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Ecotoxicological risk assessment linked to the discharge by hospitals of bio-accumulative pharmaceuticals into aquatic media: The case of mitotane

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The release of hospital wastewater into the urban sewer networks contributes to the general contamination of aquatic media by pharmaceutical residues. These residues include bio-accumulative pharmaceuticals that lead to increased risk for ecosystems because they can concentrate in organisms and food chains, and therefore reach toxic levels. In order to assess the ecotoxicological risks linked to this particular category of residues, we have developed a specific method, by combining a theoretical calculation of pollutant concentrations in organisms to estimate Body Residue (BR), and ecotoxicity biomarkers in fish cell lines, enabling the calculation of a Critical Body Residue (CBR). This method finally results in the calculation of a specific risk quotient ($Q_b = BR/CBR$), characterizing the risk linked to this type of pollutant. This method was applied to mitotane, a bio-accumulative pharmaceutical typically found in hospital wastewater, in the framework of an exposure scenario corresponding to the discharge of all the hospital wastewaters into the Rhone River which flows through the city of Lyon, France. This approach leads to risk quotients (Q_b and Q_{bg}) much higher than those found with the classical approach, i.e. $Q = PEC/PNEC$ (Predictive Environmental Concentration/Predictive Non Effect Concentration) = 0.0006. This difference in the appreciation of risk is important when using cytotoxicity as the criterion for measuring the toxicity of mitotane ($Q_b = 0.056$) and it is even greater when the criterion used is genotoxicity ($Q_{bg} = 6.8$). This study must be now consolidated by taking the biomagnification of the pharmaceuticals into consideration.

1. Introduction

Hospitals use a large variety of chemicals such as pharmaceuticals, radionuclides, disinfectants and detergents for health care, diagnostics, disinfection and research (Kümmerer et al., 1998). After application, some of these substances and non-metabolized drugs excreted by patients are found in hospital wastewaters

(Kümmerer, 2001; Langford and Thomas, 2009), and generally reach the municipal sewer network without preliminary treatment (Emmanuel et al., 2004). Thus pollutants from hospitals have been found in WWTP effluents (Brown et al., 2006; Langford and Thomas, 2009) as well as in surface waters (Sprehe et al., 2001). Among these pollutants, pharmaceuticals occupy a special place and are subject to an increasing number of studies (Langford and Thomas, 2009; Ort et al., 2010; Escher et al., 2011; Sim et al., 2011). These pharmaceuticals include bio-accumulative drugs that present a specific risk for aquatic ecosystems, because of their ability to concentrate in the organisms forming the trophic chain. A recent

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study showed that 15–20% of medicines used in hospitals are potentially bio-accumulative (Jean et al., 2012). Consequently, priority has been given to the study of 14 pharmaceuticals (Jean et al., 2012). In order to better assess the impact of these molecules on aquatic ecosystems, it is now necessary to elaborate a specific Ecological Risk Assessment methodology adapted to the discharge of these molecules into natural media.

In this article we present: (i) the steps involved in a specific methodology to assess ecological risks linked to the release of bio-accumulative pharmaceuticals in natural media, (ii) the result of its application to mitotane, one of the 14 bio-accumulative pharmaceuticals selected by Jean et al. in 2012, in a hospital discharge scenario in a large French city (case study).

2. Development of the specific ERA methodology

Our specific ERA (Ecological Risk Assessment) methodology has been developed according to the four steps defined by the United States *Environmental Protection Agency* (USE EPA) in its “Guidelines for Ecological Risk Assessment” (US EPA, 1998) (Fig. 1). This methodological framework corresponds to the international reference regarding ERA.

2.1. Formulation of the problem

The problem formulation phase is fundamental. It comprises the description of the scenario studied, the definition of priority objectives, and the formulation of the conceptual model (US EPA, 1998).

2.1.1. Description of the scenario studied

This description presents the different environmental targets potentially reached by the pollutants present in the hospital wastewater and the different related exposure pathways. Here, the main pathway of exposure concerns the discharge of the hospital wastewater into the urban wastewater, then into the WWTP, then into the river. Characterising the level of exposure of the target organisms therefore requires the assessment of the respective flows of the hospital wastewater, of the urban wastewater and of the river, in order to calculate the percentage of hospital wastewater in the river. This permits calculating the PEC (Predicted Environmental Concentration) and assessing the BR (Body Residue, i.e. the pollutant concentration in the body of the species studied). These flows can vary through a season, and even during the course of a day for urban and hospital wastewaters (Boillot et al., 2008). With concern

being given to aquatic organisms, we sought to evaluate the ecotoxicological risk for the most “critical” period, i.e. that corresponding to the maximum hospital discharge when the river is at its minimum flow level. Taking into account the bio-physico-chemical specificities of bio-accumulative molecules, we considered that the treatment of pollutants in the WWTP was non-significant regarding their elimination, which is a reasonably pessimistic assumption.

2.1.2. Definition of the priority objectives

In view of the scenario set out, the general objective of the assessment was the following: “the discharge of bio-accumulative pharmaceuticals into the public drainage system, then into the WWTP and then into the river, should not lead to effects on the organisms in the river, given their ecological, economic and societal importance”. Within this general objective, the decision was taken to first privilege the preservation of pelagic organisms living in the water column. This approach should be completed at a later stage by a risk assessment for organisms living in the sediment, given their contribution to the global ecological functioning of the river.

2.1.3. Formulation of the conceptual model

Fig. 2 summarises the different elements of the conceptual model, such as they emerge from previous formulations, and defines the sections dealt with in what follows.

3. Materials and methods

3.1. Characterisation of the exposure

The characterisation of exposure aims at determining the spatial-temporal contact between pollutants and target populations (US EPA, 1998). It includes the analysis of sources of pollutants, the transfer of the latter from their sources, and the distribution of pollutants in the environment. This analysis can be performed by using theoretical models of pollutant transfer and/or experimental results (Perrodin et al., 2011). This phase results in the determination of the PEC (Predicted Environmental Concentration).

3.1.1. Calculation of pharmaceutical concentrations in hospital wastewater

The concentration in hospital effluents is linked to the consumption of pharmaceuticals, modified by the excreted fraction, and the water consumption of the hospital of the town studied, calculated during the same period (Mullot et al., 2010).

$$\text{Effluent concentration} = \text{Quantity consumed} \times \text{Rate excreted} / \text{Volume of effluent}$$

3.1.2. Calculation of the pharmaceutical concentration in the river (PEC)

The pharmaceutical concentration in the river (PEC) is obtained by dividing the initial concentration of the hospital effluent by the dilution factor of this effluent in the sewer network of the city studied, then by the dilution factor of the latter in the river. This is carried out taking into account the conclusions of the “formulation of the problem” phase which follows: (i) there is no reduction of the bio-accumulative pharmaceutical load into the WWTP, (ii) the dilution calculation in the river is performed during the most unfavourable period, i.e. when the river is at its minimum flow level.

3.1.3. Calculation of the pharmaceutical concentration in the fish (BR)

The Body Residue (BR) corresponds to the product of the PEC multiplied by the BioConcentration Factor (BCF) ($BR = PEC \times BCF$).

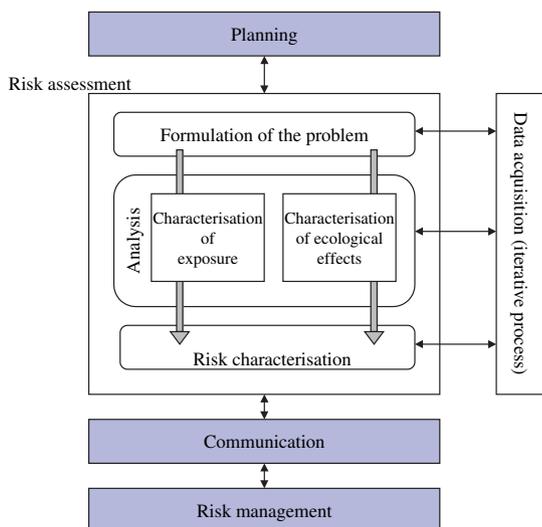


Fig. 1. General diagram of Ecological Risk Assessment (US EPA, 1998).

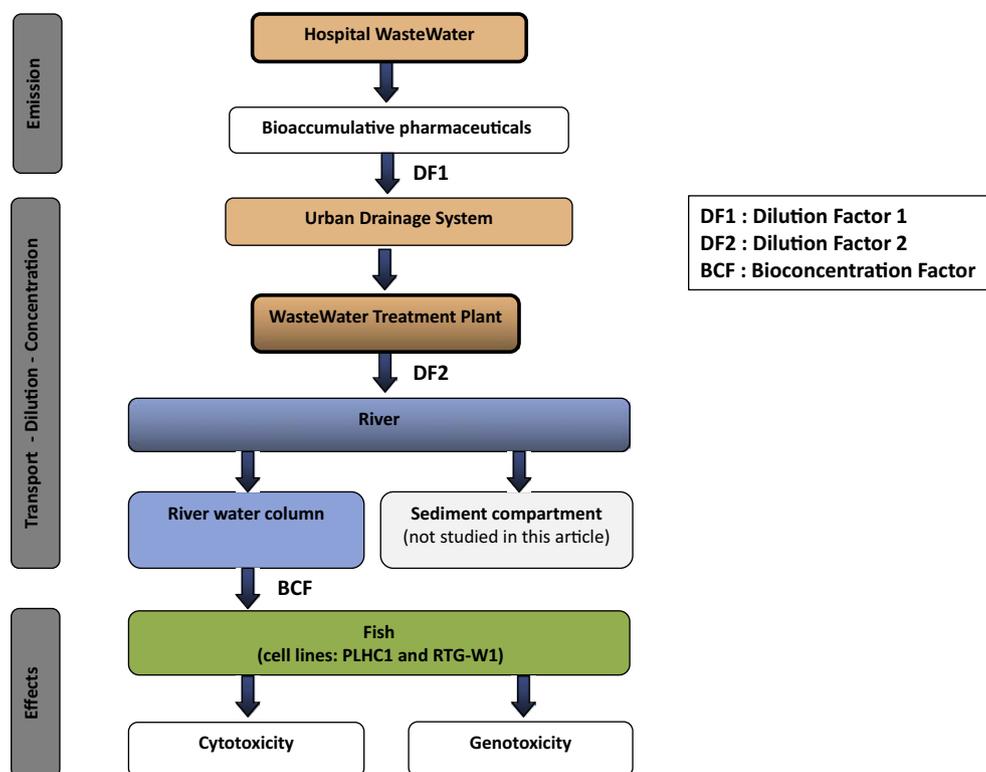


Fig. 2. Conceptual model of the scenario studied.

The BR is a unit of concentration in the body mass. The PEC is a unit of concentration in the water, and the BCF corresponds to the ratio of the concentration in the organism to the concentration in the water.

3.2. Characterisation of effects

3.2.1. Determination of the PNEC for the water column compartment

The Predicted No Effect Concentration (PNEC) is based on the result of bioassays. It is obtained from an EC10 or an EC20 value (Effect Concentration for respectively 10% or 20% organisms), or a NOEC (No Effect Concentration) or a LOEC (Lowest Effect Concentration) value, divided by a safety factor to offset the limits of an evaluation performed using only a few organisms. The recommendations in the Technical Guidance Document (TGD) of the European Chemical Bureau (ECB, 2003) can be used to adjust the value of these safety factors as a function of the nature and number of test results available.

3.2.2. Determination of the CBR for fish (experimental approach)

In this study, we chose to perform an experimental evaluation of the Critical Body Residue (CBR), which is a more realistic approach than a theoretical calculation. Our original approach was based on the identification of toxic concentrations for fish cell lines (gill and liver).

3.2.2.1. Fish cell lines. Two cell lines were used in this study. One was a model of the gill epithelium: RTG-W1 (ATCC, CRL 25-23) taken from adult rainbow trout (*Oncorhynchus mykiss*), a common freshwater fish in Europe (Bols et al., 1994). Gill cells are on the frontline of exposure to xenobiotics. The second cell line was a model of the liver epithelium. PLHC 1 (ATCC CRL-2406) is a *Poeciliopsis lucida* hepatocellular carcinoma cell line (Hightower and Larry Renfro, 1998). The cells were grown at 20 °C (RTG-W1) and at 30 °C (PLHC 1) in CO₂-free thermostatic incubators, in

Leibovitz-L15 medium, supplemented with 10%, FCS (Fetal Calf Serum) and with a mixture of antibiotics (penicillin and streptomycin).

3.2.2.2. Cytotoxicity assessment. The cytotoxicity method selected is the Cell Titer Blue method (Promega®, Charbonnières les Bains, France). The test uses the capacity of living cells to use an intracellular enzyme of the respiratory chain to convert a non-fluorescent substrate, resazurin, into resorufin. The intensity of fluorescence measured is proportional to the number of living cells. Cytotoxicity is characterized by Effect Concentrations 10 and 50 (EC₁₀, EC₅₀).

The percentages of viability were calculated as relative to the “control” cells not exposed to the pharmaceutical, according to the following formula:

$$\text{Viability (\%)} = \frac{\text{Fluorescence}_{\text{Dose}}}{\text{Fluorescence}_{\text{Control}}} \times 100$$

The range of pharmaceutical exposures must include the value under acute exposure, modelled with ECOSAR software, and those published in the document of the Canadian Ministry of the Environment and Health for the assessment of the environmental impact of chemical molecules.

In the case of mitotane, these data indicate an EC₅₀ of 22.8 mg L⁻¹ for fish, an EC₅₀ of 21.0 mg L⁻¹ for *Daphnia magna*, and an EC₅₀ of 60 mg L⁻¹ for algae. Due to the low solubility of mitotane in water, ethanol was chosen as solvent. A stock solution of 100 mg L⁻¹ of mitotane in absolute ethanol was first prepared and subsequent dilutions in complete L-15 medium were produced so that the maximum quantity of ethanol in the culture medium did not exceed 0.1%, i.e. below the toxicity threshold previously tested for the cell lines used. Cells were seeded at 200000 cells mL⁻¹ in 96 black multiwell plates (Fluonunc) 24 h prior to exposure. Then the medium was removed and replaced by the complete medium prepared with the various mitotane concentrations for the next 24 h (24 h exposure) before the CTB test was performed according to the manufacturer’s instructions. Three

independent experiments were conducted so that each included six internal replicates per plate (6 wells per dose).

3.2.2.3. Genotoxicity assessment. Genotoxicity was assessed in the form of primary DNA damage using the alkaline Comet assay (Singh et al., 1988) on the PLHC1 cell line in order to verify the existence of a possible genotoxic effect at sub-cytotoxic levels. Cells were seeded in transparent 24 well-plates 24 h prior to exposure and were exposed for the subsequent 24 h in complete L15 medium prepared with a range of sub-cytotoxic mitotane concentrations (0, 10, 50, 100, 1000 and 10000 ng L⁻¹). The Comet assay was performed in its modified version, including a digestion step with the Fpg restriction endonuclease (Formamido pyrimidine glycosylase) to enhance sensitivity. The assay was performed according to Kienzler et al. (2012). Comet scoring by image analysis was performed on 100 cells per condition (% tail intensity) and three independent replicates were performed.

3.3. Characterisation of the risk

3.3.1. Classic risk quotient Q calculation ($Q = PEC/PNEC$)

There is a wide range of possible methods of varying complexity for carrying out risk characterisation. The choice depends on the operational constraints and the available data. Rivière (1998) notes that “ecological risk can be expressed in various manners: qualitative (absence or not of risk), semi-quantitative (weak, average and high risk), in probabilistic terms (the risk is x%)”. The method known as “the quotient method” is the most widespread method for the semi-quantitative characterization of risks. Moreover, this method is that applied most often in ERA applied to residues of pharmaceuticals in water (Verlicchi et al., 2012a,b). This method consists in calculating the ratio (or risk quotient) which is expressed as a “Predicted Environmental Concentration” (PEC) divided by a “Predicted No Effect Concentration” (PNEC). When the risk quotient value “Q” is greater than 1, the risk is considered as significant, and becomes more extreme as the risk quotient increases. Conversely, the more the risk quotient falls below 1, the more the risk is regarded as low. This risk quotient method was used in our methodology.

3.3.2. Risk quotient Q_b calculation ($Q_b = BR/CBR$)

For the bio-accumulative molecules, we decided to calculate a risk quotient (Q_b), complementary to the previous one, equal to the concentration present in the organism (BR), divided by the concentration without effect (CBR): $Q_b = BR/CBR$.

4. Results

The methodology formulated above was applied to mitotane, one of the priority bio-accumulative pharmaceuticals identified by Jean et al. (2012). Mitotane is used in the last line treatment of non-surgical adenocortical carcinomas. It is a good representative of bio-accumulative hospital pharmaceuticals and their related environmental problems. This drug is not prescribed outside hospitals. Its low bioavailability to humans (38% absorption) suggests that a large part of mitotane is excreted unchanged in the hospital effluents. On a structural basis it is very close to the insecticide DDT (differing by only one chlorine atom) suggesting a potential impact on the environment.

The main data on mitotane are presented in Table 1.

The methodology formulated was implemented in a scenario corresponding to all the hospital discharges of the city of Lyon in France.

The numerical data of this scenario are presented in Table 2.

Table 1
Main data referring to mitotane.

Molecular weight	320 g mol ⁻¹
Henry constant	8.17 × 10 ⁻⁶ atm m ³ mol ⁻¹ (can be considered as non-volatile)
Water solubility	0.1 mg L ⁻¹ at 25 °C
Organic solvent solubility	Soluble in ethanol, DMSO, CarbonTetrachlorine
Partition coefficient	5.19
BCF	7330
Half-life (human)	189 d

4.1. Characterisation of exposure

4.1.1. Mitotane concentration in hospital effluent and in domestic effluent

When considering all the hospitals of the area studied, the total quantity of mitotane consumed is 1828 g year⁻¹. According to Attivi et al. the proportion of mitotane excreted unchanged in the faeces is about 64% of the dose administered (Attivi et al., 2010). On this basis, the dose released in hospital effluents is therefore 1170 g mitotane year⁻¹ (1828 g × 0.64), i.e. 3.206 g d⁻¹. In addition, the daily volume of hospital effluents is 3172 m³ d⁻¹ (0.61 × 5200). Therefore, the estimated concentration of mitotane in hospital effluents is 1.0 µg L⁻¹ (3.206 g/3 172 000 L).

The daily volume of domestic effluent, calculated on the basis of water consumption in the urban area of Lyon, is 106 815 000 L d⁻¹ (150 L inhabitant⁻¹ d⁻¹ × 712 100 inhabitants). Therefore the concentration of mitotane in domestic wastewater is on average 30 ng L⁻¹ (3206 g d⁻¹/106 815 000 L d⁻¹).

4.1.2. Mitotane concentration in the Rhône River (PEC)

The flow rate of the Rhône River during the low flow season is 800 m³ s⁻¹ (69 120 000 m³ d⁻¹). This results in a concentration in the river of 0.0464 ng L⁻¹ (30 ng L⁻¹ × 106 815 m³ d⁻¹/69 120 000 m³ d⁻¹), that is to say a PEC of 46.4 pg L⁻¹.

4.1.3. Mitotane concentration in fish (BR)

The calculation of the BR from the PEC and the BCF of mitotane gives a BR value of 340.1 ng kg⁻¹ (0.0464 × 7330).

4.2. Characterization of effects

4.2.1. Determination of PNEC

Only one document provides EC₁₀ and EC₅₀ values for mitotane, with three different trophic levels (Environnement Canada, 2009). They are available modelled data for chronic exposure in micro-algae and daphnia, and modelled fish acute exposure data. The most sensitive species is fish, for 96 h exposure with an EC₅₀ value of 1.8 µg L⁻¹. Taking into account the European TGD recommendations (ECB, 2003), we propose using a safety factor of 25 to determine the PNEC of this molecule. According to these recommendations, a safety factor of 100 must be used with two chronic tests and a safety factor of 10 must be used with three chronic tests (and three different trophic levels). As we obtained

Table 2
Numeric data of the studied scenario.

Number of inhabitants in the geographical area studied	712 100
Daily water consumption per inhabitant	150 L (inhabitant d) ⁻¹
Daily water consumption per hospital bed	0.61 m ³ d ⁻¹
Number of hospital beds in Lyon (www.chu-lyon.fr)	5200
Annual consumption of mitotane	1828 g year ⁻¹ (in 2009)

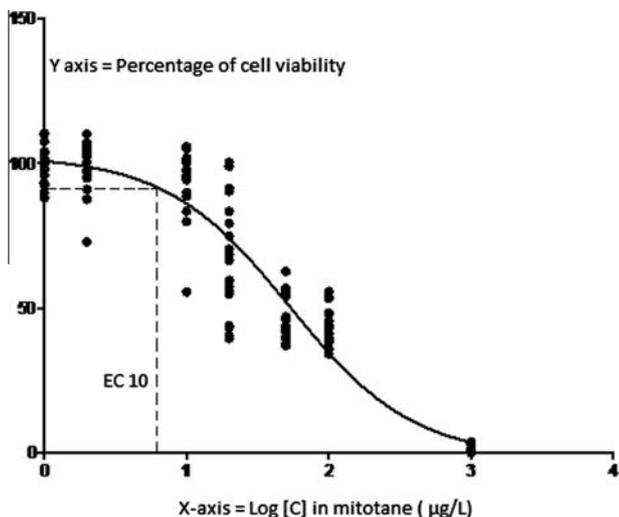


Fig. 3. Cytotoxicity of mitotane assessed by the Cell Titer Blue test on the PLHC1 cell line ($n = 3$), 24-h exposure (Hill Model, GraphPad[®]).

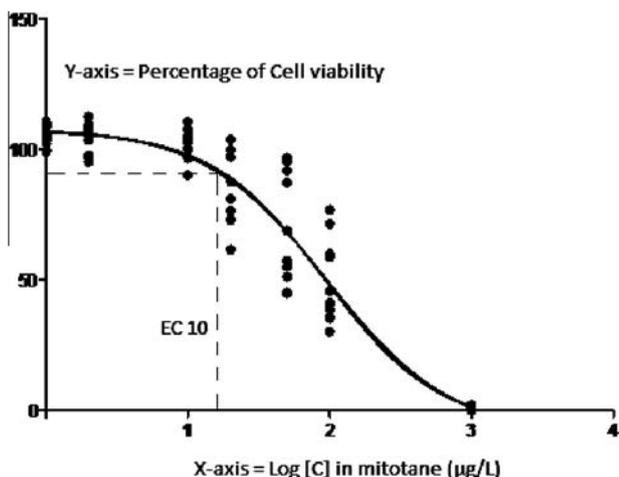


Fig. 4. Cytotoxicity of mitotane assessed by the Cell Titer Blue test on the RTG-W1 cell line ($n = 2$), 24-h exposure (Hill Model, GraphPad[®]).

ecotoxicological results for two chronic tests and one acute test, a safety factor of 25 is proposed. Thus the PNEC was equal to $0.072 \mu\text{g L}^{-1}$ ($1.8/25$), that is to say 72.0 ng L^{-1} .

4.2.2. Determination of CBR

The Critical Body Residue (CBR) was estimated experimentally on fish cell models, using two toxicity criteria: cyto- and genotoxicity.

4.2.2.1. *Cytotoxicity.* Figs. 3 and 4 show the results obtained with the two cell lines tested (RTG-W1 and PLHC 1). In both cases, there is a cytotoxic effect which increases as a function of mitotane concentration. Modelling the results obtained by the residue method led to sigmoid curves, allowing the EC_{10} and the EC_{50} values calculation.

The lowest EC_{10} value, $6.06 \mu\text{g L}^{-1}$, was used for the assessment of CBR.

4.2.2.2. *Genotoxicity.* Fig. 5 shows the primary damage to DNA measured by the Fpg-modified comet assay (percentage of DNA in the comet tail) after 24 h exposure of the PLHC1 cell line to mitotane. Damage increases as a function of concentration. The average damage level is 2.8% for the control and 9.7% for the $10 \mu\text{g L}^{-1}$ concentration. The result of the Wilcoxon test performed to compare each level of damage to unexposed cells shows that the first exposure value causing significant DNA damage is " 50 ng L^{-1} ".

4.3. Risk characterisation

4.3.1. Calculation of the risk quotient "Q"

The risk quotient "Q" (PEC/PNEC) determined using this method was equal to $6.44 \cdot 10^{-4}$ ($0.0464/72.0$), indicating a very low ecotoxicological risk to the organisms in the river.

4.3.2. Calculation of risk quotients "Qb" and "Qbg"

Risk quotient "Qb" (BR/CBR) was equal to 0.056 ($340/6060$) indicating a moderate risk to fish. This risk is significantly greater than that previously assessed with the PEC/PNEC method.

On the contrary, risk quotient "Qbg" (BR/CBR) calculated on the basis of the genotoxicity-toxicity criteria was equal to 6.8 ($340/50$), indicating a important genotoxicological risk to fish.

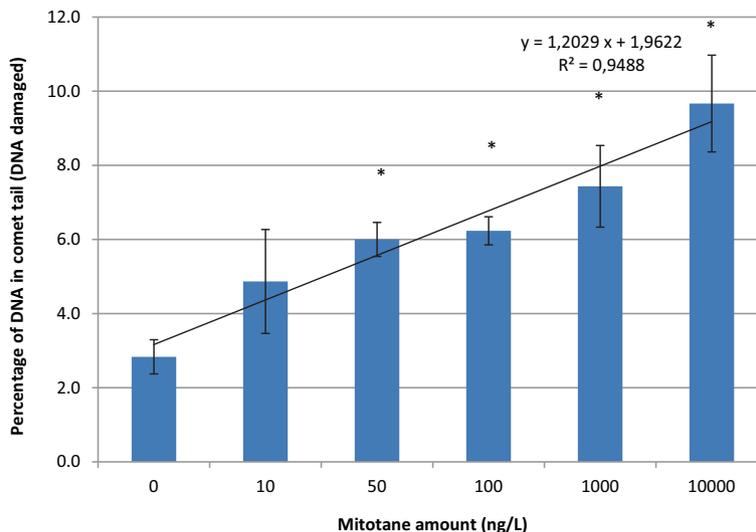


Fig. 5. Evaluation of the genotoxicity of mitotane on the PLHC 1 cell line after 24 h exposure ($n = 3$, $*p < 0.05$).

5. Discussion

5.1. The results obtained

5.1.1. Characterisation of exposure

The exposure assessment includes several successive steps, each of which has limitations: (i) The assessment of emissions, based on the consumption of pharmaceuticals in the hospital, does not take into account the possible share of pharmaceuticals consumed outside the site. (ii) The assessment of the concentration in the urban network, based on the dilution factor of the hospital effluent in the wastewater network, does not take into account the possible degradation of pharmaceuticals in the latter, which often acts as a biological reactor (Bryan Ellis and Yu, 1995; Coulibaly et al., 2002; Matthijs et al., 1995; Tanaka and Hvitved-Jacobsen, 1998). This can lead to overestimating the concentration of the pharmaceuticals at the inlet of the WWTP. In addition, the production of metabolites is excluded, although they are sometimes more toxic than the parent molecules (Kisanga et al., 2005; Landrum et al., 2003). (iii) The assessment of the concentrations downstream from the WWTP was carried out with the hypothesis of no reduction of pharmaceutical concentration in the latter (Miège et al., 2009; Gros et al., 2010; Verlicchi et al., 2012a,b). Nevertheless, a small proportion of some pharmaceuticals could be eliminated from the flow of pollutants discharged into the river. (iv) The evaluation of the pharmaceutical concentration in the water column, based on the dilution factor of the plant effluent in the river, does not take into account the probable adsorption of a fraction of certain bio-accumulative pharmaceuticals on the particles of the effluent, and therefore their sedimentation in the river (Lahti and Oikari, 2011; Löffler and Ternes, 2003; Lopez-Serna et al., 2012; Roberts and Thomas, 2006; Yang et al., 2011). Except for the production of toxic metabolites, these approximations all go in the same direction: the potential overestimation of exposure. Therefore this theoretical calculation is sufficient if the study concludes on a non-significant risk. Otherwise, the assessment should be specified for each step mentioned.

5.1.2. Characterisation of effects

The PNEC determined for mitotane was 72.0 ng L^{-1} . This value is in the same range as the PNEC established for other pharmaceuticals in hospital and urban effluents (Ferrari et al., 2003; Santos et al., 2007a; Thomas et al., 2007; Grung et al., 2008; Lin et al., 2008).

Mitotane cytotoxicity and genotoxicity are high for the fish cell lines tested. This results in a low calculated CBR related to the CBR found in the literature for other biological models and other pollutants (Lotufo, 1998; Tauxe-Wuersch et al., 2005; Thomas et al., 2007; Penttinen et al., 2011; Chandler et al., 2012).

The original approach developed in this study, based on the use of cell cultures, has many advantages compared to the theoretical approach generally proposed: (i) it is based on a real measurement and is therefore more realistic; (ii) it allows compensating for the lack of data on the EC_{10} and EC_{50} of many pharmaceuticals, especially bio-accumulative pharmaceuticals; (iii) it allows distinguishing the most vulnerable organs.

Several limitations have nevertheless been reported concerning the approach developed: (i) chronic genotoxicity, which could lead to even greater effects; (ii) its failure to take into account bio-magnification in trophic chains, which may also lead to an increased effect (Gobas et al., 1999; Zuccato et al., 2006; Kelly et al., 2009); (iii) its failure to take into account potential interactions between molecules, whether bio-accumulative or not, which can lead to synergistic or antagonistic phenomena (Lewis, 1992; Kortenkamp

and Altenburger, 1998; Emmanuel et al., 2005; Boillot and Perrodin, 2008).

5.1.3. Characterisation of risk

Risk quotients calculated with the three methods (Q, Qb and Qbg) are very different. Risk quotient "Qb" was 100 times higher than risk quotient "Q". This difference was even greater than with the risk quotient "Q" calculated for the entire aquatic ecosystem, whereas risk quotient "Qb" only concerned fish. This underlines the urgent need to take into account bio-accumulation in risk assessment studies. The difference between value "Qb" and value "Qbg" (this value was also 100 times higher) highlights the interest of taking into account genotoxicity parameters in effect assessment. In the case of mitotane, the use of such sub-acute criteria emphasises a significant risk for the scenario studied. Finally, the risk quotients calculated for mitotane highlight the ecotoxicological risks related to this molecule but also potentially related to other bio-accumulative pharmaceuticals. For these molecules, the risk quotients could also be higher than those obtained with the "classic" method for hospital effluents in several recent studies (Escher et al., 2011; Verlicchi et al., 2012a,b).

To conclude on the risk of the discharge of mitotane into rivers, it is important to note that: (i) the risk will be even greater if the discharge is done in a smaller river (the flow rate of the river which passes through the city of Lyon is very high), or in lentic ecosystems characterized by a low water renewal rate, (ii) the risk is closely related to the chemotherapy protocol applied in the hospital concerned (amount of mitotane used higher or lower than in our scenario, or even an absence of use).

5.2. The methodology developed

The strengths of the methodology developed are its ability to highlight the risks associated with the bio-accumulation of pharmaceuticals that have been relatively neglected by conventional approaches such as "PEC/PNEC". In some studies the "BR/CBR" risk quotient is used to assess risks for aquatic and terrestrial ecosystems, but generally, the evaluation of BR is founded on theoretical bases. Moreover, no existing study takes into account genotoxicity as a toxicity criterion despite the fact that many anticancer drugs exhibit such biological effects.

The weaknesses of the present methodology are stated, as is often the case in risk assessments, with the numerous approximations made during the various stages of evaluation. Nevertheless, the important point here is not the accuracy of the numerical value of the final result, but the identification and the prioritization of critical points in the scenario studied, about which it is possible to take measures to reduce the risk (nature and consumption of bio-accumulative and/or genotoxic pharmaceuticals, importance of respective flow rates of hospital effluents, municipal wastewater and rivers, etc.).

6. Conclusions

This study showed that it is possible to perform an initial assessment of the ecotoxicological risk linked to the discharge by hospitals of bio-accumulative pharmaceuticals into aquatic media, by using relatively accessible investigation resources. Other ecotoxicological risk assessment approaches have been proposed to characterise the risks related to different families of pollutants present in hospital wastewaters (Halling-Sørensen et al., 2000; Ferrari et al., 2004; Han et al., 2006; Santos et al., 2007b; Escher et al., 2011). Our approach is complementary as it takes into account the bio-accumulation of pharmaceuticals, which can sometimes be considerable.

The approach presented can, and must, be improved further in several ways. It is particularly necessary to improve the “exposure assessment” phase, in this case only carried out according to theoretical bases. This optimization is especially necessary if the risk assessed with the theoretical approach is significant. In addition, experimental tests to measure the real mitotane bio-accumulation in fish should be carried out. Biomagnification tests with laboratory trophic chains, such as the “micro-algae/daphnia/fish” chain must also be performed. Concerning the “effect assessment” stage, the evaluation of short-term genotoxicity (24 h exposure) should be supplemented by a longer study to obtain a more reliable evaluation of the genotoxic impact of mitotane. In addition, research works on the toxicity of mitotane on other cell lines (stemming from other organs and other fish species) should be carried out to consolidate the results.

The method developed will then be tested on other bio-accumulative pharmaceuticals on the list established by Jean et al. (2012). In addition, it could also be applied to household effluents as they also contain pharmaceuticals, some of which are bio-accumulative (Verlicchi et al., 2010).

Lastly, as mentioned in the formulation of the problem, the methodology developed here does not take into account risks linked to organisms living in the sediment compartment of the river. Therefore it is now necessary to undertake further work on this subject.

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