Arch Environ Contam Toxicol (2008) 55:576–583
DOI 10.1007/s00244-008-9145-2

Toxicity Assessment of the Herbicides Sulcotrione and Mesotrione Toward Two Reference Environmental Microorganisms: *Tetrahymena pyriformis* and *Vibrio fischeri*

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Received: 1 August 2007 / Accepted: 8 February 2008 / Published online: 6 March 2008

Abstract The potential toxicity of sulcotrione (2-[2-chloro-4-(methylsulfonyl)benzoyl]-1,3-cyclohexanedione) and mesotrione (2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione), two selective triketonic herbicides, was assessed using representative environmental microorganisms frequently used in ecotoxicology: the eukaryote *Tetrahymena pyriformis* and the prokaryote *Vibrio fischeri*. The aims were also to evaluate the toxicity of different known degradation products, to compare the toxicity of these herbicides with that of atrazine, and to assess the toxicity of the commercial herbicidal products Mikado® and Callisto®. Toxicity assays involved the Microtox test, the *T. pyriformis* population growth impairment test, and the *T. pyriformis* nonspecific esterase activity test. For each compound, we report original data (IC_{50} values) on nontarget cells frequently used in ecotoxicology. Analytical standards sulcotrione and mesotrione showed no toxic effect on *T. pyriformis* population growth but a toxic influence was observed on nonspecific esterase activities of this microorganism and on metabolism of *V. fischeri*. Most of the degradation products studied and the two commercial formulations showed a greater toxicity than the parent molecules. Compared with the effect of atrazine, the toxicity of these triketonic herbicides was less than in *T. pyriformis* and greater than or the same as in *V. fischeri*. Additional work is needed to obtain a more accurate picture of the environmental impact of these herbicides. It will be necessary in future experiments to study the ecosystemic levels (aquatic and soil compartments) and to assess the potential toxicity of the newly discovered degradation products and of the additives accompanying the active ingredient in the commercial herbicidal formulations.

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Introduction

Environmental risk assessment of chemicals is a major challenge arising from the intensive production and use of numerous substances considered as potential pollutants. This is particularly the case for herbicides, which are used in large amounts in agricultural, urban and domestic applications. For example, in 2001, 111,833 tons of herbicides was sold in the EU and 32,122 tons in France. In 1999, 64,702 tons of selective herbicides was used for the treatment of crops (mainly cereals and maize) in the European Union (EU) and 27,941 tons in France (http://www.epp.eurostat.ec.europa.eu). These agents can pollute nearby aquatic ecosystems through spray drift, soil leaching, or runoff, potentially leading to nontarget effects. The contamination of aquatic ecosystems by pesticide is therefore an environmental concern.

Various pesticide indicators are used in common procedures to evaluate the potential risk to aquatic ecosystems. These indexes include rate of application, environmental distribution, and soil persistence.

Numerous biotests have been developed to screen the potential toxicity of xenobiotics taking into account various criteria such as death, immobility, growth, behavior, physiological functions, and, more recently, molecular

biomarkers. Among these biotests, two unicellular organisms are considered as representative environmental (aquatic) microorganisms used in rapid bioassays: the marine luminescent bacterium *Vibrio fischeri* and the freshwater ciliated protozoan *Tetrahymena pyriformis*.

The *V. fischeri* bioluminescence inhibition assay (Microtox test) is a widely used standardized toxicity test. Its particularity consists on only small amounts of substance for several replicate tests and is not time-consuming (Kaiser and Palabrica 1991). This test is used to screen the toxicity of pure chemicals, complex mixtures, or natural samples toward a sample of prokaryotic nonphotosynthetic organisms that play critical roles in nutrient cycling in different environmental ecosystems. Significant interspecies relationships have been obtained, for certain classes of substances, between bacteria test data for *V. fischeri* and bioassay data for other organisms (Kaiser 1998).

T. pyriformis is a member of the heterotrophic free-living microfauna of aquatic ecosystems that occupies one of the first trophic levels. Together with other ciliates, it plays a key role in the transfer of matter and energy within the microbial loop and is an early warning indicator of pollution. Moreover, it is a true eukaryotic cell that can be easily cultured axenically with a short generation time in a small volume of a complex culture medium. Numerous studies have highlighted this organism as a good alternative cellular model for in vitro toxicological assessment of various chemicals including pesticides, heavy metals, pharmaceutical drugs, and organic compounds (Nilsson 1989; Sauvant et al. 1999; Bogaerts et al. 2001; Bonnet et al. 2003, 2007; Schultz et al. 2005). It has been proven to be at least as sensitive as other models commonly employed, such as established cell lines (Sauvant et al. 1994, 1995).

Until recently, atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) (Fig. 1), a member of the triazine class of herbicides, was used as a herbicide for maize crops. It was widely used as a pre- and postemergence herbicide to control broad-leaved weeds and annual grasses on both agricultural and nonagricultural soils. This compound competes with plastoquinone II at its binding site on the D1 protein, blocking electron transport from photosystem II to photosystem I (Ebert and Dumford 1976; Purcell et al. 1990). Numerous studies have shown that atrazine is toxic to bacteria and fungi in the soil (Stratton 1983; Tu 1992) and to organisms (algae, copepod, shrimp, and eastern oyster) in aquatic ecosystems (deNoyelles et al. 1982; Ward and Ballantine 1985; Pratt et al. 1988; Hoagland et al. 1993). Owing to its persistence and mobility in soils, atrazine can be found in natural aquatic environments. In France, atrazine is one of the most significant water pollutants in surface and ground water and was banned in June 2003.

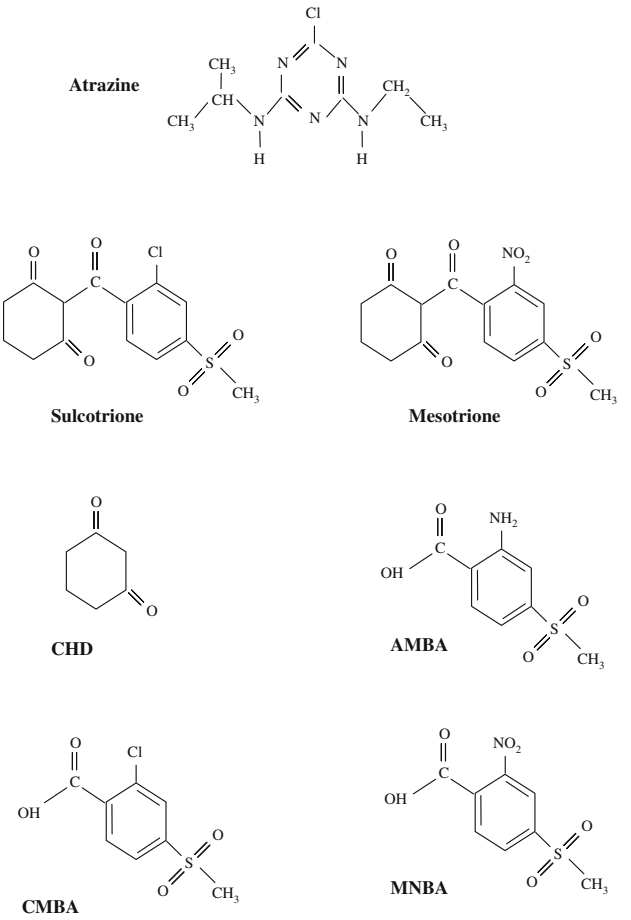

Fig. 1 Molecular structure of the herbicides and their respective degradation products

Since atrazine was banned in several European countries (although it is still widely used in the United States), new selective herbicides have been developed. Among these, the triketone class of herbicides, mainly sulcotrione (2-[2-chloro-4-(methylsulfonyl)benzoyl]-1,3-cyclohexanedione) and mesotrione (2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione), is now frequently employed as atrazine substitutes in maize crops.

Sulcotrione (Fig. 1) was introduced by Zeneca Ag Products (now Syngenta Crop Protection) and registered for use in France in 1993 (Mitchell et al. 2001). It is currently marketed in Europe by Bayer CropScience SA under the trade name Mikado®. This is a foliar-applied postemergence herbicide with a high efficacy against dicotyledonous weeds and barnyard grass. Sulcotrione affects photosynthesis by inhibiting the enzyme *p*-hydroxyphenylpyruvate dioxygenase (p-HPPD) (Schulz et al. 1993). In photosynthetic organisms, p-HPPD is a component of the biosynthetic pathway that converts tyrosine to plastoquinone and α-tocopherol (Norris et al. 1995). As a cofactor for the enzyme phytoene desaturase, plastoquinone is essential for

carotenoid biosynthesis. The subsequent decrease in carotenoid concentrations causes bleaching of foliage, necrosis, and death of sensitive plants (Wichert et al. 1999). The cellular metabolism of sulcotrione produces CMBA (2-chloro-4-methylsulfonylbenzoic acid) in plants and hydroxysulcotrione in mammals. In soil the best-known degradation pathway is hydrolysis to CMBA and CHD (1,3-cyclohexanedione) (Chaabane et al. 2005). Sulcotrione is reported as being more mobile than atrazine (Cherrier et al. 2004). Photochemistry behavior of sulcotrione was recently studied (Ter Halle et al. 2006b; Chaabane et al. 2007). It is reported by the authors of these studies that photolysis is faster at acidic pH values. Some data for sulcotrione have been obtained in fish, *Daphnia magna*, and algae (http://www.dive.afssa.fr/agritox/index.php.) but the ecotoxicological risk of this compound and of its degradation products in the environment is still poorly documented. Very recently, Chaabane et al. (2007) published a work related to the toxicity of sulcotrione on some marine microorganisms (heterotrophic bacteria).

Mesotrione (Fig. 1), a newer member of the benzoylcyclohexanedione family of herbicides, also acts by competitive inhibition of p-HPPD. This is a selective pre- and postemergence herbicide that controls most broadleaf and some grass weeds in maize crops. It was developed by Syngenta Crop Protection and registered in Europe in 2000 (Mitchell et al. 2001; Alferness and Wiebe, 2002). It is marketed under the commercial name Callisto®. The metabolite MNBA (4-methylsulfonyl-2-nitrobenzoic acid) is detected with the parent mesotrione in crops, and two degradation products, MNBA and AMBA (2-amino-4-methylsulfonylbenzoic acid), are found together with the parent compound in soil and water (Alferness and Wiebe 2002). Gledhill et al. (2001) showed that the major metabolic pathway of mesotrione in rat and mouse was hydroxylation of the aromatic ring and cleavage of the dione and aromatic rings followed by the reduction of the nitro groups (MNBA and AMBA). Recently, Durand et al. (2006a) have isolated and identified a bacterial strain able to transform mesotrione. Five degradation products, AMBA and four never described before, but also a metabolic pathway by *Bacillus* sp. 3B6 were reported (Durand et al. 2006b). Because of its recent introduction there is globally very little information in the literature concerning the ecotoxicological risk of mesotrione and its degradation products in the environment. Only a small number of data have been obtained in fish, land vertebrates, aquatic invertebrates, algae, and aquatic plants (http://www.dive.afssa.fr/agritox/index.php.; http://www.toxnet.nlm.nih.gov/.).

The aims of this study were (i) to assess the potential toxicity of sulcotrione and mesotrione, two triketonic herbicides used as atrazine substitutes, toward two nontarget representative environmental microorganisms frequently used in ecotoxicology, the eukaryote *T. pyriformis* and the prokaryote *V. fischeri*; (ii) to compare the toxicity of these compounds with that of atrazine; (iii) to assess the potential toxicity of the different degradation products to obtain a more accurate picture of the impact of a polluting agent on the environment; and (iv) to determine the toxicity of the commercial herbicidal products Mikado® and Callisto® given that under environmental conditions the respective active ingredients sulcotrione and mesotrione are formulated with various additives.

Materials and Methods

Chemical Compounds

Sulcotrione (46318; Pestanal; purity, 98.7%), mesotrione (33855; Pestanal; purity, 99.9%), and atrazine (45330; Pestanal; purity, 98.5%) were purchased from Fluka Riedel-de-Haën (Buchs, SG, Switzerland). CMBA (425010010; purity, >95%) and CHD (111170050; purity, >97%) were purchased from Acros Organics France (Noisy-Le-Grand, France). AMBA and MNBA were supplied by the Synthèse Et Étude de Systèmes à Intérêt Biologique Laboratory at Blaise Pascal University (Clermont-Ferrand, France). The commercial products Callisto® (concentrated suspension containing 100 g of mesotrione per litre) and Mikado® (concentrated suspension containing 300 g of sulcotrione per liter) were manufactured by Syngenta Crop Protection (St Cyr l'Ecole, France) and Bayer CropScience SA (Lyon, France), respectively. They were obtained from a regular agricultural supplier. For all bioassays, sulcotrione, mesotrione, and their degradation products were dissolved in DMSO. Final tested concentrations were prepared by dilutions of stock solutions, but the amount of solvent added to the cultures was always 0.5% (v/v). DMSO has no effect on *T. pyriformis* and *V. fischeri* at a final concentration of 0.5% (Bogaerts et al. 2001). The commercial products were tested without preliminary dissolution in DMSO.

Cell Culture

The ciliated protozoan *T. pyriformis*, amicronucleated strain GL, was obtained from the Biology Institute of the Carlsberg Foundation (Copenhagen, Denmark). It was grown axenically without shaking at 28°C in a proteose-peptone yeast salts (PPYS) complex medium (pH 6.8 to 7.0) containing 0.75% proteose-peptone (Difco, Detroit, MI, USA), 0.75% yeast extracts (Difco), and inorganic salts (Plesner et al. 1964). The cells were maintained in exponential growth phase by reseeding in PPYS liquid medium.

Toxicity Assays

Toxicity assays involved the Microtox test, the *T. pyriformis* population growth impairment test, and the *T. pyriformis* nonspecific esterase activity test.

Population Growth Rate Test (Experiments with T. pyriformis*)*

The *T. pyriformis* population growth impairment test (kinetic growth method) employing a tube technique based on optical density (OD) measurements was performed as previously described, with two controls (*T. pyriformis* in culture with and without 0.5% DMSO) and several grade-step increasing concentrations of one compound (Bonnet et al. 2007). Growth was monitored by measuring the OD of each tube at 535 nm every hour for 9 h. For a given experiment, five parallel replicates were realized for each sample. The reduction of cell growth in relation to an untreated control is regarded as a measure of toxicity. No variation in increase/decrease in cell size is systematically verified. The relative toxicity of the tested substances was quantified by determining the 9-h median inhibitory concentration (9-h IC_{50}), which is the concentration required to induce a 50% decrease in cell growth compared with untreated cells for the length of the experiment (9 h) (Bonnet et al. 2007). Three independent experiments were carried out to determine an average 9-h IC_{50} value.

Nonspecific Esterase Activities Test (Experiments with T. pyriformis*)*

Nonspecific esterase activities were measured as previously described (Bonnet et al. 2003). The experiment consists in the hydrolysis of fluorescein diacetate (FDA) by the esterases of *T. pyriformis* and the spectrofluorimetric quantification of the free fluorescein. The aim of this test was to determine the 45-min median inhibitory concentration (45-min IC_{50}), i.e., the concentration required to induce a 50% decrease in fluorescence compared with the untreated cells for the length of the experiment (T = 1 h + 45 min) (Bonnet et al. 2003). Three independent experiments were carried out to determine an average 45-min IC_{50} value.

Microtox Assays (Experiments with V. fischeri*)*

The *V. fischeri* bioluminescence inhibition observed in the presence of xenobiotic was measured after various exposure times (5, 15, and 30 min). All the materials for analysis (test reagent, diluents, osmotic adjusting solution, and reconstitution solution) were supplied by Azur Environmental (Carlsbad, CA, USA). For each compound, four independent experiments were performed according to the normalized procedure with a Microbics M 500 toxicity analyzer coupled to a PC using 500 DOS software for Microtox. The aim of this test was to determine the median inhibitory concentration (IC_{50}), which is the concentration required to induce a 50% decrease in bioluminescence compared with the untreated bacteria after 5, 15, and 30 min.

Results

For each test substance, at least one test using the eukaryotic cell and one test using the prokaryotic cell were taken into account. The toxicity values obtained with the different compounds, degradation products, and commercial formulations are reported in Table 1.

Influence of pH on Determination of IC_{50}

The pH was measured for all stock solutions and final tested concentrations. They were found to be relatively acidic (e.g., Callisto®, 10 g/L, pH 2.96, and Mikado®, 2 g/L, pH 3.66) in all cases except for atrazine (200 mg/L final concentration, pH 9.17). Concerning the results reported for *T. pyriformis*, the experimental protocols allow an adjustment of pH. Because the final test concentrations were diluted 200× in culture medium, the pH was in the range of 6.5–7.0. Thus the direct effects of the compounds on the eukaryotic cell were observed. In the Microtox protocol the dilution factor of the solutions was only 0.45. To differentiate the toxicity due to the pH from that due to the agents themselves, it was thus necessary to neutralize the various tested solutions, except for atrazine. We have shown in an independent experiment that solutions of pH lower than 9.7 do not decrease the bioluminescence of *V. fischeri* (data not shown). For each compound, in a second series of experiments, the testing solutions were neutralized with a $NaH_2PO_4 + Na_2HPO_4$ buffer before measurement. In the Microtox test, the values of IC_{50} obtained at 5, 15, and 30 min were similar in most cases (14 of 17 chemicals). Thus, only the results corresponding at time 15 min are reported in Table 1.

Toxicity Assessment to *T. pyriformis*

The IC_{50}s for the technical grade sulcotrione and mesotrione were large for the population growth rate test and a slight toxic influence was observed on the nonspecific esterase activities test (Table 1). The commercial products Mikado® and Callisto® were more toxic than analytical standards sulcotrione and mesotrione based on equivalent active ingredient concentrations with the two tests. The added surfactants in Mikado® and Callisto®, which are designed to enhance herbicidal efficacy, were probably responsible for their relatively greater toxicity. Concerning

Table 1 Toxicity values of the different chemicals towards *T. pyriformis* and *V. fischeri*

Chemical	Population growth rate tubes (9 h) IC_{50} (mg/L)	Nonspecific esterase activities 45 min IC_{50} (mg/L)	Microtox® 15 min IC_{50} (mg/L)
Atrazine	66.5 ± 11.4[a]	>20[a]	196.7 ± 28.5
Sulcotrione	4691.2 ± 702.1	240.2 ± 34.6	55.1 ± 5.6
Sulcotrione neutralized	–	–	194.6 ± 13.8
Mikado® eq. sulcotrione	176.3 ± 12.1	68.1 ± 0.9	12.5 ± 1.3
Mikado® neutralized eq. sulcotrione	–	–	99.9 ± 13.1
CHD	888.4 ± 29.4	114.4 ± 10.7	19.1 ± 2.8
CHD neutralized	–	–	92.3 ± 14.7
CMBA	6772.7 ± 1073.9	242.7 ± 14.9	38.8 ± 1.5
CMBA neutralized	–	–	105.1 ± 1.8
Mesotrione	7728.4 ± 1247.1	322.7 ± 43.9	43.6 ± 2.4
Mesotrione neutralized	–	–	69.2 ± 4.0
Callisto® eq. mesotrione	4.0 ± 0.1	6.7 ± 0.8	1.1 ± 0.1
Callisto® neutralized eq. mesotrione	–	–	0.9 ± 0.2
AMBA	–	>125	17.8 ± 4.0
AMBA neutralized	–	–	12.6 ± 3.0
MNBA	–	>125	37.7 ± 2.1
MNBA neutralized	–	–	312.7 ± 35.5

IC_{50} = Inhibitory Concentration 50%

[a] Bogaerts et al (2001)

the potential toxicity of sulcotrione degradation products, CMBA showed a similar toxicity to the parent compound, but a greater toxicity was noted for CHD with the two tests. The mesotrione degradation products could not be tested with the *T. pyriformis* population growth rate test because only very small quantities were available. We can only state that IC_{50} values were >125 mg/L for AMBA and MNBA with the nonspecific esterase activities test. Compared with the results for atrazine (technical grade), the toxicity was lower for all compounds, except Callisto® equivalent mesotrione in the population growth impairment test. In the nonspecific esterase activities test, Callisto® equivalent mesotrione was also found to be more toxic than atrazine. For the other chemicals we cannot draw a firm conclusion because the value for atrazine is reported as >20 mg/L (Table 1). Indeed, precipitation in the reactional medium formed by Volvic water (natural mineral water, pH = 7. It is drawn from deep inside the green ancient volcanoes of the Auvergne region in France.) was observed for final atrazine concentrations of over 20 mg/L (final concentrations are obtained after dilution from a stock solution prepared in DMSO).

Toxicity Assessment to *V. fischeri*

The comparison between neutralized and nonneutralized solutions for the same compound showed a pH influence on IC_{50} in most cases (Table 1). To assess the effects of the compounds on the prokaryotic cell directly, we consider below only the results from the neutralized experiments. The technical grade sulcotrione and mesotrione gave a non-negligible toxicity; IC_{50} values were 194.6 ± 13.8 mg/L and 69.2 ± 4.0 mg/L, respectively. The commercial products Mikado® and Callisto® were 2 and 80 times more toxic than the technical grade compound respectively, based on equivalent active ingredient concentrations. The two sulcotrione degradation products CMBA and CHD were more toxic than the parent molecule. The results were different for the mesotrione degradation products. One metabolite, AMBA was more toxic than mesotrione, while the second one, MNBA, was less toxic than the parent molecule. Compared with the results for atrazine (technical grade), the toxicity was greater for all the chemicals, except for sulcotrione and MNBA.

Discussion

In this work we report original data concerning the potential toxicity of sulcotrione and mesotrione, two herbicides used as atrazine substitutes, towards nontarget cells frequently used in ecotoxicology. To our knowledge, no *in vitro* toxicity assessment comparable to that presented here has been performed, as we have studied not only the

technical grade herbicide but also commercial products and degradation products. Until now very few studies were published on the toxicity and the fate in the environment of these new selective herbicides. Environmental risk assessment is of particular interest as herbicides can pollute nearby aquatic ecosystems and may also adversely affect human health. Although extrapolating laboratory data to the situation in field conditions is contentious, laboratory acute toxicity tests used in this work provide a first approach to estimating environmental impact and allow the potential toxicity of chemicals on representative living organisms to be assessed.

In our experiments, the commercial products Mikado® and Callisto® showed a greater toxicity than the technical grade herbicide. This was also found for glyphosate and its commercial formulation Roundup® with growth inhibition criteria in several toxicity test organisms (bacteria, microalgae, protozoa and crustaceans) (Tsui and Chu 2003; Cedergreen and Streibig, 2005). However, it is not the case for all herbicides. The results of Cedergreen and Streibig (2005), who evaluated the toxicity of ten technical grade herbicides and formulated products on the aquatic plant *Lemna minor* and the alga *Pseudokirchneriella subcapitata*, showed that the commercial product did not increase the toxicity of the technical grade compound, except for glyphosate. As our results indicate that the additives in the commercial products Mikado® and Callisto® were probably major contributors to their acute toxicity, it is essential to evaluate the toxicity of the additives in further experiments. Unfortunately, the nature of the various additives in Mikado® and Callisto® is unknown.

Globally the toxic concentrations of the herbicides to our test organisms are high to very high, sometimes more than 100 mg/L. The toxicity for target organisms (photosynthetic plants and algae) will be much lower; they have the receptor for the herbicides. The mode of action in the test organisms is probably different from that in plants and characterized by narcosis and single effect on the membrane.

Concerning the fate of sulcotrione and mesotrione in the environment, CHD, CMBA for sulcotrione, AMBA and MNBA for mesotrione are currently the only degradation products that have been evaluated for their environmental impact. However, recent studies indicate that other degradation-transformation products may be formed with these two herbicides. For sulcotrione, five photoproducts were identified by Ter Halle et al. (2006b) and Chaabane et al. (2007) but the chromone derivative product characterized by Ter Halle et al. (2006b) for photodegradation on maize cuticular wax is not reported by Chaabane et al (2007) for photodegradation in various aquatic environments. Toxicity assessment of the chromone derivative compound towards various photosynthetic and non-photosynthetic organisms is in progress in collaboration with Ter Halle et al. Concerning the study of Chaabane et al. (2007) these authors found that both active and photodegraded products of sulcotrione did not significantly reduce population growth of *Vibrio angustum*, *Deleya aquamarina* (heterotrophic bacteria) and *Synechocystis* sp. (cyanobacterium) for the range of the concentrations tested (0.1–100 μg/L). For mesotrione, Ter Halle and Richard (2006a) showed that several photoproducts are formed in natural waters but only three were identified, MNBA is one of them. Durand et al. (2006a; 2006b) gave a complete description of mesotrione biotransformation by a bacterial strain (*Bacillus* sp. 3B6). AMBA was identified as one metabolite but several other degradation products were present at relatively high concentrations. To obtain a more comprehensive picture of the impact and fate of the atrazine substitute herbicides on the environment, it would be useful to characterize the toxicity of all newly identified degradation products of these two herbicides in further experiments. In a recent work, we demonstrated that most of the photo- or biotransformation products of the systemic herbicide diuron exhibited a greater toxicity than diuron itself on the same environmental microorganisms (Bonnet et al. 2007).

Consideration of the structure–toxicity relationships yields several findings. The two selective herbicides sulcotrione and mesotrione belong to the triketone family of herbicides, which are derived from a natural phytotoxin leptospermone produced by the bottlebrush plant *Callistemon citrinus* (Mitchell et al. 2001). The structure of the two parent compounds is almost identical and three of the four degradation products studied present common characteristics (Fig. 1). The toxicities obtained with the different chemicals are nevertheless very different (Table 1). The structures of these herbicides can be separated into two parts: the benzoyl moiety and the cyclohexanedione moiety. It has been shown for the benzoyl moiety that a substituent at the 2-position on the phenyl ring is an absolute requirement for herbicidal activity, and that an additional substituent at the 4-position tends to enhance that activity (Lee et al. 1998). Our results show that the substitution of Cl in sulcotrione, at the 2-position of the benzoyl moiety, by NO_2 in mesotrione decreases toxicity towards *T. pyriformis* (population growth rate test as well as nonspecific esterase activities test), but increases it towards *V. fischeri*. With the Microtox® test, the following toxicity rank order (decreasing toxicity) is obtained for the degradation products corresponding to the benzoyl moiety: AMBA > CMBA > MNBA. For the prokaryotic cell, the NH_2 substituent in AMBA was found to be more toxic than the Cl substituent in CMBA, which was more toxic than the NO_2 substituent in MNBA. For the dione moiety, potentiation of herbicidal activity is obtained by adding substituents to the

cyclohexanedione ring (Mitchell et al. 2001). Our results demonstrate that CHD itself is, without any supplementary substituents, already toxic to the two environmental test microorganisms. A single substitution can lead to a marked change in toxicity, and our microbiotests, which reveal these differences in toxicity, are therefore very useful. When for a same compound, it has been possible in this study to use the two *Tetrahymena* tests, nonspecific esterase activities IC_{50} values were generally lower than population growth rate IC_{50} values.

To extend our knowledge of the environmental impact of these herbicides, it will be essential to make more precise studies of the ecosystemic levels with aquatic and soil compartments (biodegradation, photodegradation, hydrolysis, tolerance and activity of microorganisms). Additional works with *T. pyriformis* and *V. fischeri* will also be necessary to assess the toxicity of other newly identified degradation products.

References

Alferness P, Wiebe L (2002) Determination of mesotrione residues and metabolites in crops, soil, and water by liquid chromatography with fluorescence detection. J Agric Food Chem 50:3926–3934

Bogaerts P, Bohatier J, Bonnemoy F (2001) Use of the ciliated protozoan *Tetrahymena pyriformis* for the assessment of toxicity and quantitative structure–activity relationships of xenobiotics: comparison with the microtox test. Ecotoxicol Environ Saf 49:293–301

Bonnet JL, Dusser M, Bohatier J, Laffosse J (2003) Cytotoxicity assessment of three therapeutic agents, cyclosporin-A, cisplatin and doxorubicin, with the ciliated protozoan *Tetrahymena pyriformis*. Res Microbiol 154:375–385

Bonnet JL, Bonnemoy F, Dusser M, Bohatier (2007) Assessment of the potential toxicity of herbicides and their degradation products to nontarget cells using two microorganisms, the bacteria *Vibrio fischeri* and the ciliate *Tetrahymena pyriformis*. Environ Toxicol 22:78–91

Cedergreen N, Streibig JC (2005) The toxicity of herbicides to nontarget aquatic plants and algae: assessment of predictive factors and hazard. Pest Manag Sci 61:1152–1160

Chaabane H, Cooper JF, Azouzi L, Coste CM (2005) Influence of soil properties on the adsorption-desorption of sulcotrione and its hydrolysis metabolites on various soils. J Agric Food Chem 53:4091–4095

Chaabane H, Vulliet E, Joux F, Lantoine F, Conan P, Cooper JF, Coste (2007) Photodegradation of sulcotrione in various aquatic environments and toxicity of its photoproducts for some marine micro-organisms. Water Res 41:1781–1789

Cherrier R, Perrin-Ganier C, Schiavon M (2004) Degradation of sulcotrione in a brown soil amended with various organic matters. Agronomie 24:29–33

deNoyelles F, Kettle WD, Sinn DE (1982) The responses of plankton communities in experimental ponds to atrazine, the most heavily used pesticide in the United States. Ecology 63:1285–1293

Durand S, Amato P, Sancelme M, Delort AM, Combourieu B, Besse-Hogan P (2006a) First isolation and characterization of a bacterial strain that biotransforms the herbicide mesotrione. Lett Appl Microbiol 43:222–228

Durand S, Légeret B, Martin AS, Sancelme M, Delort AM, Besse-Hoggan P, Combourieu B (2006b) Biotransformation of the triketone herbicide mesotrione by a *Bacillus* strain. Metabolite profiling using liquid chromatography/electrospray ionization quadrupole time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 20:2603–2613

Ebert E, Dumford SW (1976) Effects of triazine herbicides on the physiology of plants. Residue Rev 65:1–103

Gledhill AJ, Jones BK, Laird WJ (2001) Metabolism of 2-(4-methylsulphonyl-2-nitrobenzoyl)-1,3-cyclohexanedione (mesotrione) in rat and mouse. Xenobiotica 31:733–747

Hoagland KD, Drenner RW, Smith JD, Cross DR (1993) Freshwater community responses to mixtures of agricultural pesticides: Effects of atrazine and bifenthrin. Environ Toxicol Chem 12:627–637

Kaiser KLE, Palabrica VS (1991) *Photobacterium phosphoreum* toxicity data index. Water Pollut Res J Can 26:361–431

Kaiser KLE (1998) Correlations of *Vibrio fischeri* bacteria test data with bioassay data for other organisms. Environ Health Persp 106:283–591

Lee DL, Knudsen CG, Michaelay WJ, Chin H, Nguyen NH, Carter CG, Cromartie TH, Lake BH, Shribbs JM, Fraser T (1998) The structure–activity relationships of the triketone class of HPPD herbicides. Pestic Sci 54:377–384

Mitchell G, Barlett DW, Fraser TEM, Hawkes TR, Holt DC, Townson JK, Wichert RA (2001) Mesotrione: a new selective herbicide for use in maize. Pest Manag Sci 57:120–128

Nilsson JR (1989) *Tetrahymena* in cytotoxicology: with special reference to effects of heavy metals and selected drugs. Eur J Protistol 25:2–25

Norris SR, Barrette TR, DellaPenna D (1995) Genetic dissection of carotenoid synthesis in *Arabidopsis* defines plastoquinone as an essential component of phytoene desaturation. Plant Cell 7:2139–2149

Plesner P, Rasmussen L, Zeuthen E (1964) Techniques used in the study of synchronous *Tetrahymena*. In: Zeuthen E (eds) Synchrony in Cell Division and Growth. Intersciences Press, New York, NY, pp 534–565

Pratt JR, Bowers NJ, Niederlehner BR, Cairns J Jr (1988) Effects of atrazine on freshwater microbial communities. Arch Environ Contam Toxicol 17:449–457

Purcell M, Leroux GD, Carpentier R (1990) Atrazine action on the donor side of photosystem II in triazine-resistant and susceptible weed biotypes. Pest Biochem Physiol 37:83–89

Sauvant MP, Pépin D, Bohatier J, Grolière CA, Veyre A (1994) Comparative study of two in vitro models L-929 fibroblasts and *Tetrahymena pyriformis* (GL) for the cytotoxicological evaluation of packaged water. Sci Total Environ 156:159–167

Sauvant MP, Pépin D, Grolière CA, Bohatier J (1995) Effects of organic and inorganic substances on the cell proliferation of L-929 fibroblasts and *Tetrahymena* toxicological bioassays. Bull Environ Contam Toxicol 55:171–178

Sauvant MP, Pépin D, Piccinni E (1999) *Tetrahymena pyriformis*: A tool for toxicological studies. Chemosphere 38:1631–1669

Schultz TW, Netzeva TI, Roberts DW, Cronin MTD (2005) structure–toxicity relationships for the effects to *Tetrahymena pyriformis* of aliphatic, carbonyl-containing, α,β-unsaturated chemicals. Chem Res Toxicol 18:330–341

Schulz A, Ort O, Beyer P, Kleinig H (1993) SC-0051, a 2-benzoyl-cyclohexane-1,3-dione bleaching herbicide, is a potent inhibitor of the enzyme *p*-hydroxyphenylpyruvate dioxygenase. FEBS Lett 318:162–166

Stratton GW (1983) Interaction effects of permethrin and atrazine combinations towards several nontarget microorganisms. Bull Environ Contam Toxicol 31:297–303

Ter Halle A, Richard C (2006a) Simulated solar irradiation of mesotrione in natural waters. Environ Sci Technol 40:3842–3847

Ter Halle A, Drncova D, Richard C (2006b) Phototransformation of the herbicide sulcotrione on maize cuticular wax. Environ Sci Technol 40:2989–2995

Tsui MTK, Chu LM (2003) Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. Chemosphere 52: 1189–1197

Tu CM (1992) Effect of some herbicides on activities of microorganisms and enzymes in soil. J Environ Sci Health Part B 27:695–709

Ward GS, Ballantine L (1985) Acute and chronic toxicity of atrazine to estuarine fauna. Estuaries 8:22–27

Wichert RA, Townson JK, Bartlett DW, Foxon GA (1999) Technical review of mesotrione, a new maize herbicide. In Proc Brighton Crop Prot Conference: Weeds, BCPC, Farnham, Surrey, UK, Vols 1–3, pp 105–110