended for human consumption, for which maximum residue limits (MRLs) in animal tissues have been defined. In addition, some compounds for veterinary use are also authorised as biocides (insecticides and/or pesticides), with MRLs being set in plants.
- The compound's physico-chemical properties and behaviour in the environment are key elements, used to assess the fate of pharmaceuticals in water (Annex II), in particular:
  - the molecular structure that can be used to predict certain degradation pathways, the volatility described by the vapour pressure at 20°C and the Henry constant;
  - the compound's mobility, described by its water solubility, the ionisation potential (pKa), log D or Dow (water-soluble form at pH 7) and the octanol/water partition coefficient (Kow) characterising its hydrophilic/hydrophobic nature;
  - the adsorption onto organic matter, particularly in soil, water or activated sludge from wastewater treatment plants, expressed by the Koc value;
  - the adsorption onto soil, expressed by the Kd: related to the soil's characteristics (texture), the presence or absence of clay, the particle size of the constituents;
  - the formation of complexes with divalent cations (calcium, magnesium) or the transition elements present in the environment (iron, manganese, etc.);
  - the half-lives for abiotic degradation (hydrolysis, photolysis) and biodegradation;
  - the photosensitivity.

2.2 Module B: Identification of relevant metabolites formed in humans or animals

Some drugs are not metabolised in the body and are eliminated in their original form, but most are transformed into several conjugated or non-conjugated metabolites. Metabolites may be inactive or have a certain activity, and there are therefore cases in which the metabolite contributes to the drug's activity. In humans and animals, the metabolic pathways are generally well known and documented in the scientific literature.

Pharmacokinetics identifies the ways in which the compound is eliminated from the body before being discharged into water: parent compound and/or metabolites. To determine the existence of relevant metabolites, it is important to know: (i) the percentage of excretion of the parent compound and major metabolites (especially conjugated metabolites that are likely to return to the parent compound when reactivated in the environment), (ii) the primary analysis of the biological effects of the metabolites.

As an example, in the case of pesticides, the rules adopted at European level define as relevant metabolites those accounting for more than 5% of the parent compound and that are likely to induce the same biological activity as the parent compound or to have toxicological properties that are considered severe (European Commission, 2003).

For the relevant metabolites identified, a health risk assessment should also be conducted.

It should be noted that when these metabolites are released into the water by humans or animals, they may, just like the parent compound, undergo degradation processes generating transformation products (Module C).

2.3 Module C: Identification of relevant transformation products formed in the environment and in water treatment plants

In the environment, in wastewater treatment plants (WWTP) or in drinking water treatment plants, biotic (aerobic or anaerobic) or abiotic (hydrolysis, photolysis, etc.) degradation processes, and interactions with treatment products and processes (chlorination, ozonation, etc.) can generate transformation products. The chemical structure of the compound may give some indications of this, but studies may be needed to characterise the structures and effects of any transformation products potentially formed.

Unlike metabolism, environmental transformations usually lead to the formation of a large number of by-products that will be different depending on the nature of the degradation processes studied: abiotic or biotic natural degradation or degradation during water disinfection processes such as chlorination or ozonation. Under these conditions, the studies used to identify the structures of the transformation products are complex and there are currently very few available data in the scientific literature on the environmental transformation pathways of drugs.

If necessary, in the field of medicinal products, a health risk assessment should also be conducted for the relevant transformation products identified.

2.4 Module D: Assessment of human exposure *via* drinking water

The routes by which human and veterinary pharmaceuticals are introduced into drinking water are shown in Figure 2-1 of Appendix 2.

In order to assess human exposure, it is important to know the concentrations (maximum and median) of the compound in drinking water. Failing this, and with sufficient caution, an estimate can be used for a semi-quantitative assessment of the concentration in drinking water.

2.4.1 Use of the available data

There are a limited number of studies investigating the presence of pharmaceuticals in drinking water and some are presented in Appendix 4. If they are to be used, it is important to take the following into account:

- the analytical methods used should be validated for the matrices studied;
- the results of international studies cannot easily be transposed to the case of France. This is because the use of medicinal products (compounds and quantities used), as well as the water treatment processes, may vary from country to country;
- a significant amount of data must be used in order for the results to be interpreted with objectivity and rigour;

- the results can only be regarded as representative of French drinking water distribution units if the sampling plan was developed with this objective in mind.

2.4.2 Theoretical calculation of the expected concentration in drinking water (semi-quantitative data)

In 2010, the working group had proposed using a theoretical calculation of the predicted concentration in drinking water considering the two cases presented below. Since then, this approach has been tested and the results are shown in Section 2.4.2.3.

Assays should preferably be conducted on representative samples using a suitable sampling strategy. In the absence of data, modelling provides theoretical semi-quantitative information on the concentration in drinking water.

2.4.2.1 Case 1: known concentration of the compound in water used as a resource for the production of drinking water

The processes that may influence the fate and behaviour of certain pharmaceuticals in water resources are detailed in Appendix 3.

- If there is robust information available for estimating the fate and behaviour of the compound in the studied treatment system or in several categories of systems considered to be standard models, a theoretical reduction should be calculated that can then be verified by analysis.
- Otherwise, a worst-case scenario should be used, i.e. it should be considered that the drinking water has the same concentration as the resource.

2.4.2.2 Case 2: unknown concentration in the resource

If there are no available data on contamination of the resource, a predicted environmental concentration (PEC) can be calculated. This calculation is required in Marketing Authorisation (MA) dossiers by Directives 92/18/EEC for veterinary medicines (with some exceptions) and 2001/83/EC for human medicines. If it is not provided in the MA dossier, or if it needs to be adjusted, it can be calculated according to the EMA guidelines defined for human (EMEA, 2006) and veterinary (EMEA, 2008) medicines.

As with Case 1, the compound's fate in the drinking water treatment plant can be taken into account.

2.4.2.3 Feedback on the theoretical calculation of the predicted concentration

In order to assess the reliability of the theoretical calculation of the predicted concentration in drinking water ($PC_{drinking\ water}$), these calculations were performed for the compounds screened for during the national analysis campaign for pharmaceuticals in drinking water conducted by the Nancy Laboratory for Hydrology (ANSES, 2011).

For human medicines, the $PC_{drinking\ water}$ was estimated using a formula derived from the calculation formula defined by the EMEA (2006) on the basis of calculations from the European Commission's (2003) technical guide.

Due to the number of different cases to be taken into account for a veterinary drug or its metabolite (animal species treated, type of agriculture, route of administration), the guidelines do not specify a single calculation formula. A formula for calculating the $PC_{drinking\ water}$ was therefore extrapolated from those used in the MA dossiers.

The calculated $PC_{drinking\ water}$ values were compared with the maximum concentrations measured in drinking water for the quantified compounds, with limits of quantification for compounds detected but not quantified, or with limits of detection for compounds that were not detected. The findings were as follows:

- The formula is not reliable:

  - Compounds for which the formula appears to give a satisfactory result were mainly undetected compounds.
  - The differences between measured and calculated concentrations were greater for heavily consumed compounds.
  - Generally, the formula overestimated the expected concentrations.
- Some parameters are not available for all compounds, or the existing values vary greatly depending on the source (e.g. the Kd).
- Some parameters are dependent on the local situation (e.g. the fraction removed during water treatment) and set a punitive value by default that leads to the $PC_{drinking\ water}$ being overestimated.

The tested formulas are too general and do not provide a reliable estimate of concentrations of pharmaceuticals in drinking water at the national level. **Assays performed in drinking water should therefore be preferred to estimates.**

2.4.3 Obtaining quantitative data by measuring the concentration in drinking water

If the predicted concentrations show a likelihood of residues deemed significant being present, and/or if the compound is considered to be biologically active at very low doses, a quantitative assessment should be conducted using measurements taken on site.

To measure the concentration of the compound in drinking water (Appendix 5), the following are needed:

- extraction and analytical methods described and validated according to the applicable standards and with acceptable levels of uncertainties (maximum 50% of intra-laboratory uncertainties - Directive 2009/90/EC laying down technical specifications for chemical analysis and monitoring of water status);
- standard solutions certified for the studied compound (parent compounds, relevant metabolites or transformation products);
- limits of detection and quantification that are relevant for the expected concentrations;
- laboratories that are competent for screening substances in trace amounts in the water matrix (e.g. possessing approvals for water quality monitoring, accreditation, etc.) and that participate in inter-laboratory tests in order to determine the variability in the measurements and ensure comparability of results.

An analysis campaign can only be implemented on the basis of an appropriate sampling plan with regard to the objective sought (e.g. representative samples from French drinking water distribution units, a description of the environment of sampling sites, etc.), enabling the rigorous processing of the data generated, and statistical analyses in particular. Sometimes the small number of analyses conducted due to a limited budget does not allow any satisfactory conclusions to be drawn.

Several chronic exposure scenarios can be developed for each population:

- worst-case scenario considering the maximum measured or predicted concentration,
- scenario based on the median concentration.

This approach is applied to the studied compound and, if applicable, to its identified relevant transformation products or metabolites (Module B and C).

These first four modules, illustrated in Figure 1, indicate the possibility of assessing human exposure to a parent compound, metabolite or transformation product. For the latter two, there are currently very few available data on drinking water.

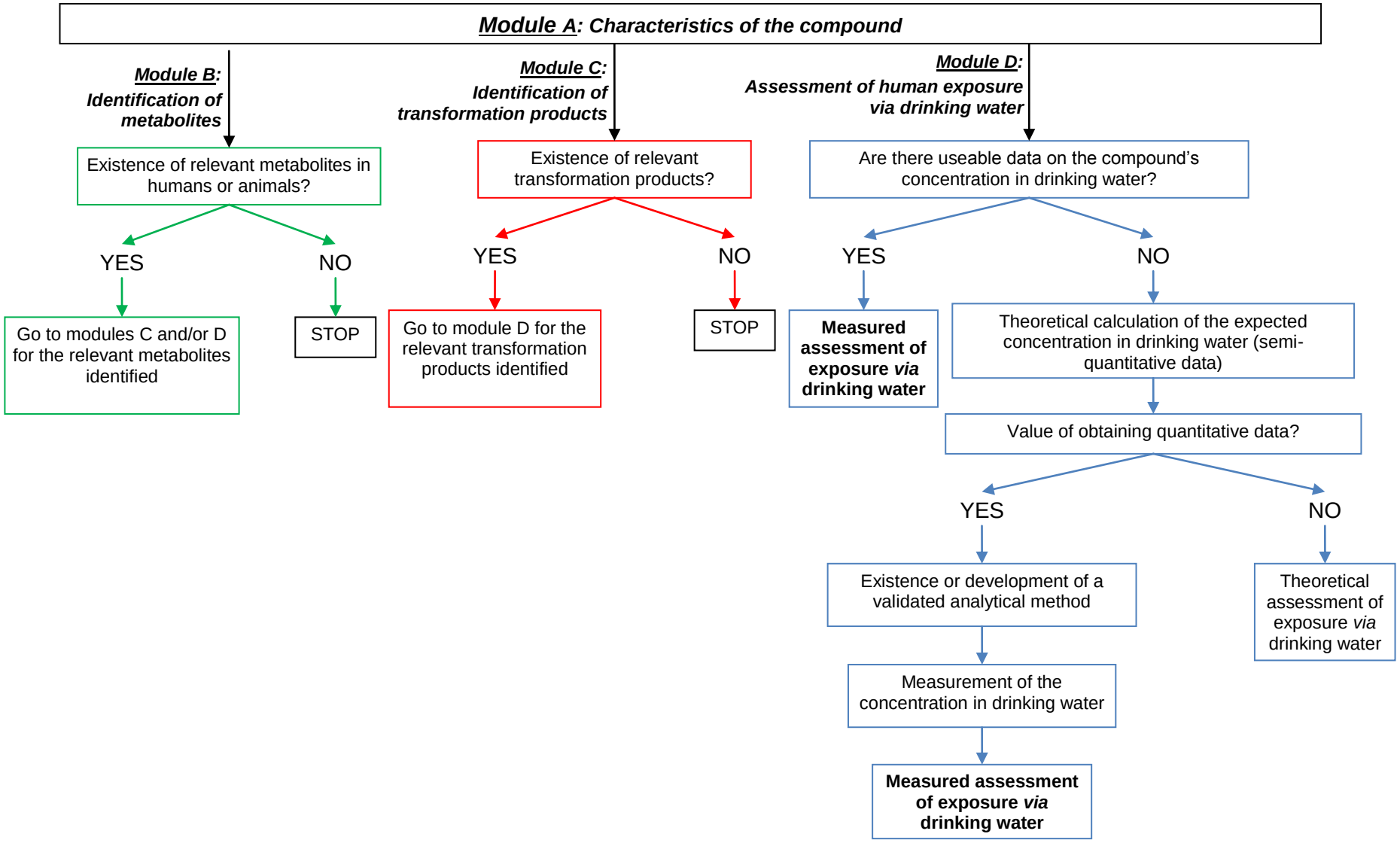


Figure 1. The four modules leading to the assessment of human exposure to pharmaceuticals (parent compound, metabolites or transformation products) *via* drinking water

2.5 Module E: Biological effects

The biological effects should be documented for the parent compound and the relevant metabolites and transformation products identified during the implementation of modules B and C.

Drugs are compounds whose biological effects have been studied in depth. Before they can be placed on the market, their therapeutic and undesirable effects are identified and described in the MA dossier. In the course of their use, pharmacovigilance can discover effects that were not previously identified. Although they are not always available, there are many sources of data on the health effects of drugs, including:

- MA dossiers and drug monitoring agency databases (ANSM, ANMV, EMA, etc.);
- documents from health and safety agencies (WHO, US-EPA, RIVM, Health Canada, etc.);
- the scientific literature.

For the HRA, the study of a contaminant's biological effects should typically enable the acute, sub-chronic and chronic toxicities to be described, and should define the type of toxicity (i.e. with or without a threshold). In addition to the data traditionally identified, for a drug's active substances and metabolites, if relevant, the study of the biological effects must also indicate the modes of action related to the therapeutic use and the associated posologies.

If several compounds have identical effects and mechanisms of action indicating an additive effect, this must be taken into account in the HRA (US EPA, 2007a). If there is any doubt, the expert appraisal must decide on a case-by-case basis whether or not the additive nature of the effects should be taken into consideration.

2.6 Module F: Determination of toxicity reference values

2.6.1 Process for establishing a toxicity reference value

A compound's toxicity reference value (TRV) is usually specific to a determined critical effect, route of administration and duration of exposure.

2.6.1.1 Threshold toxic effects

In the event of oral exposure, the TRV is defined as the estimated amount of the substance to which an individual can theoretically be exposed for a specified period without the occurrence of any adverse health effects.

The TRV is expressed in terms of the weight of compound per kilogram of body weight per day (mg/kg/d).

This TRV is obtained from a critical dose, which can be a maximum (no observed adverse effect level, NOAEL), a minimum (lowest observed adverse effect level, LOAEL) or a benchmark dose (BMD), which is then divided by uncertainty factors (UF) to obtain an acceptable level of safety for humans.

$$\text{TRV} = \frac{\text{Critical dose}}{\text{UF}}$$

This TRV is not a toxicity threshold but a level of exposure deemed acceptable because it does not lead to the manifestation of any adverse effect.

For the WHO (2011), setting values for the uncertainty factors requires expert judgment and careful consideration of the available scientific evidence. These factors should take into account, among other things, variability between species and between individuals and uncertainties related to experimental protocols (Table I).

Table I. Examples of uncertainty factors in calculating guideline values (WHO, 2011).

Type of uncertainty	Uncertainty factor
Interspecies variability (extrapolation to humans of data obtained in animals)	1 to 10
Intraspecies variability (individual variability between humans)	1 to 10
Relevance of studies	1 to 10
Nature and severity of the effect	1 to 10

For the WHO and EPA, when the uncertainty factor is greater than 1000, the GVs are regarded as "provisional" to emphasise the high level of uncertainty inherent in these values.

2.6.1.2 Non-threshold toxic effects

Toxic effects without a threshold correspond mainly to genotoxic, mutagenic and/or carcinogenic effects. This indicates an excess risk, i.e. an increase in the likelihood of the occurrence of the effect compared to a situation in which there was no exposure to the substance.

The TRV for these substances can be defined as the dose corresponding to the additional probability, compared to an unexposed subject, that an individual will develop cancer if exposed for his/her entire life to a unit dose of the carcinogen. The index primarily used is the slope factor (SF) expressed in $(mg/kg/d)^{-1}$.

2.6.2 Application of the approach to pharmaceuticals

Although the biological effects of drug active substances have been studied in detail, the data needed for following the traditional HRA approach do not always exist or may be unavailable, in particular for determining the TRV. As a result, the method offers several alternatives depending on the data available. Whenever possible, Cases 1 or 2 should be preferred because they are based on toxicological data specific to the compound.

In the absence of these data, it is nevertheless possible to approximate the TRVs **with caution** based on the minimum daily posology, which should be combined with specific UFs (Case 3).

Lastly, a probabilistic approach established from toxicological databases (threshold of toxicological concern) can be used (Case 4).

2.6.2.1 Case 1 – Use of a TRV that has been validated by national or international organisations

For some active substances, TRVs have been validated in the MA dossier, by health and safety organisations or published in the scientific literature. If robust TRVs corresponding to the route and duration of exposure identified in the previous steps do exist, they can be used for calculating the GV.

The criteria for selecting the values to be used are mainly the origin of the data (animal or human) and their availability, the uncertainty factors applied when establishing them, the consistency of the route of exposure and the duration of experimentation compared to what is being studied, and the reputation of the organisation that developed them (US EPA, WHO, etc.).

For certain veterinary medicines

In accordance with the regulatory provisions in force (Article 6 of Directive 2001/82/EC as amended, transposed by Article L.5141-5-2 of the French Public Health Code), compounds contained in veterinary medicines intended for animal species producing food intended for human consumption shall be subject to the determination, at Community level, of a maximum residue limit (MRL). This is the maximum level of residues (of active substance, excipient, metabolite) resulting from the use of a veterinary medicines, legally authorised in or on foodstuffs intended for human consumption.

For each compound in question, the determination of an MRL is subject to an assessment by the European Medicines Agency (EMA), whose findings are made public. Establishing an MRL is subject to a decision of the European Commission.

For the active substances concerned, the dossier on the MRLs describes how the acceptable daily intake (ADI) is developed. This ADI is an estimate of the amount of active substance and/or its metabolites, expressed in µg/kg of body weight, that can be ingested daily over a lifetime without appreciable risk to humans. It is the same for adults and children.

If this ADI meets the criteria defined in the previous paragraph, it can serve as a basis for the HRA.

2.6.2.2 Case 2 – Use of toxicology studies from the MA dossier or the scientific literature

Where there are no existing validated TRVs, data from toxicological studies published in peer-reviewed journals or accessible pharmacotoxicological studies from the MA dossier will be used. The selection of the reference (or pivotal) study must take into account its robustness (sample size, number of doses tested, one or more species studied, etc.) and the accessibility of the procedure and data. At the selected dose, UFs are applied to obtain the TRV_{tox}.

2.6.2.3 Case 3 – Use of the minimum daily posology

The minimum daily posology is the lowest dose that can be used therapeutically in one day. It is expressed in mg/kg/d.

In the absence of a TRV and a robust or accessible toxicological study, the minimum daily posology for humans can be used as a critical dose, as suggested by some authors (Australian guidelines for water recycling, 2008; Bull *et al.*, 2011; DWI, 2007). This approach should not be used for carcinogenic or mutagenic compounds such as cytotoxic drugs.

At the minimum posology used as a critical dose, UFs are applied and, given the use of the minimum dose instead of a LOAEL, an additional UF is added to obtain the $TRV_{posology}$.

2.6.2.4 Case 4 – Threshold of toxicological concern (TTC)

For substances found at low concentrations and for which specific toxicological data prove inadequate or inaccessible for conducting a conventional toxicological assessment, the threshold of toxicological concern (TTC) approach can be used to propose a level of exposure below which the compound of interest poses only a negligible risk to human health. The TTC is based on a probabilistic approach and the conditions for determining it differ depending on whether the compounds studied have a deterministic or stochastic effect (AFSSA, 2005; Kroes *et al.*, 2000; Munro *et al.*, 2008). This approach has limitations for certain toxicological effects (allergy, reproductive toxicity, immunotoxicity, etc.).

However, in the absence of data enabling one of the above three cases to be used, the lowest TTC threshold, determined for mutagenic/carcinogenic substances, will be applied, namely TRV_{TTC} = **0.15 µg/person/day**.

2.7 Module G: Determination of a guideline value

The WHO defines the guideline value (GV) as an estimate of the concentration of a compound in drinking water that presents no risk to the health of a person consuming this water for 70 years. Conventionally, the starting point for setting a GV is the compound's TRV.

2.7.1 Threshold toxic effects

For compounds with threshold toxic effects, the GV for water is calculated taking into account the body weight and water consumption of the target population:

$$GV = \frac{TDI \times b.w. \times P}{C}$$

where:

- *b.w.* is the body weight;
- *C* is the daily consumption of drinking water;
- *P* is the proportion of the TDI attributed to drinking water, because this is not usually the only source of exposure for humans, so only a part of the TDI is attributed to water intake.

For calculating the GV, three types of populations should be taken into account: adults, children and infants. For these three populations, the WHO recommends the use of the following values (AFSSA, 2007; WHO, 2011):

- for adults: body weight of 60 kg and water consumption of 2 L/d;
- for children: body weight of 10 kg and water consumption of 1 L/d;
- for infants: body weight of 5 kg and water consumption of 0.75 L/d.

These values are rather conservative. According to France's second individual and national survey on food consumption (AFSSA, 2009a; ANSES - OCA, 2010; Cartier *et al.*, 2012), the average consumption of tap water is about 600 mL per day for an adult. Consumers of around 2 litres per day are considered major consumers (Table II).

Table II. French water consumption data (mean, median and P95, in g/d) for adults and children (from AFSSA, 2009a; ANSES - OCA, 2010; Cartier *et al.*, 2012)

Population	Body weight (in kg)				Total tap water consumption (in g/L)			
	Mean	P 5	Median	P 95	Mean	P 5	Median	P 95
Adults (aged 18 to 79)	70	49	69	94	714.6	75.5	576.9	1812.9
Children (aged 3 to 17)	38	16	34	69	346.4	25.6	259.7	943.4

By default, 20% of TDI is attributed to water (WHO, 2011).

The GV to be used for the HRA is the one calculated according to the most conservative approach.

Case of veterinary active substances with a MRL

Calculating MRLs for active substances used in the composition of veterinary medicinal products intended for species producing food intended for human consumption involves the standard composition of the "household shopping basket" (an internationally recognised standard). It is therefore possible to determine the fraction of the ADI that is "consumed" in terms of foods of animal origin and to deduce the remaining margin which can then be used to adjust *"P",* the proportion of TDI attributed to drinking water.

2.7.2 Non-threshold toxic effects

In the case of toxic effects with no threshold, these GVs are concentrations in drinking water associated with an excess cancer risk of 10^{-6} for a lifetime (one additional case of cancer in a population of 1,000,000 people consuming drinking water containing the substance in question at a concentration equal to the GV for 70 years).

$$GV = \frac{IER}{PF} \times \frac{b.w.}{C}$$

where:

- IER is the individual excess risk;
- SF is the slope factor;
- b.w. is the body weight;
- C is the daily consumption of drinking water.

To determine the GV, the IER is set at 10^{-6}, the daily water consumption set at 2 L and the body weight set at 60 kg.

To take into account any specific susceptibility of infants and young children, the US EPA proposes applying an additional factor of 10 for the period of life from birth to 2 years and an additional factor of 3 for the period from 2 to 15 years (US EPA, 2005).

2.7.3 Use of the threshold of toxicological concern

From the threshold set at 0.15 µg/person/day, the GV calculated for a consumption of 2 L of water per day is therefore: GV_{TTC} = 75 ng/L.

To summarise

Figure 2 presents the proposed approach for calculating the GV showing the selection of cases to be used based on the available data.

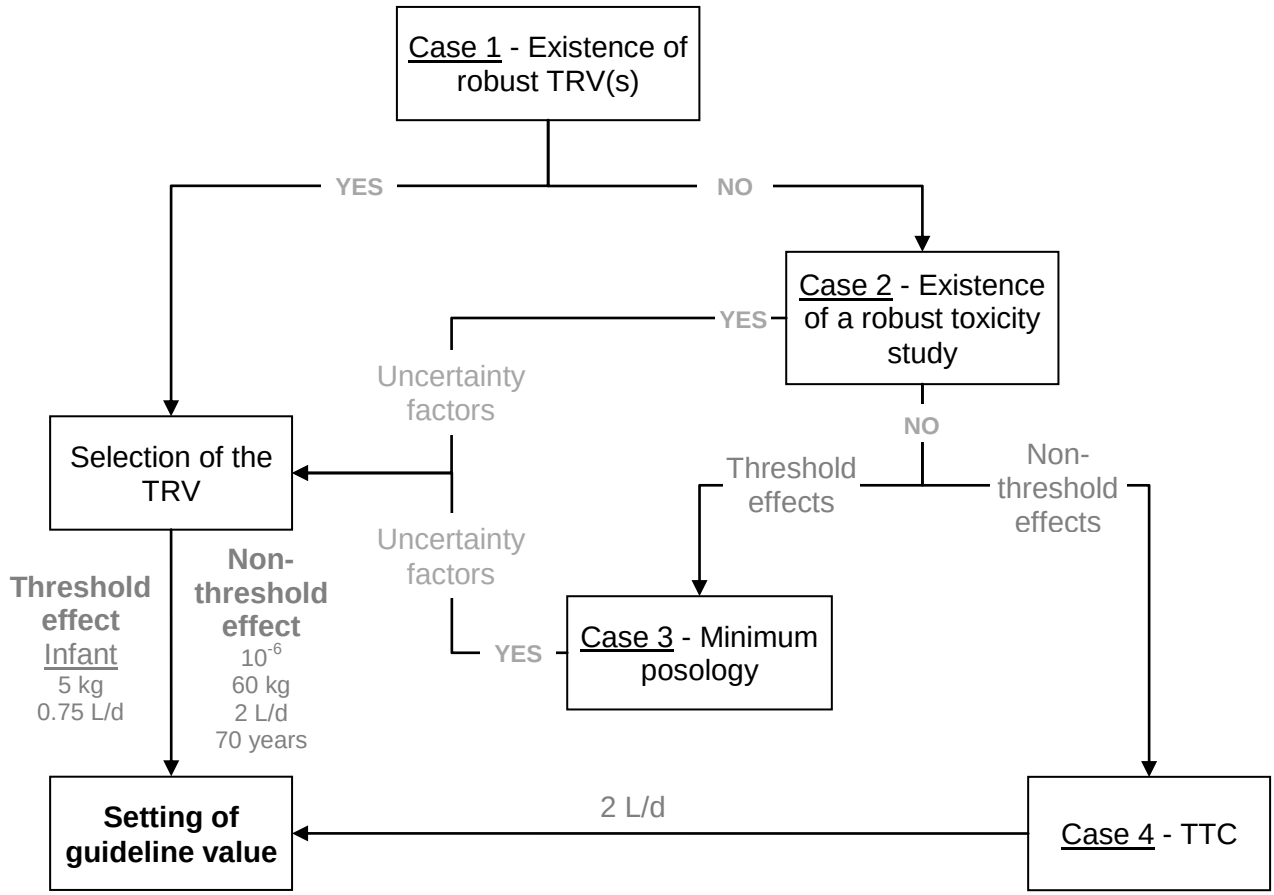


Figure 2. Diagram of the process for setting the guideline value adopted for pharmaceuticals in drinking water

2.8 Module H: Risk assessment

The risk is estimated by the safety margin (SM) corresponding to the ratio of the GV selected in module G to the level of exposure identified in module D (measured or estimated concentration).

$$SM = \frac{GV}{C_{max}}$$

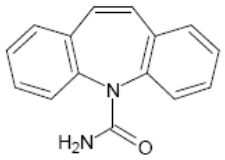
If this ratio is greater than 1, the risk is regarded as negligible or acceptable. If the ratio is less than 1, the health risk is regarded as significant. The higher the safety margin, the lower the risk.

3 Application of the general method to an active substance used in human medicine and detected during the 2011 national analysis campaign: carbamazepine

3.1 Module A – Characteristics of carbamazepine

Carbamazepine (CBZ) is a derivative of dibenzazepine which has antiepileptic, neurotropic and psychotropic properties. It is indicated in the treatment of epilepsy (generalised tonicoclonic and partial seizures), in trigeminal and glossopharyngeal neuralgias, and in manic or hypomanic excited states. In France, the first marketing authorisation application for this compound dates back to 1983. It has been marketed since 1988, initially under the name Tegretol®. Table III presents the characteristics of CBZ.

Table III. Characteristics of carbamazepine (HSDB, 2007; IPCS, 1999; SRC, 2011)

Parameter	Value
CAS number	298-46-4
Chemical formula	$C_{15}H_{12}N_20$
Structural formula	
Presentation	white to yellow-white crystalline powder
Chemical class	dibenzazepine
Use	principal: anticonvulsant
Molar mass	236 $g.mol^{-1}$
Henry constant	$1.08.10^{-10}$ $atm.m^3.mol^{-1}$ at 25°C
Vapour pressure	$1.84.10^{-7}$ mmHg at 25°C
Melting point	190 to 193°C
Water solubility	18 mg/L at 25°C
pKa	13.9
Log Dow (water-soluble form at pH 7)	2.25
Log K_{ow}	2.45 (molecular form)
K_{oc}	510 3870[a]
K_d	1.4 to 4.4[b]
Formation of complexes with divalent cations (Ca^{2+}, Mg^{2+}) or transition elements found in the environment (Fe, Mn, etc.)	Not specified
Abiotic degradation half-life (hydrolysis, photolysis)	Non-hydrolysable[c] 110 days by photolysis[c]
Biodegradation half-life	31 days (obtained from a solution of 2g of carbamazepine per litre)[d]

[a]Jones *et al.*, 2002; [b]Beausse, 2004; [c]De Laurentiis *et al.*, 2012; [d]Khan and Ongerth, 2004

3.2 Module B - Identification of relevant metabolites formed in humans or animals

CBZ is largely metabolised by the liver, primarily by oxidation leading to the production of a single pharmacologically active metabolite, 10,11-epoxycarbamazepine (10,11-epoxyCBZ) (ANSM, 2012). The plasma concentration of the metabolite during long-term treatment of epileptic patients varies between 5 and 81% of that of the parent drug (Bertilsson, 1978). It is almost completely metabolised into an inactive metabolite, trans-10,11-dihydroxy-10,11diolcarbamazepine, which can be conjugated to form O-glucuronides, however, it is excreted in the urine mainly in a non-conjugated form (Figure 3). This pathway may also lead to the formation of minor metabolites from the aromatic hydroxylation of CBZ. Only 1% of the 10,11-epoxyCBZ formed is excreted unchanged. The quantitatively most important metabolite in urine is trans-10,11-dihydroxy-10,11diolcarbamazepine (Bertilsson, 1978).

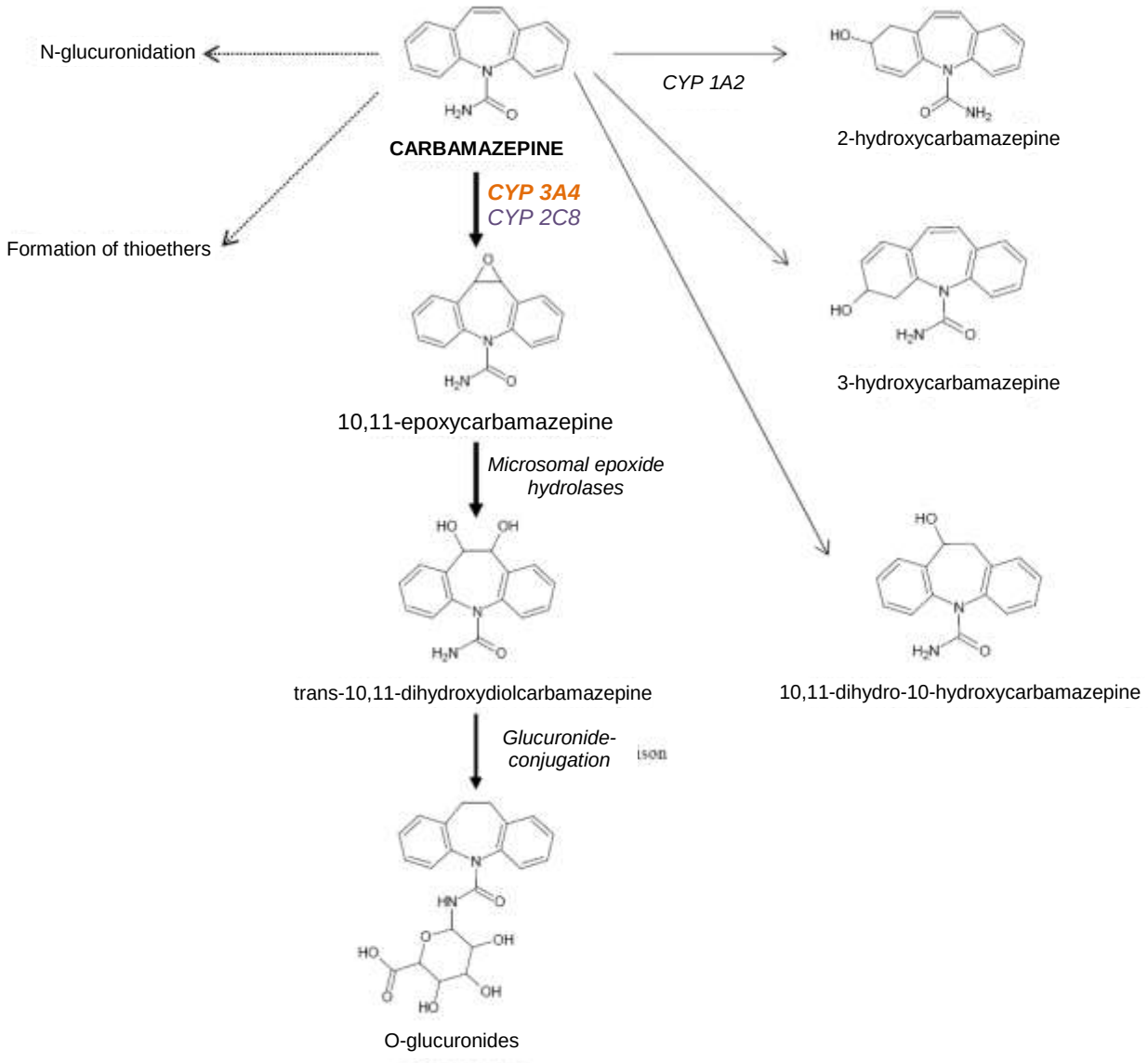


Figure 3. Main metabolic pathways of carbamazepine (Amore *et al.*, 1997; Miao and Metcalfe, 2003; Mockenhaupt *et al.*, 2005)

Among the metabolites of CBZ, 10,11-epoxyCBZ is considered relevant because it is pharmacologically active, unlike trans-10,11-dihydroxy-10,11diolcarbamazepine.

3.3 Module C - Identification of relevant transformation products formed in the environment

Several degradation products of CBZ have been identified, which are either formed in the environment (Figure 4) or during water treatment (Figure 5). Studies have identified many structures but have not been able to quantify the different degradation products.

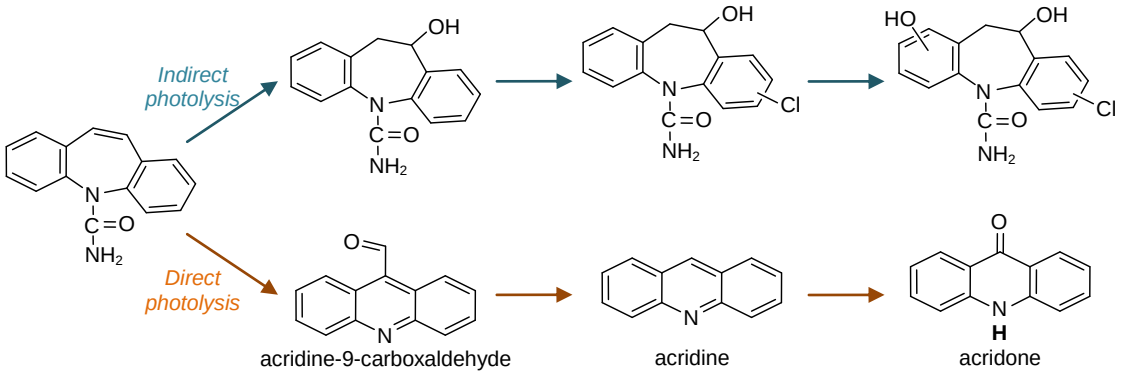
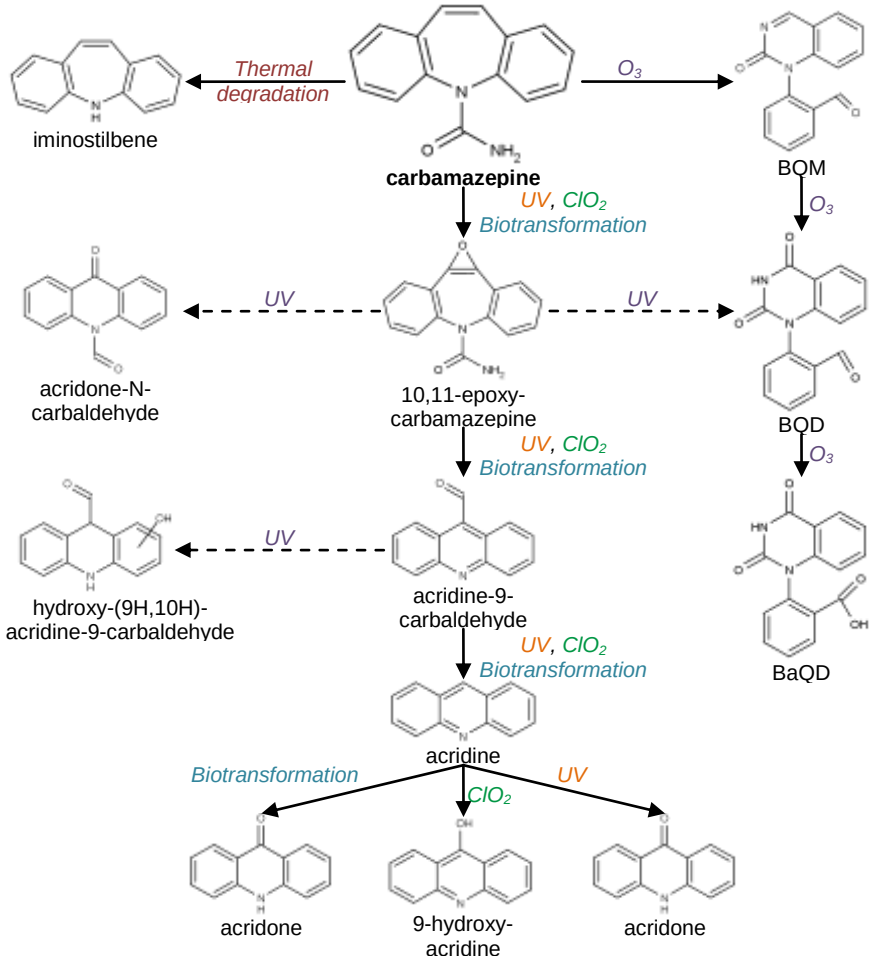


Figure 4. Degradation pathways of carbamazepine by photolysis in estuarine waters (Chiron *et al.*, 2006)

A portion of the conjugated metabolites of CBZ and 10,11-epoxyCBZ is deconjugated in sewage treatment plants (activated sludge). 10,11-epoxyCBZ, a metabolite of CBZ, has been identified in the laboratory as capable of being produced by the action of the various processes used in water treatment (biotransformation, UV, chlorine dioxide).

Among these CBZ transformation products, acridine and acridone have documented genotoxic effects (Bleeker *et al.*, 1999), although their occurrence in the environment is rare, often below the LOQ, and cannot be attributed solely to the environmental transformation of CBZ.



BQM: 1-(2-benzaldehyde)-4-hydro-(1H,3H)-quinazoline-2-one; BQD: 1-(2-benzaldehyde)-(1H,3H)-quinazoline-2,4-dione; BaQD: 1-(2-benzoic acid)-(1H,3H)-quinazoline-2,4-dione

Figure 5. Degradation pathways of carbamazepine in laboratory conditions by various processes used in water treatment (Ikehata *et al.*, 2006 and Kosjek *et al.*, 2009)

Among the degradation products of CBZ, the follow-up to the HRA will focus on 10,11-epoxyCBZ, which is one of its main degradation products and is specific to the compound.

3.4 Module D – Assessment of human exposure *via* drinking water

A few publications report the transient presence of CBZ in drinking water at maximum concentrations of the order of a tenth of a nanogram per litre (Mompelat *et al.*, 2009; Stackelberg *et al.*, 2004; Togola and Budzinski, 2008).

During its national analysis campaign of pharmaceuticals in drinking water, the LHN screened for CBZ and 10,11-epoxyCBZ in 285 treated water samples collected throughout French territory in 2011 (ANSES, 2011 - Table IV).

- CBZ was quantified (LOQ = 5 ng/L) in 4% of the analysed samples, with a maximum concentration of 33 ng/L measured in drinking water.
- The maximum level of its major metabolite, 10,11-epoxyCBZ, was 6 ng/L. This metabolite was quantified at a frequency of 7.6% (LOQ = 1 ng/L).
- 8.7% of the samples had a quantifiable concentration for at least one of the two compounds. In a given sample, the sum of the concentrations of CBZ and 10,11-epoxyCBZ was a maximum of 40 ng/L.

Table IV. Results from assaying of carbamazepine and 10,11-epoxycarbamazepine in drinking water in France (ANSES, 2011)

Compounds	n	Limit of detection (ng/L)	Limit of quantification (ng/L)	Frequency of detection (>LOD)	Frequency of quantification (>LOQ)	Maximum level (ng/L)
Carbamazepine	285	1.5	5	9.0%	4.0%	33.4
10,11-epoxycarbamazepine	285	0.3	1	14.9%	7.6%	6.2
CBZ + 10,11-epoxyCBZ	285	-	-	17.0%	8.7%	39.7

As these results give just a snapshot of contamination of drinking water by CBZ and 10,11-epoxyCBZ, it is not possible to assess the population's actual exposure to these compounds. A "worst-case" assessment is therefore conducted by assuming daily exposure to the maximum concentration.

3.5 Module E – Biological effects

3.5.1 Pharmacological mechanism of action (ANSM, 2012)

CBZ acts mainly on the voltage-dependent sodium channels, with its other mechanisms of action being only partially understood. In addition, the decrease in glutamate release and the stabilisation of neuronal membranes can essentially explain its antiepileptic effects. The antimanic properties of CBZ appear to be due to the decrease in regeneration of dopamine and norepinephrine.

Anticonvulsant and antineuralgic properties have also been demonstrated for 10,11-epoxyCBZ (Bertilsson and Tomson, 1986; Reynolds, 1996). In mice, the antiepileptic and neurotoxic effects are proportional to the sum of concentrations of CBZ and its metabolite 10,11-epoxyCBZ (Bourgeois and Wad, 1984).

3.5.2 Pharmacokinetics (ANSM, 2012; Vidal®, 2012)

CBZ is almost completely absorbed after oral administration. The peak plasma concentration is reached between 2 h and 12 h after administration of a single dose, depending on the posology form (oral suspension or tablet). The binding rate of CBZ to plasma proteins is 70% to 80%.

Virtually all of the active substance is metabolised by the liver. Cytochrome P_{450} 3A4 has been identified as the main enzyme involved. In children, the kinetics of metabolism is faster than in adults.

Most CBZ is excreted in the urine, almost exclusively as metabolites with about 1% being excreted unchanged. Some is excreted in the faeces. With monotherapy, after administration of a single dose, the elimination half-life of the unchanged substance in plasma is about 36 hours, whereas after repeated administration, this is achieved on average after only 16 to 24 hours, depending on the duration of treatment.

During pregnancy, the free fraction of CBZ is increased and it can cross the placenta. CBZ and 10,11-epoxyCBZ pass into breast milk, where concentrations are between 25 and 60% of the plasma concentration.

3.5.3 Toxicology

3.5.3.1 Effects in humans at therapeutic doses

- *Undesirable effects (ANSM, 2012)*

Many undesirable effects have been reported for CBZ at the concentrations used therapeutically, especially while treatment is being introduced. These include central nervous system (convulsions, ataxia, dizziness, drowsiness, agitation, confusion, involuntary movements, etc.), haematological (leukopenia, thrombocytopenia, hypereosinophilia, etc.), liver (isolated increase in gamma-glutamyl tranpeptidase, increase in alkaline phosphatase, etc.), cardiovascular (tachycardia, hypotension, conduction disturbances, etc.), respiratory (including respiratory depression) and gastrointestinal disorders (including nausea and vomiting).

In addition, serious and sometimes fatal skin reactions (including Lyell's and Stevens-Johnson syndrome) have been reported during treatment with CBZ. The risk of these events occurring is about 10 times higher for populations in some Asian countries.

- *Toxicity to reproduction and development*

CBZ is classified as Category D toxic to reproduction by the FDA: 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 the use of the drug in pregnant women despite potential risks.

Numerous scientific publications report that exposure to antiepileptic drugs during pregnancy is associated with an increased risk of major congenital abnormalities in the offspring (Eadie, 2008; Harden *et al.*, 2009; Perucca, 2005).

There are many data on women, mostly epileptic, exposed to CBZ during pregnancy. In recent studies, the overall frequency of foetal malformations is either identical to that of the general population (2 to 3%) (Harden *et al.*, 2009; Morrow *et al.*, 2006), or slightly increased (Meador *et al.*, 2008; Samren *et al.*, 1999).

CBZ increases the frequency of neural tube closure abnormalities, mainly *spina bifida* (Jentink *et al.*, 2010). The risk period is between 4 and 6 weeks of gestation. Cardiac malformations, hypospadias, cleft lip and/or palate and hypoplasia of the distal phalanges and nails have also been described without the associations being confirmed.

Tomson *et al.* (2011) recently demonstrated an increase in the frequency of major birth defects in children of women treated during pregnancy with more than 1000 mg/d of CBZ compared to those treated with less than 400 mg/d of CBZ.

To date, the effects on psychomotor development of children exposed to CBZ *in utero* are controversial, even if the overall results are reassuring when children are monitored until the age of about 10 years (Harden *et al.*, 2009).

3.5.3.2 Studies in animals

- *Toxicity after repeated administration*

MA dossiers mention toxicity studies in animals after repeated administration of CBZ. LOAELs in rats ranging from 50 to 200 mg/kg/day have been reported, while in dogs NOAELs are between 50 to 100 mg/kg/day and LOAELs between 100 and 300 mg/kg/day (cited by Houeto *et al.*, 2012).

However, very few studies of the chronic toxicity of CBZ at doses below therapeutic doses have been reported in the scientific literature.

Cunningham *et al.* (2010) attribute the appearance of hepatocellular carcinomas in female rats (study described below) to increased metabolic activity of the liver and its possible enzyme induction. The corresponding LOAEL is 25 mg/kg/d (cited by Cunningham *et al.*, 2010).

- *Toxicity to reproduction and development*

Animal experiments show a teratogenic effect of CBZ (ANSM, 2012) that can induce testicular atrophy, aspermatogenesis, increased resorptions, increased skeletal and visceral abnormalities, decreased foetal weight, decreased weight gain of litters during lactation, etc. These effects were observed at doses toxic to the mother with LOAELs generally of about 200 mg/kg/d (Cunningham *et al.*, 2010; Vorhees *et al.*, 1990).

- *Mutagenicity - carcinogenicity*

CBZ administered to Sprague-Dawley rats for two years in the diet at doses of 25, 75 and 250 mg/kg/d resulted in a dose-dependent increase in the incidence of hepatocellular tumours in females and benign testicular interstitial cell adenomas in males. CBZ should therefore be regarded as carcinogenic in Sprague-Dawley rats (Novartis, 2012). For hepatocellular carcinomas in female rats, the LOAEL is 25 mg/kg/d (cited by Cunningham *et al.*, 2010).

At present, there are no data enabling the results obtained in rats to be transposed and to confirm CBZ's carcinogenicity to humans (ANSM, 2012; Novartis, 2012).

In vitro studies on the mutagenicity of CBZ have mostly yielded negative results while those on clastogenesis give contradictory results (Awara *et al.*, 1998; Celik, 2006; Flejter *et al.*, 1989; Schaumann *et al.*, 1985; Sinués *et al.*, 1995).

10,11-epoxyCBZ is neither mutagenic (Glatt *et al.*, 1983), nor cytotoxic *in vitro* and *in vivo* (Frigerio and Morselli, 1975). However, this epoxy can be genotoxic by binding to macromolecules (Ehrenberg and Hussain, 1981).

3.6 Module F – Determination of the TRV

In order to test the HRA method proposed in Section 2, the TRV was determined using several approaches.

Considering the lack of toxicity studies specific to 10,11-epoxyCBZ, the fact that this metabolite has the same pharmacological activity as CBZ, and the presence in the body of 10,11-epoxyCBZ during studies on CBZ in humans and animals, the TRV_{tox} and $TRV_{posology}$ were first determined for the sum of the two compounds (CBZ + 10,11-epoxyCBZ).

3.6.1.1 Finding a critical dose using toxicological studies from the MA dossier or the scientific literature

To our knowledge, there is no existing TRV that has been validated by national or international agencies for CBZ or 10,11-epoxyCBZ. Data from toxicity studies in animals have therefore been used.

The LOAEL of 25 mg/kg/d for hepatocellular carcinomas in female rats exposed to CBZ via the diet for two years was used as the critical dose for the determination of a TRV (Cunningham *et al.*, 2010; Novartis, 2012). The following uncertainty factors were added to this LOAEL:

- 10 for the use of a LOAEL instead of a NOAEL;
- 10 for interspecies variability;
- 10 for intraspecies variability.

A **TRV_{tox} of 25 µg/kg/d** for the sum of CBZ + 10,11-epoxyCBZ is therefore obtained.

3.6.1.2 Use of the minimum daily posology

The minimum posology of CBZ is 100 mg/day in adults as a starting dose for the prevention of relapses in manic-depressive psychosis (ANSM, 2012). Considering that the body weight of an adult is 60 kg, the benchmark dose is 1.67 mg/kg/d.

An uncertainty factor of 1000 is applied to this dose, broken down as follows:

- 10 for the use of a LOAEL instead of a NOAEL;

- 10 for intraspecies variability;
- 10 as an additional UF for the use of a minimum dose.

A **$TRV_{posology}$ of 1.67 µg/kg/d** for the sum of CBZ + 10,11-epoxyCBZ is therefore obtained.

3.6.1.3 TTC

According to the TTC approach, a threshold of **0.15 µg/person/day** (0.0025 µg/kg/day) would be protective for each of the compounds taken independently.

3.7 Module G - Determination of a guideline value

The GVs for CBZ and 10,11-epoxyCBZ in drinking water corresponding to the TRVs established in the previous module were calculated according to the method proposed in Section 2. They are presented in Table V.

The GV_{tox} to be used for the HRA for CBZ + 10,11-epoxyCBZ is the most protective, i.e. **33 µg/L** established for the "infants" scenario.

Table V. Summary of TRVs and GVs for carbamazepine and 10,11-epoxycarbamazepine obtained with three different methods

TRV selection method	Compound(s) concerned	Population	TRV	Body weight (kg)	Daily water consumption (L)	Share of the ADI attributable to water	GV (µg/L)
Toxicological studies	CBZ + 10,11-epoxy-CBZ	Adult	TRV_{tox} = 25 µg/kg	60	2	20%	GV_{tox} = 150
		Child		10	1	20%	GV_{tox} = 50
		Infant		5	0.75	20%	GV_{tox} = 33
Minimum daily posology	CBZ + 10,11-epoxy-CBZ	Adult	$TRV_{posology}$ = 1.67 µg/kg	60	2	20%	$GV_{posology}$ = 10
		Child		10	1	20%	$GV_{posology}$ = 3
		Infant		5	0.75	20%	$GV_{posology}$ = 2
TTC	CBZ	General	Threshold = 0.15 µg/pers/d	-	2	-	GV_{TTC} = 0.075
	10,11-epoxy CBZ						

As an exercise and to assess the method, the HRA was also conducted with the $GV_{posology}$ of 2 µg/L for CBZ + 10,11-epoxyCBZ and individually for each of the compounds, with the GV_{TTC} of 0.075 µg/L.

3.8 Module H – Health risk assessment

The safety margins for CBZ and 10,11-epoxyCBZ calculated with the maximum concentrations measured during the national campaign and the GVs from module G, are shown in Table VI.

Table VI. Safety margins for carbamazepine and 10,11-epoxycarbamazepine in drinking water

	Compound(s) concerned	C_{max} in µg/L	GV in µg/L	SM

Toxicological studies	CBZ + 10,11-epoxy-CBZ	0.040	33	**825**
Minimum posology	CBZ + 10,11-epoxy-CBZ	0.040	2	**50**
TTC	CBZ	0.033	0.075	**2.3**
	10,11-epoxyCBZ	0.006		**12.5**

Regardless of the HRA method used, the safety margins are greater than 1. Thus, in view of current knowledge, the health risk associated with the ingestion of CBZ and 10,11-epoxyCBZ *via* drinking water at the exposure doses known in France is regarded as negligible.

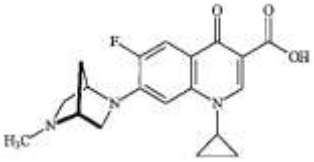
4 Application of the general method to an active substance used in veterinary medicine and detected during the 2011 national analysis campaign: danofloxacin

The HRA related to the presence of danofloxacin in drinking water was based on a critical study of the toxicological dossier for danofloxacin mesylate (ANMV, 1992), on reports by JECFA (FAO, 1997; JECFA, 1998; WHO, 1997) and the FDA (FDA, 2002) and on summary reports by the EMA (EMEA; EMEA, 1997; EMEA, 1998a; EMEA, 1998b; EMEA, 1999; EMEA, 2002). Scientific publications supplemented this information.

4.1 Module A – Characteristics of danofloxacin (FAO, 1997)

Danofloxacin is an antibiotic of the fluoroquinolone class used exclusively in veterinary medicine. It has marketing authorisation for use in cattle, sheep, pigs, goats and poultry, among others, for oral, subcutaneous, intramuscular or intravenous administration. Danofloxacin may be marketed as danofloxacin mesylate. The main physico-chemical characteristics of danofloxacin and danofloxacin mesylate are presented in Table VII.

Table VII. Physico-chemical characteristics of danofloxacin and danofloxacin mesylate

Parameter	Danofloxacin	Danofloxacin mesylate	Source
CAS number	112398-08-0	119478-55-6	
Chemical formula	$C_{19}H_{20}FN_3O_3$	$C_{19}H_{20}FN_3O_3 – CH_3O_3S$	FAO, 1997
Structural formula			FAO, 1997
Molar mass (g/mol)	357	453	FAO, 1997
Henry constant	Not specified	Not specified	
Vapour pressure	Not specified	$< 7.10^{-7}$ mmHg	FDA, 2002
Water solubility (g/L)	172 – 205	156 at pH 5 0.07 at pH 7 1.06 at pH 9	FAO, 1997 FDA, 2002
pKa	8.46[a]	6.22 and 9.43	FDA, 2002
Log K_{ow}	2.4 at pH 7[a]	0.14 at pH 5 0.39 at pH 7 0.22 at pH 9	FDA, 2002
Log K_{oc}	Not specified	4.9 to 5.8	FDA, 2002
K_d	Not specified	2280 to 3800	FDA, 2002
Formation of complexes with divalent cations (Ca^{2+}, Mg^{2+}) or transition elements found in the environment (Fe, Mn, etc.)	Not specified	Not specified	
Degradation half-life by hydrolysis	Not specified	Stable at pH 5 to 9 after 5 days at 50°C	FDA, 2002
Degradation half-life by photolysis	Not specified	2.6 to 24 min (depending on pH)	FDA, 2002
Biodegradation half-life	Not specified	91 to 143 days in soil	FDA, 2002

[a]Hu *et al.*, 2007

4.2 Module B - Identification of the metabolites of danofloxacin

Excretion of danofloxacin after administration is considered to be similar for target species and laboratory animals. It occurs mainly via urine and faeces, with about 80% being excreted unchanged, and less than 20% excreted in the form of the main metabolite, desmethyldanofloxacin. Danofloxacin-N-oxide and the β-glucuronide conjugate of danofloxacin can also be found. Figure 6 presents the main metabolites of danofloxacin (EMEA, 1999).

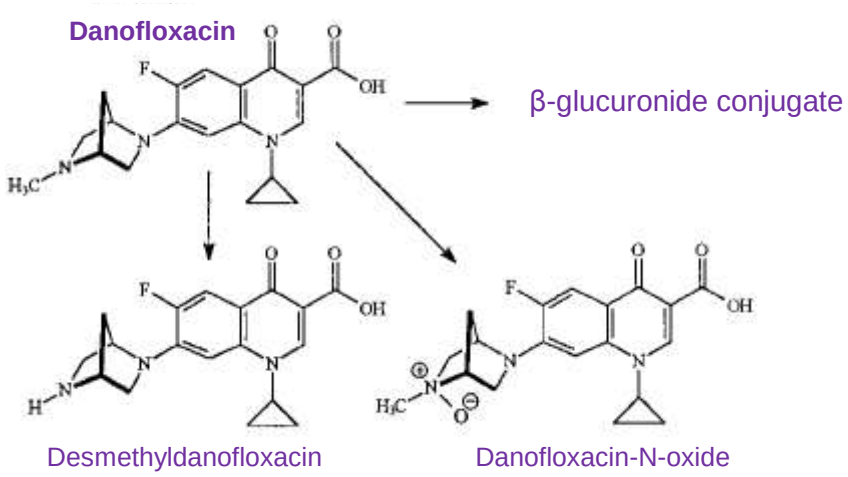


Figure 6. Main metabolites of danofloxacin, from FAO (1997)

4.3 Module C - Identification of transformation products

There is little information on the degradation of danofloxacin in the environment or in water treatment plants.

Danofloxacin is photosensitive; Figure 7 shows its degradation pathways by photolysis. Its half-lives in purified water, freshwater or seawater, under a light intensity (290-420 nm) of 0.83 mW/cm^2 are estimated at between 1.7 min and 7.8 min (Ge *et al.*, 2010); in river water under solar radiation it is about 1 min (Sturini *et al.*, 2012). The main transformation product identified is desmethyldanofloxacin (Ge *et al.*, 2010).

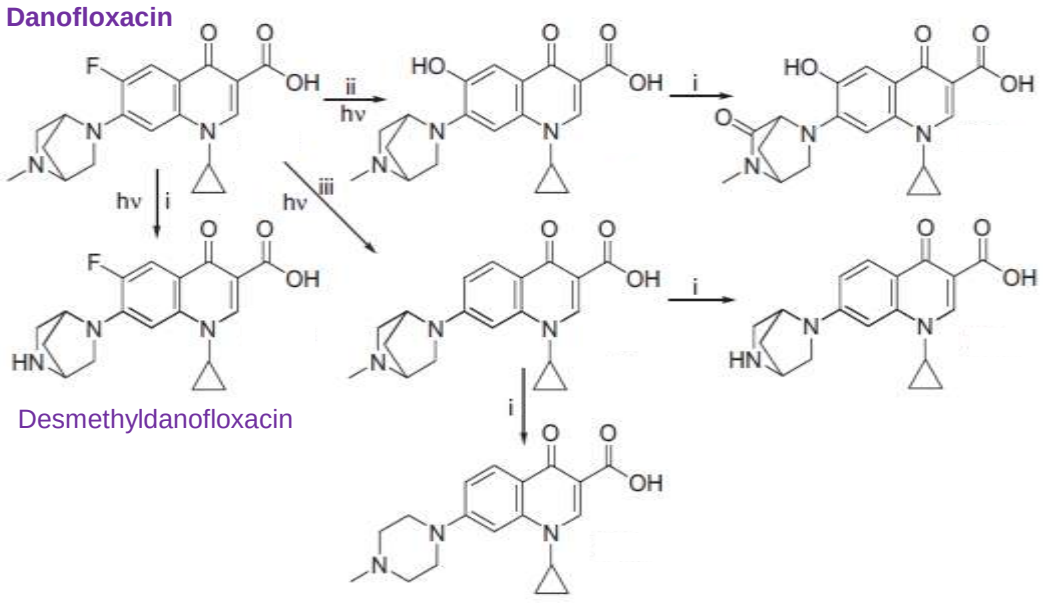


Figure 7. Degradation products of danofloxacin by photolysis (from Sturini *et al.*, 2012)

Liu *et al.* (2011) identified 11 degradation products for danofloxacin, including desmethyldanofloxacin (Figure 8), by hydrolysis, oxidation and photolysis in laboratory conditions.

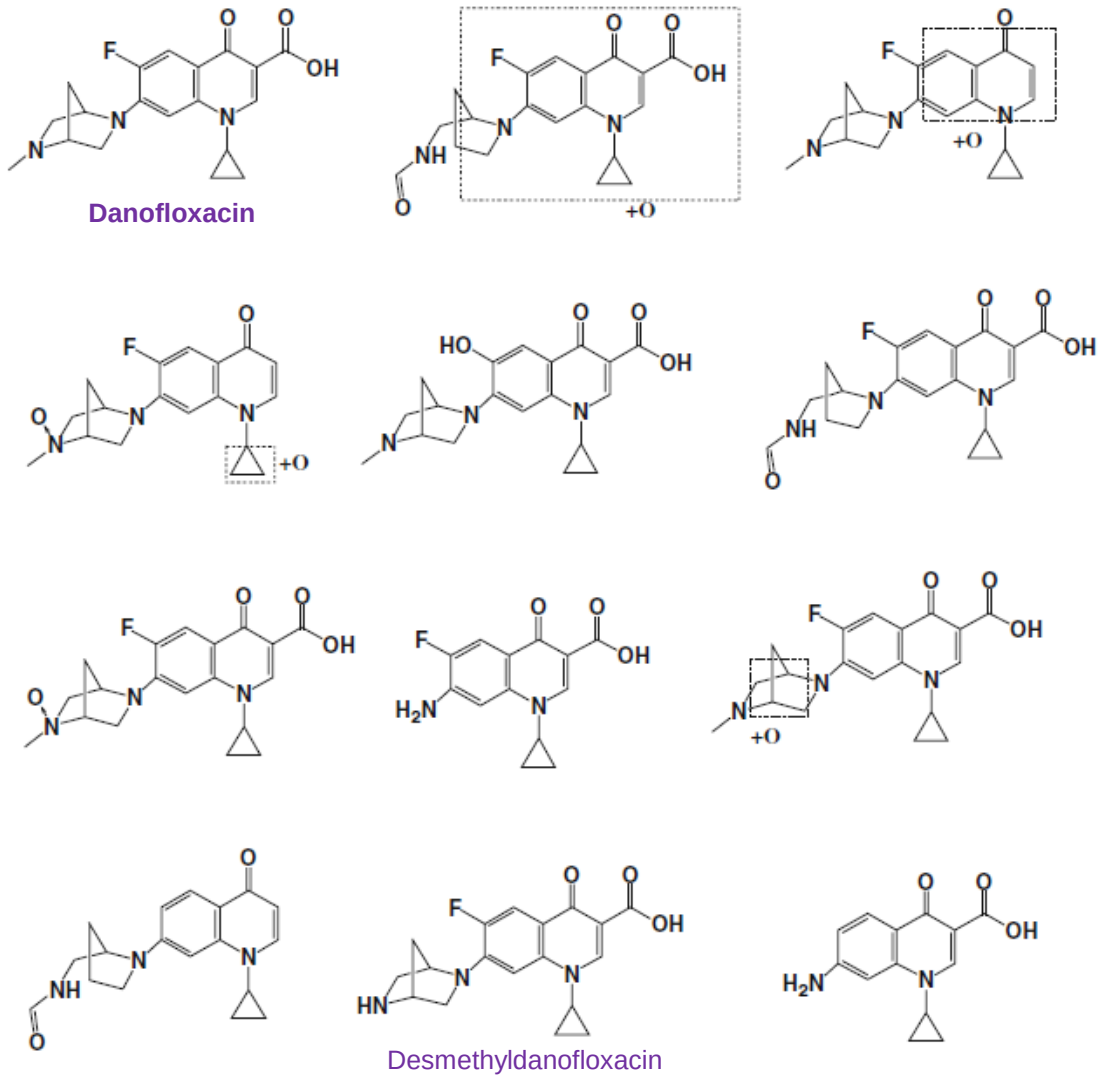


Figure 8. Degradation products of danofloxacin by hydrolysis, oxidation and photolysis (from Liu *et al.*, 2011)

4.4 Module D - Assessment of human exposure *via* drinking water

During the national analysis campaign of pharmaceuticals in drinking water, the LHN screened for danofloxacin in 285 treated water samples collected throughout French territory in 2011 (ANSES, 2011). Danofloxacin was detected in 10 samples and quantified in two samples at 34 and 57 ng/L (Table VIII).

Table VIII. Results from assaying of danofloxacin in drinking water in France (ANSES, 2011)

Compound	Number of samples	Limit of detection (ng/L)	Limit of quantification (ng/L)	Frequency of detection (>LOD)	Frequency of quantification (>LOQ)	Maximum level (ng/L)
Danofloxacin	285	8	25	3.5%	0.8%	57

As these results give just a snapshot of contamination of drinking water by danofloxacin, it is not possible to assess the population's actual exposure to this compound. A "worst-case" situation is therefore used that assumes daily exposure to the maximum concentration measured.

In the absence of data on the concentration of desmethyldanofloxacin in drinking water, the assessment of exposure to desmethyldanofloxacin could not be performed.

4.5 Module E - Biological effects

4.5.1 Mechanism of action

Fluoroquinolones act on bacterial DNA by preventing its replication; quinolones bind to the ends of the DNA strands, which can no longer pair up. The formation of a DNA-quinolone complex is irreversible and results in the death of the bacteria.

4.5.2 Pharmacokinetics

Danofloxacin is rapidly and completely absorbed (80-100%) orally in monogastric species. Maximum serum concentrations are reached approximately one hour after ingestion. Fluoroquinolones are distributed widely in tissues; they penetrate well in the bronchial secretions, bones and cartilage, as well as in the prostate. They are partially metabolised by the liver and excreted in active form in bile and urine. Urinary concentrations can be as much as 10 times higher than those in plasma.

4.5.3 Toxicity (WHO, 1997)

The median lethal doses (LD_{50}) for danofloxacin and desmethyldanofloxacin by oral route in rats or mice are between 1500 and 2000 mg/kg. Toxicity is manifested by central nervous system disorders.

Exploratory studies conducted for obtaining MAs have identified several types of toxicity and can be used to calculate no observed adverse effect levels (NOAEL).

A NOAEL of 6.25 mg/kg/d was established for danofloxacin for **tubular nephropathies** in rats, based on studies in rats exposed *in utero* and during lactation and for an additional 3 months via feed at doses of up to 150 mg/kg/d.

A NOAEL of 2.4 mg/kg/d has been reported for danofloxacin for **arthropathies** from a 3-month study in young dogs exposed orally to repeated doses of up to 25 mg/kg. For the same effect, a NOAEL of 0.25 mg/kg/d has been shown for desmethyldanofloxacin, based on a 3-month study in young dogs exposed orally to repeated doses of up to 10 mg/kg. In dogs, desmethyldanofloxacin is more toxic than the parent compound and could be the metabolite responsible for the toxicity of the active substance on cartilage. The appearance of lesions could be related to a particular sensitivity of the animal associated with metabolic detoxification of danofloxacin, since a dose-response relationship is generally not shown.

Danofloxacin is not teratogenic in rats or mice. Multi-generation studies in rats, mice and rabbits have shown toxic effects of danofloxacin on reproduction (maternal and foetal toxicity) with NOAELs of 6.25, 100 and 7.5 mg/kg in rats, mice and rabbits, respectively.

Studies conducted over two years in mice and rats have not demonstrated any carcinogenicity. Danofloxacin is not mutagenic either *in vitro* or *in vivo.* Although desmethyldanofloxacin induces unscheduled synthesis of DNA *in vitro,* this genotoxic potential does not appear to persist *in vivo*.

4.6 Module F - Determination of toxicity reference values

As an exercise and to assess the HRA method proposed in Section 2, the TRV was determined according to several different cases.

4.6.1 Danofloxacin

4.6.1.1 Use of a TRV that has been validated by national or international organisations

As danofloxacin is used in the composition of drugs intended for animal species producing food intended for human consumption, an ADI has been established in MRL dossiers by JECFA and the EMA.

In 1997, the EMA calculated an ADI of 24 µg/kg/d of body weight by applying a safety factor of 100 to the NOAEL of 2.4 mg/kg/day. The factor of 100 is justified by the fact that young dogs are the most susceptible species to arthropathies induced by quinolones and humans are relatively insensitive to this effect (EMEA, 1997).

JECFA established a maximum ADI for danofloxacin of 20 µg/kg/d based on the NOAEL of 2.4 mg/kg/day. A factor of 100 was applied to this NOAEL. The value obtained was rounded to one significant figure in accordance with JECFA practice (WHO, 1997).

The TDI used for follow-up to the HRA is **24 µg/kg/d.**

4.6.1.2 Use of the minimum daily posology

As danofloxacin is used only in veterinary medicine, there is no minimum daily posology for humans.

4.6.1.3 TTC

According to the TTC approach, a threshold of **0.15 µg/person/day** (0.0025 µg/kg/day) would be protective.

4.6.2 Desmethyldanofloxacin

4.6.2.1 Use of a TRV that has been validated by national or international organisations

In the EMA dossier on MRLs for danofloxacin, an ADI of 2.5 µg/kg/d has been calculated for desmethyldanofloxacin. As the critical effect is identical to that of danofloxacin, a safety factor of 100 was also applied (EMEA, 1997).

4.6.2.2 Use of the minimum daily posology

Desmethyldanofloxacin is a metabolite and not an active substance, and there is therefore no posology for this compound.

4.6.2.3 TTC

According to the TTC approach, a threshold of **0.15 µg/person/day** (0.0025 µg/kg/day) would be protective.

4.7 Module G - Determination of a guideline value

4.7.1 Danofloxacin

The GVs in drinking water for danofloxacin are calculated for adults, children and infants, in accordance with the HRA method proposed (Table IX).

Table IX. Calculation of $GV_{drinking\ water}$ for danofloxacin depending on age groups

TRV selection method	Population	TRV	Body weight (kg)	Daily water consumption (L)	Share of the ADI attributable to water	GV (µg/L)
TDI	Adult	TDI = 24 µg/kg	60	2	20%	**GV_{TDI} = 144**
	Child		10	1	20%	**GV_{TDI} = 48**
	Infant		5	0.75	20%	**GV_{TDI} = 32**
TTC	General	Threshold = 0.15 µg/pers/d	-	2	-	GV_{TTC} = 0.075

The GV_{TDI} to be used for the HRA is the most protective, namely the one established for the "infants" scenario of **32 µg/L**.

In order to test the approach, the HRA will also be conducted with the GV_{TTC}.

Adjusting the share of the TDI attributable to water

As danofloxacin is a drug intended for animal species producing food intended for human consumption, MRLs have been defined in the MA dossiers (Table X).

Table X. Acceptable daily intake and maximum residue limits for danofloxacin (EMEA, 2002)

ADI = 24 µg/kg		
Animal species	**MRL µg/kg**	**Target foodstuffs**
Cattle, sheep, goats, poultry*	200 100 400 400	Muscle Fat Liver Kidneys
All food-producing species except cattle, sheep, goats and poultry	100 50 200 200	Muscle Fat Liver Kidneys
Cattle, sheep, goats	30	Milk

* Use is prohibited for animals whose eggs are used for human consumption.

In 2002, in the summary of its report on MRLs extended to all food-producing species, the EMA stated that with the values set for the MRLs, in Europe, daily dietary intake of danofloxacin should not exceed 52% of the ADI (EMEA, 2002). The share of the TDI attributable to water can be adjusted in order to take account of these factors.

4.7.2 Desmethyldanofloxacin

Table XI presents the GVs in drinking water for desmethyldanofloxacin calculated for adults, children and infants.

Table XI. Calculation of $GV_{drinking\ water}$ for desmethyldanofloxacin depending on age groups

TRV selection method	Population	TRV	Body weight (kg)	Daily water consumption (L)	Share of the ADI attributable to water	GV (µg/L)
TDI	Adult	TDI = 2.5 µg/kg	60	2	20%	**GV_{TDI} = 15**
	Child		10	1	20%	**GV_{TDI} = 5**
	Infant		5	0.75	20%	**GV_{TDI} = 3**
TTC	General	Threshold = 0.15 µg/pers/d	-	2	-	GV_{TTC} = 0.075

In accordance with the HRA method proposed, the most protective GV_{TDI} of 3 µg/L, which corresponds to the "infant" scenario, is used for the HRA.

In order to assess the approach, the HRA will also be conducted with the GV_{TTC}.

4.8 Module H – Risk characterisation

4.8.1 Danofloxacin

The safety margin associated with danofloxacin in drinking water, calculated with the maximum concentration measured during the national campaign and the most conservative GV, is:

$$SM_{DJT} = \frac{GV_{TDI}}{C_{max}} = \frac{32}{0.057} = \mathbf{561}$$

As this safety margin is greater than 1, the health risk associated with the ingestion of danofloxacin *via* drinking water is regarded as negligible.

If the TTC had had to be used, the safety margin would have been:

$$SM_{TTC} = \frac{GV_{TTC}}{C_{max}} = \frac{0.075}{0.057} = \mathbf{1.3}$$

As this safety margin is greater than 1, the health risk associated with the ingestion of danofloxacin *via* drinking water would also have been regarded as negligible.

4.8.2 Desmethyldanofloxacin

In the absence of any exposure estimation, it was not possible to complete the HRA for desmethyldanofloxacin.

5 Conclusions

This report proposed an approach for assessing the health risks associated with the presence of pharmaceuticals in drinking water based on a classic protocol. If toxicology data are not available, alternative proposals can be applied such as the minimum daily posology or the threshold of toxicological concern (TTC).

Applying this method to carbamazepine and danofloxacin has brought to light some limitations.

- In terms of exposure, there are few available robust data on the contamination of drinking water in France by pharmaceuticals, and especially by their metabolites and transformation products. The LHN's study, which served to characterise French exposure to CBZ and danofloxacin, despite being of good quality, gives only a snapshot of French contamination and does not include spatial and temporal variations.
- Assessing the chronic toxicity of the active substances is hampered by a lack of data, mainly on drugs for human use, which are either non-existent or inaccessible. In addition, it is difficult to extrapolate the data from MA dossiers and pharmacovigilance to doses much lower than the therapeutic doses and to the general population. The minimum daily posology is used with an additional uncertainty factor of 10. The TTC approach, despite being more protective, is not based on toxicological effects specific to the compound, and can therefore be used only as a management tool.

All of these limitations mean that a quantitative risk assessment is difficult and highlight a need for chronic toxicity studies on pharmaceuticals, their metabolites and transformation products, with a view to being able to establish TRVs.

Despite the limitations identified, it was possible to conduct HRAs for carbamazepine and its metabolite 10,11-epoxycarbamazepine, and for danofloxacin. They indicate a negligible risk following the ingestion of these compounds *via* drinking water with adequate safety margins regardless of the assessment methods used and in view of the available analytical and toxicological data.

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6.2 Regulations

Commission Directive 92/18/EEC of 20 March 1992 modifying the Annex to Council Directive 81/852/EEC on the approximation of the laws of Member States relating to analytical, pharmacotoxicological and clinical standards and protocols in respect of the testing of veterinary medicinal products.

Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy.

Directive 2001/82/EC of the European Parliament and of the Council on the Community code relating to veterinary medicinal products.

Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use.

Commission Directive 2009/90/EC of 31 July 2009 laying down, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, technical specifications for chemical analysis and monitoring of water status.

6.3 Standards

NF X 50-110 (May 2003) Quality in Expertise - General Requirements of Competence for Expert Appraisals. AFNOR (classification index X 50-110).

APPENDICES

Appendix 1 – Formal request letter

Ministry of Health and Sports

Paris, 28 July 2009

Directorate General for Health
Sub-directorate for the Prevention of risks associated with the environment and food
Water Quality Bureau

DGS/EA 4 No 298

Person in charge of the dossier:
Géraldine GRANDGUILLOT
Tel 01 40 56 54 18 / Fax 01 40 56 50 56
Email: geraldine.grandguillot@sante.gouv.fr

The Director General for Health
to
The Director General of the French Food Safety Agency (AFSSA)
Department for the evaluation of nutritional and health risks / Water risk assessment unit

and

The Director General of the French Health Products Safety Agency (AFSSAPS)
Directorate for the assessment of medicinal and biological products / Department for risk assessment and surveillance, and information / Toxicology department

Subject: Assessment of the health risks associated with the presence of carbamazepine in water intended for human consumption (drinking water)

Our Ref: Dossier no 090025 (to be used in any correspondence) and related to:

1. DGS Dossier no 060004: request for scientific and technical support from the Director General for Health (DGS) to the French Food Safety Agency (AFSSA) for the drafting of a measurement protocol relating to the presence of pharmaceuticals in drinking water, dated 16 January 2006;
2. DGS/AFSSA Agreement (2006-2008) signed on 28 November 2006, for conducting research work on the topic of the health risks associated with the presence of active chemicals of human and veterinary drug origin in drinking water;
3. Correspondence of 18 July 2008 communicating the initial results of the measurement campaigns for pharmaceuticals in drinking water.

On the initiative of the DGS, exploratory “pilot” campaigns for measuring endocrine disruptors, with priority given to human and veterinary medicines with an endocrine disrupting effect in resources and drinking water, were implemented in three catchment areas, between 2006 and 2008, by the regional directorates for health and social affairs (DRASS) that coordinate these catchments, and with funding from the water agencies, in some cases.

At the same time, and in accordance with my request to AFSSA for scientific and technical support and the agreement signed between our organisations, dated 2006 and referenced above, this Agency, in conjunction with the French Health Products Safety Agency, defined the criteria for prioritising active chemicals of human and veterinary drug origin in the environment, is collecting reference data on these substances and is organising a national analysis campaign for these substances in resources and drinking water (the AFSSA Laboratory for study and research in hydrology).

In this context, the results of the exploratory campaigns have been sent to AFSSA, by correspondence dated 18 July 2008, mentioned in the reference, in order to obtain a factual summary of them and to take into account feedback from this initial "pilot" experiment for the development of the protocol for the national campaign.

These results reveal the widespread presence of carbamazepine, in particular, in drinking water, at relatively consistent concentrations from one catchment to another. As suggested by you, it does indeed seem relevant that this compound should act as a "sentinel", mainly due to its persistence in the environment, which was mentioned in particular in the report from the European KNAPPE* project, and be used as a "tracer" for the continuation of your agencies' joint work.

At this stage, I would like to again call on your joint expertise for the interpretation of the results of these exploratory measurement campaigns, in order to specify the health risks associated with situations in which carbamazepine is present in drinking water, with a view to defining management measures for these situations.

This initial health risk assessment, based on the results of the exploratory measurements for carbamazepine, will also enable you to test and refine the risk assessment methodology to be used for interpreting the results of the national campaign under way.

I hereby confirm, therefore, that the following dossier has been created and recorded at the DGS under the number 090025:

ASSESSMENT OF THE HEALTH RISKS ASSOCIATED WITH THE PRESENCE OF CARBAMAZEPINE IN WATER INTENDED FOR HUMAN CONSUMPTION

Jocelyne BOUDOT
Sub-Director for the Prevention of
risks associated with the environment and food

Appendix 2 – Uses and sources

1- Uses

A wide variety of compounds are used for therapeutic and medical diagnosis purposes. Their scope is very broad: medicinal products affecting metabolism and hormones, psychotropic drugs, antibiotics, anti-cancer drugs, etc. The wide range of therapeutic targets explains the variability of their chemical structure. This variability exists between different classes of drugs, but may also exist within each therapeutic category (e.g. neuroleptics or antidepressants). It generates very different physico-chemical properties (solubility, volatility, biodegradability, etc.) that determine their metabolism in the body and environmental fate.

In France, more than 3000 active substances are currently available in human medicine. The French consume comparatively large quantities of medicinal products, far more than their European counterparts.

With regard to veterinary medicinal products, more than 300 active substances are used. Two classes constitute the majority of these compounds: antibiotics and antiparasitics.

The consumption data available for these two types of uses are described in the AFSSA report on the prioritisation of pharmaceuticals of interest for the analysis of resources and treated drinking water (AFSSA, 2008).

2 - Sources and routes of entry of pharmaceuticals into the aquatic environment

Medicinal products for human and veterinary use differ in their chemical nature, the amounts used, the ways in which they are introduced into the environment, and their geographical distribution. The ways in which these substances are introduced into the water environment are shown in Figure 2-1, although for the exposure assessment, only their medical use (the main route of introduction) was taken into account.

Human medicines, after ingestion, injection or application to the skin, mucous membranes and skin appendages, are excreted in wastewater systems as the parent compound or metabolites. In the case of urban systems, which will combine waste from patients treated at home, at the workplace or in care establishments, the effluents loaded with residues are usually treated by a wastewater treatment plant or by an independent household sanitation unit before entering the aquatic environment. The efficiency and reliability of existing treatments will determine the level of risk for environmental release. Another route of introduction of these substances is improper disposal of unused or expired medicinal products directly into wastewater systems or household waste treated in non-hazardous waste storage facilities (landfill sites). The spreading of sludge from municipal and industrial wastewater treatment plants can also be a source of introduction into the aquatic environment.

Veterinary medicines concern both pets (1/3 of sales) and livestock (2/3 of sales), and are used for curative, preventive or zootechnical purposes. Drugs for pets are overwhelmingly administered in individual treatments and mainly in urban areas. Conversely, drugs for livestock are mostly used in mass treatment.

When veterinary drugs are eliminated (as the parent compound or metabolite) by the faecal or urinary route, they enter the environment immediately, when the animals are grazing, or after a delay, when manure and slurry are spread. A peculiarity of veterinary drugs is the existence of directly administered treatments in the environment. This is the case with veterinary drugs intended for aquaculture that are poured directly into breeding tanks. This is also the case with dilute solutions for dipping sheep: after use, the bath water is discharged into the surrounding environment. In all of these cases, veterinary medicines enter aquatic resources without first going through a treatment plant.

It is important to note that some compounds are used in both human and veterinary medicine; this is particularly the case with certain antibiotics (amoxicillin, erythromycin, etc.).

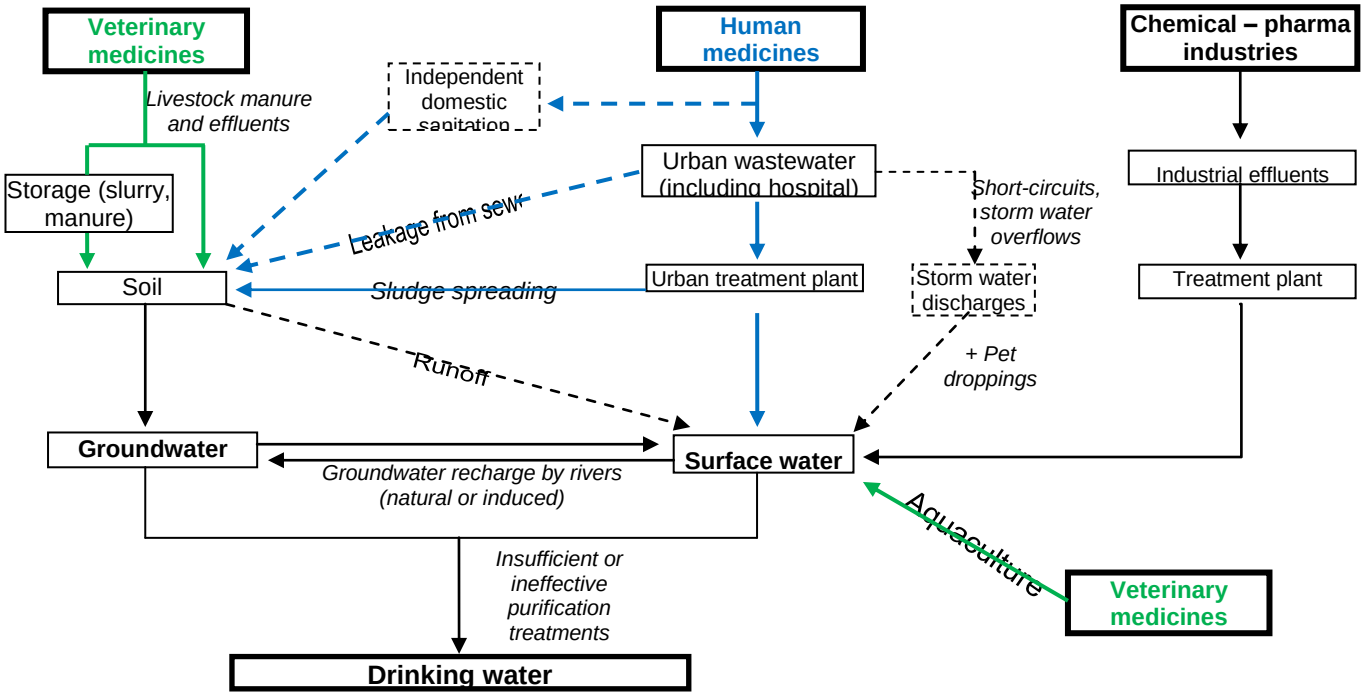
Finally, the plants that manufacture the active substances (human or veterinary drugs), which are usually equipped with treatment plants for their liquid waste, may also contribute to the introduction of pharmaceuticals into the aquatic environment. Mixing and packaging units, which generally work with dry ingredients, generate less liquid waste.

Other sources

It is possible that the presence of a compound in the environment may not be solely the result of the use of a medicinal product containing it. For example, some substances are both parent compound and metabolite, as is the case for example with oxazepam, which is used as such but is also the metabolite of many other benzodiazepines (clorazepate, diazepam, etc.).

Some compounds may also have non-medical uses that affect the concentrations likely to be found in the environment. This is particularly the case with antiparasitics used in veterinary medicine, products that constitute nearly a quarter of sales of veterinary drugs. Certain medicated antiparasitic active substances have also been developed as insecticides for use in plant health (Virlouvet, 2006).

It is also interesting to note the case of compounds with a natural source. This is the case with naturally excreted steroid hormones (oestradiol, progesterone, etc.), as well as with other compounds such as dopamine or salicylic acid.



Dotted line: minor routes (waste corresponding to solid waste other than sludge is not included in this figure); Green: drugs for veterinary use; Blue: drugs for human use; Black: medicines for human and/or veterinary use

Figure 2 - 1. Routes by which medicines for human and veterinary use are introduced into water intended for human consumption

Appendix 3 – Fate of pharmaceuticals in sewage systems, the environment and water treatment units

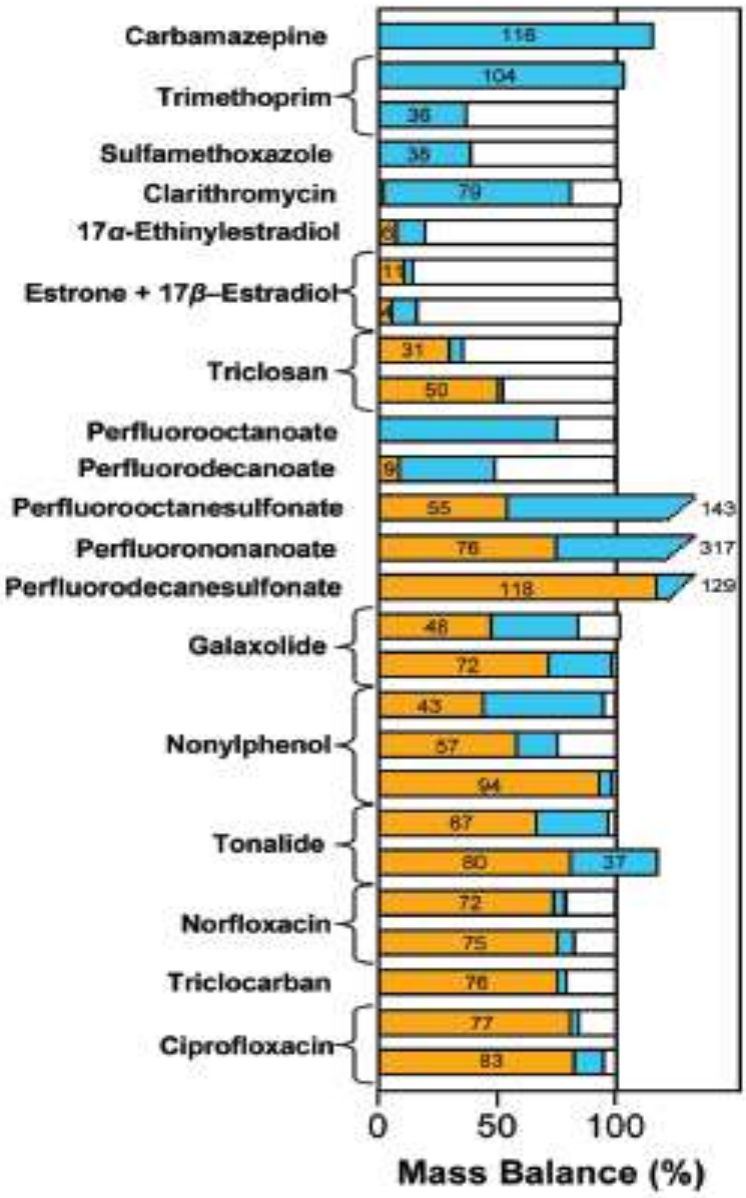
1- Fate of pharmaceuticals in the environment

The fate of pharmaceuticals in treatment plants or in the environment (soil, groundwater, surface water and sediment) depends on the compound's physico-chemical and environmental characteristics:

- The molecular structure, which can be used to predict certain degradation or transformation pathways,
- The volatility, described by the vapour pressure at 20°C and the Henry constant,
- The compound's mobility, described by its water solubility, ionisation potential (pKa), log D or Dow (water-soluble form at pH 7) and Kow partition coefficient (characterising its hydrophilic/hydrophobic nature),
- Interactions with the environmental components (water, soil and sediment matrices), which can modify the physico-chemical behaviour of pharmaceuticals.
    - *Adsorption onto organic matter* (in soil, water or activated sludge from wastewater treatment plants) expressed by the Koc value (Chefetz *et al.*, 2008; Poerschmann and Kopinke, 2001),
    - *Adsorption onto soil* expressed by the Kd: related to the soil's characteristics (texture), the presence or absence of clay, the particle size of the constituents (Gielen *et al.*, 2009; Kay *et al.*, 2005; Scheytt *et al.*, 2005; Yu *et al.*, 2009),
    - *The formation of complexes* with divalent cations (Ca^{2+}, Mg^{2+}) or the transition elements present in the environment (Fe, Mn, etc.) (Park *et al.*, 2002),
- Abiotic degradation processes:
    - *Hydrolysis,* described by the hydrolysis constant. The efficacy of the reaction depends on the functional groups present in the compound. Hydrolysis causes a partial chemical transformation and thus generates transformation products.
    - Direct or indirect *photodegradation,* described by the photodegradation half-life (or photolysis constant). Photodegradation processes cannot cause the complete mineralisation of the compound in environmental conditions. They therefore generate transformation products.
        - Direct photodegradation (sunlight absorbed by the compound): the parameters used to characterise the phenomenon are the UV-visible absorption spectrum and the photochemical efficiency (Meite *et al.*, 2010). The photochemical behaviour of the pharmaceuticals can vary greatly and the compound's chemical structure can give some indication of the nature of the degradation products formed.
        - Indirect photodegradation: environmental constituents such as organic matter or certain ions absorb sunlight and generate reactive species (oxidant states, free radicals, electrons, etc.) that can induce the transformation of compounds (Richard and Canonica, 2005). This transformation pathway is often overlooked because it is of lesser importance but it can generate different degradation products from those described for the hydrolysis, direct photodegradation or biodegradation pathways.

- Biodegradation processes
  - *Biodegradation* (aerobic or anaerobic) described by the half-life ($T_{1/2}$). This is the partial transformation (complete mineralisation is very rarely observed) of pharmaceuticals into intermediate transformation products (Heidler and Halden, 2008).
  - In surface water, most groundwater and wastewater treatment systems, the processes occurring tend to be aerobic, whereas in sediments, confined groundwater, sealed-off bank areas, private sewage pre-treatment systems and animal manure storage areas, the anaerobic pathway predominates.
  - The literature data show greatly varying half-lives that are not determined by the pharmacological class the compound belongs to. However, the presence of certain functional groups can give some indications about the transformation products formed.
  - Looking more closely at wastewater treatment systems, biodegradation is due to predominantly biological processes using aerobic means with either free cultures (e.g. activated sludges, lagooning) or fixed cultures (e.g. trickling filters, bioreactors). Over the past five years, research in this area has increased. A meta-analysis focusing mainly on the fate of drugs in sewage treatment plants was conducted in 2008 on more than a dozen publications (Heidler and Halden, 2008). Figure 3-1 summarises this synthesis, which is based on mass balances. The distribution between the solid phase (sludge, yellow histogram) and liquid phase (blue histogram) varies greatly, but as a general rule, pharmaceutical substances are found mainly in the liquid phase. Several studies show that biological treatment fosters the partial degradation of these substances. Identification of the transformation products is currently improving.
- Bioaccumulation
  - Bioaccumulation of pharmaceuticals may occur for a limited number of compounds (e.g. lipophilic compounds). This phenomenon that affects biofilms, algae or aquatic fauna can then enrich the sludge and sediment and cause the subsequent release of these compounds.

Some of these processes can cause the parent compounds to be reactivated from the metabolites. Indeed, in treated wastewater, some pharmaceuticals (e.g. carbamazepine) are sometimes found in higher concentrations when leaving the plants than when arriving. Even if this observation partly results from measurement uncertainty or failure to take into account the water's residence time in the plant (for sampling), it seems highly likely that the reformation of the compound in the plant by reactivation from its metabolites, for example (deconjugation), contributes significantly to this phenomenon (Farré *et al.*, 2008; Heidler and Halden, 2008).



Blue: fraction found in the treated water
White: fraction lost mainly by degradation
Orange: fraction found in sludge

Figure 3 - 1. Compilation of mass balances for organic compounds in wastewater published in peer-reviewed journals (from Heidler and Halden, 2008).

With regard to the transfer of drug compounds into groundwater and their fate there, as with all environmental processes, the physico-chemical characteristics are evidently crucial. Nevertheless various external factors can influence these transfers such as the input level of products (at the ground, surface or subsurface), and the way in which they are added: concentrated in the case of veterinary treatments in the field, or diluted when combined with surface water (groundwater recharge) or with liquid (e.g. treated wastewater) or solid effluent (e.g. spreading sludge).

The nature of the ground (mainly particle size and mineralogy) at the product application site is also crucial. Accordingly, adsorption on the soil is related to the soil's characteristics (texture), the presence or absence of clay and the particle size of constituents (Gielen *et al.*, 2009; Kay *et al.*, 2005; Scheytt *et al.*, 2005; Yu *et al.*, 2009). Moreover, according to Rauch-Williams *et al.* (2010), adsorption onto organic matter depends on the type of organic matter present in the soil.

If the product is added to the ground surface, before reaching the groundwater, it must infiltrate the unsaturated part of the aquifer and first of all the soil horizons. A delay in infiltration or retention of products may then be observed. Chefetz *et al.* (2008) thus report the retention of carbamazepine and diclofenac in the first 5 to 15 cm of soil. In tests on columns, Scheytt *et al.* (2006) also show a transfer delay factor[1] which is 1.84 for carbamazepine, 2.51 for propyphenazone, 3.00 for ibuprofen and 4.8 for diclofenac.

Conclusion

The physico-chemical and environmental characteristics (degradation half-lives) of a compound are essential for predicting its behaviour and fate in the environment. These parameters can be interrelated.

For each relevant compound it is crucial to have details of its physico-chemical characteristics and degradation half-lives in environmentally realistic experimental conditions that are relevant to the situations studied (surface water, groundwater, soil, etc.).

The possibility of reactivation of the parent compounds from the metabolites should also be considered.

Behaviour with respect to the drinking water treatment processes

In the drinking water treatment systems, organic products are eliminated from water in two main ways: by retention or transformation.

2.1 - Retention treatments: the compound is removed from the water, the main problem then becomes waste management

- *Selective or non-selective adsorption treatments:* adsorption onto an inorganic or organic substrate and bioadsorption onto biofilms
  - Physico-chemical clarification: adsorption onto iron/aluminium hydroxide floc or powdered activated carbon.
  - An understanding of the log K_{OW} related to the polarity of the compound and its ionic or molecular nature helps predict the efficacy of adsorption.
  - The adsorbed compound remains in the filter but can be biotransformed by selection of bacteria.
  - Biological clarification: adsorption onto biofilms expressed by the Kd: adsorbent (biofilm)/water partition coefficient (Jones *et al.*, 2002).
  - The likelihood of the compound being biodegraded is important. Biodegradation can lead to deconjugation of conjugated metabolites and the reactivation of the parent compound.
- Membrane retention treatments: nanofiltration or reverse osmosis.
- While for reverse osmosis most organic compounds with more than 6 carbon atoms are successfully retained, this is not true for nanofiltration, where the polarity of the compound, its molecular weight, vapour pressure and steric hindrance play a major role. Only tests on

[1] Expresses how much a contaminant is delayed in its transport in water due to its adsorption on organic matter in soil.

pilot plants can provide information on the relative efficacy of each type (brand) of membrane used.

- *Forced aeration treatments (stripping):* are used to transfer the volatile compound from the water to the atmosphere. The parameters to be taken into account are the Henry constant and the vapour pressure at 20°C or at the water temperature. The risk of secondary reactions in the compound to be removed is virtually nonexistent. Additional procedures may be needed to prevent air pollution.

2.2 - Transformation treatments: the compound is transformed, often partially, and enters the water with its degradation products

- Abiotic degradation processes:
  - Oxidation treatments. Disinfection treatments with ozone, chlorine or chlorine dioxide can lead to the formation of new compounds (ozonides, organochlorines, etc.). The importance of this route will depend on the pharmaceuticals' reactivity with the oxidising agent, the doses applied and the contact time. Studies should be done on a case-by-case basis to identify the degradation products.
  - Photodegradation reactions. UV radiation is sometimes used for water disinfection. At the radiation doses used (400 J/m^2), the risk of adverse reactions is often very low or even zero. However, this will depend on the sensitivity of the compound to UV radiation. Some photosensitive compounds will undergo significant photodegradation (Kim and Tanaka, 2009; Meite *et al.*, 2010). Studies should be conducted on a case-by-case basis to identify the photoproducts likely to form.
- Biodegradation processes:
  - The development of biofilms in various structures receiving water undergoing purification (sand or carbon filters, etc.) can enable biodegradation phenomena as described above.

Conclusion

Advanced treatment processes such as adsorption, biodegradation and/or retention, alone or in combination, can eliminate trace compounds, although their cost/benefit ratio remains to be determined. To do this, it is essential to know the reaction constants with chlorine or ozone type oxidants and UV radiation at wavelengths used in water treatment.

Studies are needed to evaluate the transformation products potentially formed and compile a list that may be required as part of the control and surveillance of drinking water quality.

Appendix 4 - Concentrations of pharmaceuticals in drinking water

In water resources (surface and groundwater), the observed concentrations generally range from the ng/L to several µg/L. Thus, in surface water, maximum concentrations of up to 10 µg/L for paracetamol (Kolpin *et al.*, 2002), 8.5 µg/L for iopromide (Perez and Barcelo, 2007) or 5 µg/L for ibuprofen (Hilton *et al.*, 2003) have been measured.

Concerning the occurrence of pharmaceuticals in water intended for human consumption (drinking water), relatively few studies are available in the international literature and most only concern active substances and not metabolites. Some of them have shown the presence of drugs in drinking water at concentrations that may exceed one microgram per litre for ibuprofen (Loraine and Pettigrove, 2006).

Table 4 - I gives a non-exhaustive list of the results of screening in drinking water for a number of drugs (international literature).

The results of international studies cannot, however, be easily transposed to the case of France. This is because the organisation of sanitation, as well as the use of medicinal products, may vary according to the country (Andreozzi *et al.*, 2003). As an example, with regard to overall consumption of drugs, France has the highest per capita sales of pharmaceutical products in Europe (Clerc *et al.*, 2006). Differences in the type of compounds consumed can also be observed between countries. These can be explained by:

- Consumption and prescription habits that are particularly influenced by national recommendations. For example, statins (lipid lowering) account for 11% of prescriptions in France and only 2% in Germany (Le Pen *et al.*, 2007).
- The prevalence of disease may differ greatly from one country to another. As an example, in 2000, the prevalence of diabetes was 2.9% of the population in France and 7.4% in Italy (Wild *et al.*, 2004).

Studies concerned with the presence of drugs in drinking water have been conducted in France. For instance, Bruchet *et al.* (2005) measured concentrations of 21 antibiotics and contrast agents in treated water in the Paris region. Only four contrast agents were quantified, with a maximum concentration of 60 ng/L for iopromide. Togola and Budzinski (2008) analysed 17 compounds in water from the south of France. Eight of these compounds were quantified in drinking water, at concentrations of up to 210 ng/L for paracetamol.

As part of the first French National Environment & Health Action Plan (PNSE 1) and at the request of the DGS, the regional directorates for health and social affairs (DRASS), in conjunction with the catchment agencies, conducted drug residue measurement campaigns in water used for the production of drinking water and in drinking water, in three catchment areas, between 2006 and 2008 (AFSSA, 2009b). Although these campaigns were unable to provide results representative of the national situation, it may be possible to use the maximum concentrations in a worst case scenario for the health risk assessment. However, these results are still difficult to interpret and need to be consolidated at the national level.

In 2009-2010, AFSSA conducted a national campaign (mainland France, Corsica and overseas départements) to analyse pharmaceuticals in water, with the financial support of the DGS. A prioritisation approach was developed and used to select 72 compounds of interest (active substances and metabolites) to be screened for in water (AFSSA, 2005). All the major therapeutic classes in human and veterinary medicine were represented. Analytical methods were developed and validated for all these substances. Over 500 samples were taken in resources (surface and groundwater) and drinking water to obtain a map of the presence of pharmaceuticals in these types of water in France.

Table 4 - I. Review of concentrations (ng/L) of certain drugs screened for in tap water around the world

Therapeutic use	Compound	Maximum concentration (ng/l)	Country	Reference
Hormone	**Ethinyloestradiol**	22.5 < 5 4 < 0.5 (LOQ) 0.5	Germany UK UK Germany Germany	Rurainski *et al.*, 1977 Aherne *et al.*, 1985 Aherne and Briggs, 1989 Ternes, 2001 Kuch and Ballschmiter, 2001
	Oestradiol	2.1 <1 (LOD)	Germany Canada	Kuch and Ballschmiter, 2001 Boyd *et al.*, 2003
Anti-cancer	**Cyclophosphamide**	< 0.02 (LOD) < 10 (LOQ) < 60 (LOD)	Italy Germany Canada	Zuccato *et al.*, 2000 Ternes, 2001 Tauber and Stevenson, 2003
	Bleomycin	13	UK	Aherne *et al.*, 1990
Antibiotic	**Erythromycin**	< 0.03 (LOD) < 100 (LOD) < LOQ	Italy USA France	Zuccato *et al.*, 2000 Stackelberg *et al.*, 2007 Bruchet *et al.*, 2005
	Tylosin	1.7 < LOQ	Italy France	Zuccato *et al.*, 2000 Bruchet *et al.*, 2005
Non-steroidal anti-inflammatory and analgesic	**Ketoprofen**	< 5 (LOQ) not determined < 90 (LOD) 3 8	Germany Taiwan Canada France Finland	Ternes, 2001 Lin *et al.*, 2005 Tauber and Stevenson, 2003 Togola and Budzinski, 2008 Vieno *et al.*, 2005
	Ibuprofen	< 0.5 (LOD) 3 not determined < 90 (LOD) 0.6 < 18 (LOD) 8.5 1350	Italy Germany Taiwan Canada France USA Finland USA	Zuccato *et al.*, 2000 Ternes, 2001 Lin *et al.*, 2005 Tauber and Stevenson, 2003 Togola and Budzinski, 2008 Stackelberg *et al.*, 2004 Vieno *et al.*, 2005 Loraine and Pettigrove, 2006
	Paracetamol	210.1 < 36 (LOD) < 9 (LOD)	France USA USA	Togola and Budzinski, 2008 Stackelberg *et al.*, 2007 Stackelberg *et al.*, 2004
	Diclofenac	2.5 < 10	France Germany	Togola and Budzinski, 2008 Heberer, 2002
Anti-epileptic	**Carbamazepine**	30 not determined 24 43.2 140 258	Germany Taiwan Canada France USA USA	Ternes, 2001 Lin *et al.*, 2005 Tauber and Stevenson, 2003 Togola and Budzinski, 2008 Stackelberg *et al.*, 2007 Stackelberg *et al.*, 2004
Contrast agent	**Iopamidol**	60 82	France France	Bruchet *et al.*, 2005 Paffoni *et al.*, 2006

LOD: Limit of detection; LOQ: Limit of quantification

Appendix 5 – Methods of analysing pharmaceuticals in water

There are currently no standardised methods for individual assaying of pharmaceutical compounds in water. However, in addition to numerous scientific publications and expert reports in this area, standardised multi-residue methods using solid-phase extraction (SPE) and analysis by high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) have been published by the US EPA (2007b) and more recently by the French consortium Aquaref, 2009.

Analysing drugs in water presents the same major challenge as that of analysing trace micropollutants such as pesticides, plasticisers, solvents, etc. and faces a number of difficulties, including:

- the wide variety of chemical classes and the need to screen for the metabolites and transformation products,
- the very low concentration levels,
- the presence of numerous interfering substances in water, especially in waste water.
- the limit of quantification (LOQ) depends on the compound, the analytical equipment, and also the method of calculating the LOQ. It is usually in the range of 1 to 50 ng/L for clean water.

In France, information collected as part of the inter-laboratory test developed by AFSSA[2] in 2009 revealed that, to date, about four laboratories have been accredited for the analysis of pharmaceuticals in water, but more than 20 private or public laboratories, offering analysis or research, have developed or are in the process of developing an analytical method (mainly HPLC/MS/MS). Expanded interlaboratory uncertainties (k = 2) vary in drinking water, from 47 to 157% depending on the compound.

[2] The inter-laboratory test organised by AFSSA in 2009 involved 31 laboratories and concerned 12 drug compounds.

Appendix 6 – Links mentioned in the experts' public declarations of interest

This section presents the links declared by the experts in their public declarations of interest. It states firstly how these links were analysed in relation to the field to which the formal request refers, and secondly how they have been managed with regard to any potential risks of conflict of interest.

The public declarations of interest are updated by the experts each time there is a change in their situation.

During the expert appraisals, the links of interest are reviewed in light of the agenda at the beginning of each meeting.

REVIEW OF THE SECTIONS OF THE PUBLIC DECLARATION OF INTEREST (PDI)

IF	Financial interest in the capital of a company
IP-A	Occasional services: others
IP-AC	Occasional services: consulting activities
IP-CC	Occasional services: conferences, seminars, training activities
IP-RE	Occasional services: expert reports
IP-SC	Occasional services: scientific work, tests, etc.
LD	Lasting or permanent relationships
PF	Financial participation in the capital of a company
SR	Other unpaid relationships (relating to a family member)
SR-A	Other unpaid relationships
VB	Activities resulting in a payment to the budget of an organisation

FOR THE EXPERT COMMITTEE

LAST NAME	**First name** ***Sections of the PDI*** Description of the interest	**Date of declaration of interests**
ANSES analysis:	*if declared relationship*	
ANDRES	**Yves**	04/01/2013
ANSES analysis:	*No declared relationship to the field of the request*	
BOUDENNE	**Jean-Luc**	08/12/2012
ANSES analysis:	*No declared relationship to the field of the request*	
CABASSUD	**Corinne**	20/03/2012
ANSES analysis:	*No declared relationship to the field of the request*	

CARRÉ	**Jean**	22/11/2012
ANSES analysis:	*No declared relationship to the field of the request*	
CHUBILLEAU	**Catherine**	28/01/2013
ANSES analysis:	*No declared relationship to the field of the request*	
CORREC	**Olivier**	03/12/2012
ANSES analysis:	*No declared relationship to the field of the request*	
DAGOT	**Christophe** ***IP-AC*** Management consulting - SIPIBEL	03/12/2012
ANSES analysis:	*No risk of conflict of interest relating to the topic of the formal request.*	
DUBROU	**Sylvie**	04/12/2012
ANSES analysis:	*No declared relationship to the field of the request*	
HÉDUIT	**Alain**	13/10/2011
ANSES analysis:	*No declared relationship to the field of the request*	
HUMBERT	**Jean-François**	23/11/2012
ANSES analysis:	*No declared relationship to the field of the request*	
JOYEUX	**Michel** ***LD*** Eau de Paris	03/12/2012
ANSES analysis:	*No risk of conflict of interest relating to the topic of the formal request.*	
LE BÂCLE	**Colette**	03/05/2011
ANSES analysis:	*No declared relationship to the field of the request*	
LE CANN	**Pierre**	29/11/2012
ANSES analysis:	*No declared relationship to the field of the request*	
LÉVI	**Yves** ***VB*** Médiflux research programme with thesis (Eau de Paris, SUEZ-Environnement + public consortium) Payments of apprenticeship taxes (Suez-Environnement, Sanofi, CCD, Pall, SITA)	03/12/2012
ANSES analysis:	*No risk of conflict of interest relating to the topic of the formal request.*	
MATHIEU	**Laurence**	29/08/2012
ANSES analysis:	*No declared relationship to the field of the request*	

MAZELLIER	**Patrick**	30/11/2012
ANSES analysis:	*No declared relationship to the field of the request*	
MUDRY	**Jacques**	28/11/2012
ANSES analysis:	*No declared relationship to the field of the request*	
PONTIÉ	**Maxime**	21/12/2012
ANSES analysis:	*No declared relationship to the field of the request*	
POURCHER	**Anne-Marie**	18/12/2012
ANSES analysis:	*No declared relationship to the field of the request*	
TARDIF	**Robert**	23/11/2012
ANSES analysis:	*No declared relationship to the field of the request*	
TREMBLAY	**Michèle**	19/11/2012
ANSES analysis:	*No declared relationship to the field of the request*	
WELTÉ	**Bénédicte** ***LD*** Eau de Paris	03/12/2012
ANSES analysis:	*No risk of conflict of interest relating to the topic of the formal request.*	

FOR THE WORKING GROUP

LAST NAME ANSES analysis:	First name ***Sections of the PDI*** Description of the interest *if declared relationship*	Date of declaration of interests
CASELLAS	**Claude** ***VB*** Research contract with Sanofi-Aventis (less than 0.5% of the team budget) *No risk of conflict of interest relating to the topic of the formal request.*	09/05/2011
CARRÉ	**Jean** *No declared relationship to the field of the request*	22/11/2012
CHIRON	**Serge** *No declared relationship to the field of the request*	26/05/2011
JOYEUX	**Michel** ***LD*** Eau de Paris *No risk of conflict of interest relating to the topic of the formal request.*	03/12/2012
LÉVI	**Yves** ***VB*** Médiflux research programme with thesis (Eau de Paris, SUEZ-Environnement + public consortium) Payments of apprenticeship taxes (Suez-Environnement, Sanofi, CCD, Pall, SITA) *No risk of conflict of interest relating to the topic of the formal request.*	03/12/2012
MAZELLIER	**Patrick** *No declared relationship to the field of the request*	30/11/2012
MONTIEL	Antoine *No declared relationship to the field of the request*	20/03/2012
ROSIN	Christophe *No declared relationship to the field of the request*	27/09/2010

French Agency for Food, Environmental
and Occupational Health & Safety
27-31 avenue du général Leclerc
F - 94701 Maisons-Alfort Cedex
www.anses.fr

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