



## Uptake visualization of deltamethrin by NanoSIMS and acute toxicity to the water flea *Daphnia magna*

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### ABSTRACT

The objective of this study was to investigate the uptake of deltamethrin, an insecticide, by *Daphnia magna* neonates by SIMS and to compare these findings with results based on established toxicity tests.

Young daphnids (aged <24 h) were exposed to 0, 50 and 200  $\mu\text{g L}^{-1}$  (ppb) deltamethrin. Mobile, immobile and dead animals were enumerated after 24 and 48 h following OECD 202 [OECD 202, 2004. *Daphnia* sp., acute immobilisation test, guideline for testing of chemicals] guidelines. The animals were embedded in epoxy resin, cut into semi-thin sections (500 nm) and placed on silicon supporters. NanoSIMS 50 (Cameca) images were made from tissues of the intestine for carbon, nitrogen (measured as CN), phosphorus and bromine. To distinguish between relative concentrations of bromine in the guts from different exposure concentrations of deltamethrin, a carbon normalization method was carried out.

Both deltamethrin concentrations and time showed a significant effect on immobilization and mortality of the daphnids ( $P < 0.0001$ ). Bromine from deltamethrin could be visualized by NanoSIMS in all exposed gut tissues (gut wall, microvilli layer, peritrophic membrane). Highest deltamethrin concentrations following  $^{12}\text{C}$  normalization were found in animals exposed to 200  $\mu\text{g L}^{-1}$  deltamethrin, followed by 50  $\mu\text{g L}^{-1}$  and the control.

NanoSIMS 50 was successfully used as a supplemental technique for elucidating the relation between the uptake and localization of deltamethrin and its toxicity to *D. magna*. These results highlight the potential usefulness of NanoSIMS to detect marker elements of xenobiotic compounds within exposed organisms, to compare relative exposure concentrations, and to locate these compounds at their original tissue location.

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### 1. Introduction

Environmental contamination resulting from the use of various pesticides by farmers remains a current problem. One of the frequently used pesticides is deltamethrin (Fig. 1), an insecticide belonging to the group of pyrethroids, employed against agricultural pests. As a result of yearlong application, it can be found in many environmental matrices, especially in water, sediments, plants and animals (Pawlisz et al., 1998).

Deltamethrin is a suspected endocrine disruptor (Garey and Wolff, 1998) and the toxicity of deltamethrin has already been investigated employing different animals such as frogs, fish, insects, mussels (Mulla et al., 1978; Muir et al., 1985; Thybaud, 1990; Kontreczky et al., 1997) but also *Daphnia magna* (Ruiquin et al., 1989; Day and Maguire, 1990), which is frequently used as a test species in water. To investigate its way of action, and to study the accumulation of deltamethrin, animal serum, blood, ur-

ine (Yao et al., 1992) and tissue samples such as liver as well as water and sediment samples have been analyzed using gas chromatography-electron capture detection (GC-ECD) and HPLC (Leng et al., 2003; Xue et al., 2005). While these methods allow for quantification of the pollutant, they are not able to produce information on the location of accumulation in the tissue or even in sub-cellular compartments. Thus, information on how the compound is taken up by cells, such as in the intestine, and its further distribution cannot be obtained by these methods. NanoSIMS analysis is a new technique to visualize substances at their original cell location, including the uptake of deltamethrin in aquatic organisms (Eybe et al., 2008). The NanoSIMS 50 (Cameca) is a secondary ion mass spectrometer with a lateral resolution of approximately 50 nm. The high sensitivity, high lateral resolution, and high transmission at high mass resolution for secondary ions make the NanoSIMS 50 a powerful tool for investigating elemental composition of biological samples. Distribution maps of several elements and their isotopes can be obtained simultaneously from the same sample area by raster scanning the sample surface with the primary ion beam, in conjunction with using a multi-detector. Thus, the main

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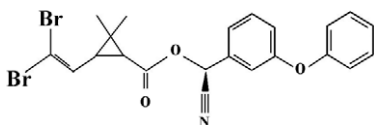


Fig. 1. Chemical structure of deltamethrin.

advantage of this technology is the ability to observe the distribution of a variety of elements (five at the same time) at their original location.

In this study our objectives were (a) to investigate the acute toxicity of deltamethrin to *D. magna* in a toxicity test that was adapted from the OECD 202 (guideline for testing chemicals, acute immobilization test); (b) to visualize the uptake of deltamethrin using the NanoSIMS 50 within the intestinal tissues of the same *D. magna* animals used in the toxicity test, as this area was the best identifiable and constitutes a formerly well-described tissue and (c) to compare the findings of both investigations.

## 2. Methods

### 2.1. Toxicity test

The water flea *D. magna*, an aquatic crustacean, is an appropriate organism to study the toxicity and the uptake of deltamethrin, as it is directly exposed to pesticide contaminated water in the environment and uptake does not depend on factors such as eating habits. Furthermore, it is already an established and frequently used organism for toxicity tests. The experiment was adapted from the OECD 202 guideline for testing chemicals (OECD, 2004), and constitutes an acute immobilization test. In short: young daphnids, from third brood progeny, aged less than 24 h at the start of the test, were exposed to deltamethrin at 50 and 200  $\mu\text{g L}^{-1}$  for a period of 48 h. Immobilization was recorded at 24 and 48 h.

Twenty-five animals, divided into five groups of five animals each, were used at each concentration. The animals were kept in glass beakers containing 70 mL test solution, loosely covered with aluminium foil to reduce reactions of deltamethrin induced by direct light. Volvic mineral water was used as water for the daphnids during breeding and the test period. Average conditions were as follows: dissolved oxygen: 8.9  $\text{mg L}^{-1}$ , pH 8.3, hardness 61.6  $\text{mg L}^{-1}$  as  $\text{CaCO}_3$  (before and after the test). The temperature during the test was 21.9 °C (measured in ambient air) and an illumination cycle of 16 h light and 8 h darkness was followed. The animals were not fed during the study.

Deltamethrin (Sigma–Aldrich, Seelze, Germany) stock solution was prepared with ethanol (1%) and millipore water. To exclude an influencing effect of ethanol on the test outcome, ethanol concentration was adjusted to 0.04% for each exposure group including the controls.

Daphnids that were able to swim were considered mobile, animals which still moved their antennae but did not swim within 15 s after gentle shaking were considered immobile and daphnids that did not move anymore were considered dead.

### 2.2. *D. magna* raising

The *D. magna* Straus culture in our laboratory was set up based on individuals sampled in a wastewater stabilization pond of Differdange (Luxembourg) in the year 2000 (Cauchie et al., 2000). Daphnids from the third brood progeny were raised in conditions similar as in the tests, apart from a regular feeding with the green algae *Desmodesmus subspicatus* (SAG 86.81, Culture Collection of Algal Laboratory, Trebon, Czech Republic) at a minimum concentration of  $10^5$  cells  $\text{mL}^{-1}$ .

### 2.3. NanoSIMS 50 sample preparation

After elucidating the mobility of the animals at the end of the exposure experiments, whole *D. magna* bodies were embedded in an epoxy resin after chemical fixation: The first fixation was done with 5% glutaraldehyde in 0.1 M phosphate buffered saline (PBS) over night. Glutaraldehyde was removed and animals were washed in pure PBS. The daphnids were then fixed with 1% osmium tetroxide for 1 h. After an additional washing step with PBS (0.1 M) and dehydration with five dose escalating acetone concentrations (30%, 50%, 70%, 90%, and 100% aqueous acetone), the animals were embedded in epoxy resin (Epon 812 substitute) and hardened for 24 h at 60 °C. The samples were cut to 500 nm semi-thin sections (Leica ultracut UCT, Le Pecq Cedex, France) and placed on silicon supporters (Siltronix, Archamps, France) for NanoSIMS 50 analysis. The samples were stored in plastic boxes at room temperature in the dark until analysis. For each exposure group (control, 50 and 200  $\mu\text{g L}^{-1}$  deltamethrin) a minimum of three animals were measured by NanoSIMS.

### 2.4. NanoSIMS 50 analysis

Measurements were performed with the NanoSIMS 50 (Cameca, Gennevilliers Cedex, France) in the raster imaging mode. A  $\text{Cs}^+$  primary ion beam (impact energy of 8 keV and intensity of 2 pA) was used for rasterizing over a  $40 \times 40 \mu\text{m}^2$  area on the sample surface with a spatial resolution of 100 nm (spot size). The images were recorded with  $256 \times 256$  pixels, with an acquisition time of 30 ms  $\text{pixel}^{-1}$  and with a parallel detection of four different negative secondary ions. For *D. magna* samples,  $^{12}\text{C}$  (for later carbon normalization),  $^{12}\text{C}^{14}\text{N}$ ,  $^{31}\text{P}$  and  $^{81}\text{Br}$  signals were simultaneously collected with four electron multipliers. Despite the high mass resolution power, combined with a high transmission of the NanoSIMS 50 ( $\Delta M/M > 4000$  for a transmission of 70%), the  $^{81}\text{Br}$  isotope (49.5% natural abundance) was preferred to the  $^{79}\text{Br}$  (50.5%), due to a possible mass interference with  $^{31}\text{P}^{16}\text{O}_3$  clusters. Mass calibration of Br, carried out on a daily base, was achieved using standard references (NaBr, Sigma–Aldrich, Seelze, Germany).

### 2.5. Carbon normalization

NanoSIMS images from comparable compartments of the gut wall and microvilli regions were manually digitized using the ENVI™ imaging software (ITT Corporations, Boulder, USA) for each exposure group. To obtain comparable Br intensity values independent from daily variations of the NanoSIMS signal intensity, the ratio of Br to C intensity counts (Br/C) was calculated based on the assumption of a relatively constant  $^{12}\text{C}$  content in the sufficiently large regions used for signal normalization. Digital masks of tissues, sufficiently enriched in Br (gut wall and microvilli layer) were defined using the CN images, since they provided both, the highest contrast and grey value ranges. These masks were then overlaid on the normalized Br images to extract the areas of interest. For each image (three images from different animals per exposure group), 200 normalized intensity values were selected randomly and used for further statistical comparisons. In addition to comparing different concentrations in different exposure groups, the accumulation of Br in the microvilli layer was compared to the gut wall (gut cells without microvilli).

### 2.6. Statistics

Statistical analyses were carried out by SPSS version 16.0 (Chicago, IL). After testing homogeneity of variance and normal distribution of the toxicity data, a linear mixed model was constructed with state of mobility (mobile, immobile, dead) as the dependent

variable and time, concentration and the individual *D. magna* nested within concentration as independent variable. Following the Fischer *F*-test, post hoc tests (Fischer protected LSD-tests) were performed to detect differences between the three concentrations. A *P*-value of <0.05 (two sided) was considered statistically significantly different.

For comparing relative deltamethrin concentrations in the differentially exposed water flea groups, a Kruskal–Wallis test was carried out as data was not normally distributed. This test was followed by carrying out all individual pairwise comparisons (Man–Whitney *U*-tests). To limit the risk of making at least one type I error at the overall significance level ( $\alpha = 0.05$ ) the Šidák–Bonferroni correction (Shaffer, 1995) was applied to correct  $\alpha$  for each single pairwise test. Considering three pairwise tests, this resulted in a corrected significance level ( $\alpha_{\text{corr}}$ ) of  $1-(1-0.05)^{1/3} = 0.017$  for each test. For comparing the microvilli area with the gut wall, pairwise Wilcoxon tests for dependent comparisons were carried out at each level of exposure at the  $\alpha_{\text{corr}}$  defined above.

### 3. Results

#### 3.1. Toxicity

The numbers of mobile, immobile and dead animals for the different exposure times and exposure concentrations are displayed in Table 1.

In general, both, time and concentration, showed a significant impact on the mobility of the animals ( $P < 0.0001$ , respectively). However, a significant interaction of time and concentration was found ( $P = 0.0003$ ). A significantly higher immobility and mortality in the animals was observed at  $t = 48$  h of exposure versus  $t = 24$  h ( $P < 0.02$ ) for both 50 and 200  $\mu\text{g L}^{-1}$  exposure groups. However, while both 50 and 200  $\mu\text{g L}^{-1}$  deltamethrin exposure resulted in significantly higher immobility and mortality as compared to the control ( $P < 0.0001$ , respectively), the results of 50  $\mu\text{g L}^{-1}$  exposure versus 200  $\mu\text{g L}^{-1}$  exposure were only significantly different at 24 h ( $P = 0.03$ ) and not at 48 h, as most animals were dead at this time already.

#### 3.2. NanoSIMS 50 investigation

Br could clearly be detected in all observed guts of exposed animals, except from the control samples (Figs. 2–4). The highest concentrations of Br were found in the peritrophic membranes of the exposed guts and in incorporated food particles (*D. subspicatus*, algae) that were fed to the animals directly after hatching before separating into the test beakers for the exposure studies. The intestinal walls of the exposed animals contained also visible amounts of Br, whereas the control animal guts contained considerably less to no detectable Br.

Carbon normalization revealed significant differences of Br concentrations for the different exposure groups (both within the gut wall layer as well as within the microvilli layer). The mean normalized intensity values (Br/C) are displayed in Table 2. The highest concentrations of Br were found in *D. magna* guts that were exposed to 200  $\mu\text{g L}^{-1}$  deltamethrin followed by 50  $\mu\text{g L}^{-1}$  and the

control. Although the mean normalized intensity values (Br/C) in the gut wall appeared slightly higher than in the microvilli layer, pairwise Man–Whitney *U*-tests showed that the differences in Br concentrations between the different layer types were not significant (with exception of the 50  $\mu\text{g L}^{-1}$  exposed animals). When plotting measured intensity values versus exposure concentrations, a straight line was obtained ( $R = 0.9996$ ), which would be in line with a simple diffusion mechanism of deltamethrin into tissues.

Even though animals were randomly distributed and prepared in an identical way for NanoSIMS investigation, the guts of the examined animals (50 and 200  $\mu\text{g L}^{-1}$  deltamethrin) were found to be smaller than the guts of the control samples. Guts of the control animals, as determined by the NanoSIMS, had an average diameter of 102.7 ( $\pm 29.6$ )  $\mu\text{m}$  whereas the exposure groups showed average diameters of 50.1 ( $\pm 11.5$ ) for 50  $\mu\text{g L}^{-1}$  and 40.6 ( $\pm 5.8$ )  $\mu\text{m}$  for 200  $\mu\text{g L}^{-1}$  ( $n = 3$ , respectively). Furthermore, the peritrophic membranes in the control guts were always intact and the layer thickness was pronounced, whereas the peritrophic membranes in the exposed animals were often not visible, thinner or destroyed, as well as not in touch with the microvilli layer.

### 4. Discussion

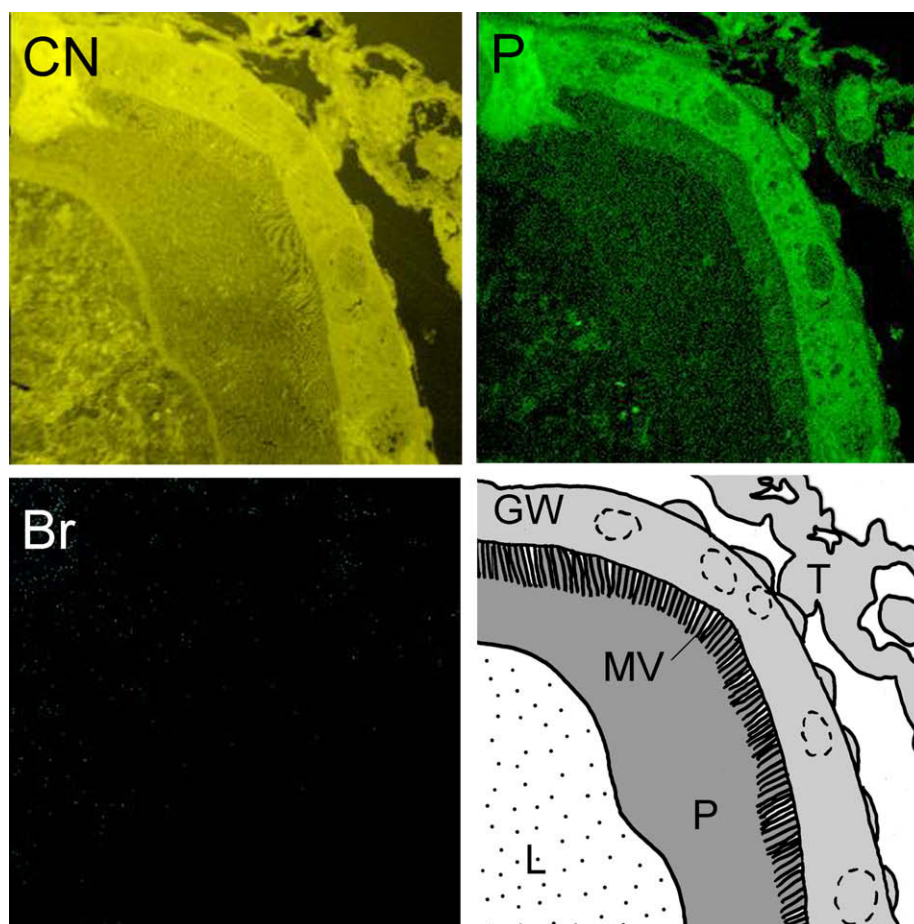
As in a number of previous studies investigating pesticides, *Daphnia* sp. showed to be a suitable indicator for water contamination (Angeler et al., 2005; Lopes et al., 2007; Ren et al., 2007), and our studies based on the OECD toxicity test suggested clear mobility differences between the different exposure concentrations and times. In addition, the investigation of *D. magna* guts with the NanoSIMS 50 following pesticide exposure allowed for studying the distribution of bromine from deltamethrin within the gut tissues, and demonstrated its suitability for comparing different exposure concentrations following  $^{12}\text{C}$  normalization.

In this study we focused on investigating the gut of *D. magna*, as this organ was the most accessible for separation and monitoring by NanoSIMS 50, as it constitutes the largest tissue identifiable in water fleas, and its fine structures have already been described (Schultz and Kennedy, 1976). The insecticide deltamethrin modifies the gating characteristics of voltage-sensitive sodium channels and delays their closure, a protracted sodium influx ensues which lowers the action potential threshold (Bradberry et al., 2005). As an effective insecticide, deltamethrin can be assumed to harm water fleas, which are phylogenetically closely related to insects. In this study, deltamethrin showed already toxic effects after 24 h of exposure: The proportion of unaffected animals did not change after 24 h of exposure; however, the percentage of dead animals increased from 24% to 76% (50  $\mu\text{g L}^{-1}$ ) and from 44% to 76% (200  $\mu\text{g L}^{-1}$ ) after 48 h. More unaffected animals were found after 48 h (8%) with 50  $\mu\text{g L}^{-1}$  of exposure versus 200  $\mu\text{g L}^{-1}$  (4%). Other toxicity studies investigating the immobilization of *D. magna* neonates (aged < 24 h) reported an  $\text{EC}_{50}$  of 30–90  $\mu\text{g L}^{-1}$  (ppb) for deltamethrin within 48 h (Day and Maguire, 1990). Although we did not determine the  $\text{EC}_{50}$ , we observed a definite effect on neonate immobilization at already 50  $\mu\text{g L}^{-1}$ . Thus, the concentrations causing toxic effects (immobility, death) are in agreement with for-

**Table 1**

Number of mobile, immobile and dead animals after 24 and 48 h of exposure to different concentrations of deltamethrin.

|                                       | After 24 h exposure |          |      | After 48 h exposure |          |      |
|---------------------------------------|---------------------|----------|------|---------------------|----------|------|
|                                       | Mobile              | Immobile | Dead | Mobile              | Immobile | Dead |
| Control                               | 25                  | 0        | 0    | 25                  | 0        | 0    |
| 50 $\mu\text{g L}^{-1}$ deltamethrin  | 2                   | 17       | 6    | 2                   | 4        | 19   |
| 200 $\mu\text{g L}^{-1}$ deltamethrin | 1                   | 13       | 11   | 1                   | 5        | 19   |



**Fig. 2.** Gut of *D. magna* (control) after 48 h of exposure to deltamethrin in water. Elements shown are nitrogen (as CN), phosphorus and bromine, as obtained by the NanoSIMS 50. Brightness indicates higher concentration. Raster size 40  $\mu\text{m}$ , acquisition time 30 min. GW: gut wall, L: lumen with food particles, MV: microvilli layer, P: peritrophic membrane, T: tissue remains.

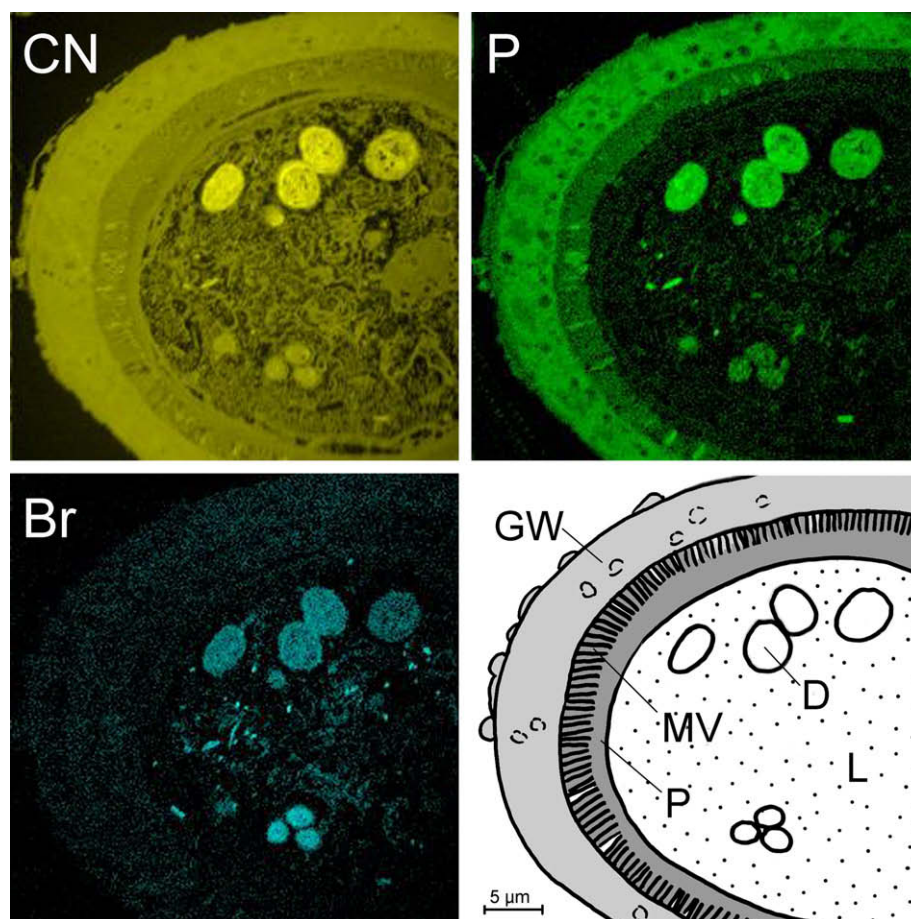
mer studies. Compared with water fleas, other aquatic organisms showed similar sensibilities to deltamethrin: Toxicity studies on toads, frogs, northern pikes and pond snails revealed mean  $\text{LC}_{50}$  of 11.9–500  $\mu\text{g L}^{-1}$  after 48 h of exposure (Rao et al., 1983; Thybaud, 1990; Salibian, 1992; Sahay and Agarwal, 1997).

In general, the NanoSIMS results are in good agreement with the results from the toxicology experiment, showing increased accumulation of bromine derived from deltamethrin containing two atoms of bromine, with increased exposure concentration. The uptake of substances from aquatic organisms can normally occur via food uptake and the gut (biomagnification) as well as directly from the water (bioconcentration). In the present study, it was found that the pesticide entered the gut of the water fleas during 48 h of exposure, which was associated with significant observed toxic effects in the animals (high number of immobilized or dead *D. magna*). The highest concentration of Br was found in the peritrophic membrane but also in the food that remained in the gut of neonates, such as present algae. It is possible that during exposure, the concentration of Br (deltamethrin) was higher in the gut lumen compared to the food particles, but was washed out during the embedding procedure afterwards. A possible pathway of deltamethrin into the gut could therefore be via food particles, alternatively, it can also be assumed that the pesticide was absorbed from the gut lumen into the surrounding tissue directly by diffusion, both pathways seem possible and would be in line with the relatively high lipophilicity of deltamethrin and the obtained curve of uptake. Deltamethrin exhibits, like all pyrethroid

pesticides, a highly nonpolar nature of low water solubility as well as a high octanol–water partition coefficient ( $\log K_{ow} = 6.1$ ), and can therefore accumulate in (lipid) tissues of living organisms (Mirfazaelian et al., 2008). In fish, bioconcentration factors (BCF) of deltamethrin ranged from 360 and 6000 (Laskowski, 2002).

To distinguish different Br concentrations in the guts exposed to different concentrations of deltamethrin, we chose the carbon normalization method, which was already reported earlier (Fragu et al., 1988; Follet-Gueye et al., 1998; Lechene et al., 2006). The brightness of NanoSIMS images is already an indication of the number of atoms detected. However, due to signal intensity variations that arise during the measurements, it is not always accurate to compare the quantity of elements between different samples, especially when measured on separate days. The theory behind this normalization is, that the concentration of common elements (such as C, N) remains usually quite similar in one cell or tissue type. In our study, carbon was originating from the sample tissue itself and the resin. Thus, it was assumed that the ratio of bromine to carbon intensity (counts) reflects more accurately the accumulation of bromine in the tissues and cancels out between-measurement effects. Although the real concentration of Br within the tissues cannot be determined, this method allows for comparing the Br uptake between different samples.

Carbon normalization confirmed what was already assumed based on visual image brightness. Significant differences of Br concentrations in the tissues between the exposed groups and the control (for gut wall and microvilli layer) were disclosed. Furthermore,



**Fig. 3.** Gut of *D. magna* after 48 h exposure to deltamethrin ( $50 \mu\text{g L}^{-1}$ ) in water. Elements shown are nitrogen (as CN), phosphorus and bromine, as obtained by the NanoSIMS 50. Brightness indicates higher concentration. Raster size  $40 \mu\text{m}$ , acquisition time 30 min. D: *Desmodesmus subspicatus* (algae), GW: gut wall, L: lumen with food particles, M: microvilli layer, P: peritrophic membrane.

it actually showed a significant higher concentration in the animals exposed to  $200 \mu\text{g L}^{-1}$  deltamethrin compared to the  $50 \mu\text{g L}^{-1}$  group, which was not detectable by comparing the brightness visually. These results were linked to the observed rates of immobilization and death: whereas the control guts showed little or no bromine and all control animals were still mobile after 48 h, the animals exposed to 50 and  $200 \mu\text{g L}^{-1}$  showed significant higher toxic responses and a clearly visible Br signal that was dependent on the exposure concentration.

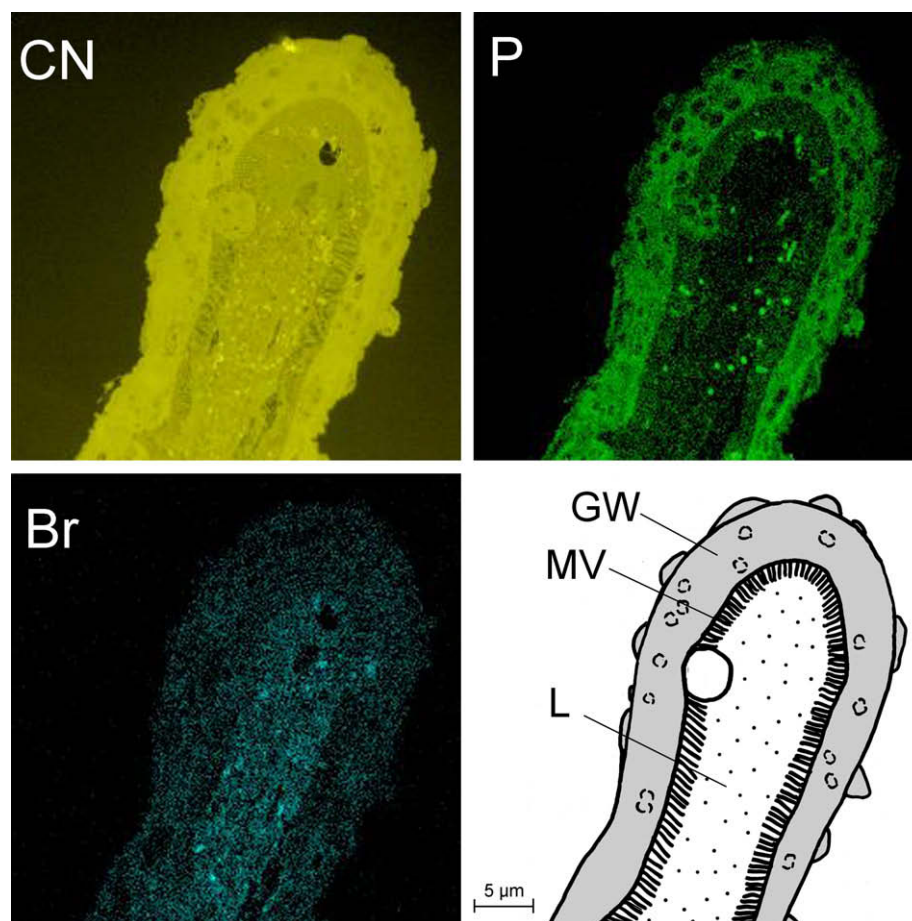
The microvilli layer as a part of inner gut wall cells tended to show lower mean intensity values (Br/C) compared to the gut wall layer. It may be assumed that these lower mean values were generated due to differences in resin/tissue ratio, as between single microvilli existed areas of pure resin not containing tissue and hence no Br. This example shows that the comparison between different tissue types may imply difficulties, because, in addition to interindividual differences of xenobiotic uptake, different tissues may contain dissimilar concentrations of carbon, which may therefore result in inaccurate interpretation.

Besides immobilization and lethality, in this study deltamethrin also showed an effect on the diameter of the observed guts as well as on the layer thickness and integrity of the peritrophic membranes, which normally contain enzymes and protect the tissue from mechanical injury (Bolognesi et al., 2002). This layer was frequently found removed from the microvilli layer in the exposed guts ( $50$  and  $200 \mu\text{g L}^{-1}$  deltamethrin), whereas the control samples showed always an intact peritrophic membrane connected

to the microvilli border in all observed animals. It can be speculated that the insecticide deltamethrin, that leads to increased action potentials (Bradberry et al., 2005) caused a contraction of the gut tissue, and therefore a smaller diameter, possibly resulting in destruction of the peritrophic membrane. However, it can also not be excluded that a possible alteration of ion channels or of the membrane itself caused the shrinkage in the gut tissues not until the embedding procedure.

Deltamethrin has a half-life ranging between 17 and 110 d (Las-kowski, 2002), and was assumed to be stable during the exposure trial. However, following intake by the water fleas, metabolites could have been formed which were then detected by the NanoSIMS. Three main metabolites of deltamethrin have been reported, including dibromovinyl-dimethylcyclopropane carboxylic acid, which is the only one containing bromine. Dibromovinyl-dimethylcyclopropane carboxylic acid was already used as a biological monitoring parameter for deltamethrin exposure of workers spraying pesticides (Yao et al., 1992). Whether these metabolites are formed in *D. magna* remains unknown, and a differentiation with NanoSIMS within this study was not possible.

Due to the usage of the chemical embedding technique, it was also not possible to exclude wash-out effects on the overall distribution of bromine in the tissues. An alternative technique, based on freeze-drying, has been suggested (Edelmann, 2002). While having the advantage to minimize compositional changes during preparation, this technique also possesses disadvantages, such as being more costly, the whole process of sample preparation being



**Fig. 4.** Gut of *D. magna* after 48 h exposure to deltamethrin ( $200 \mu\text{g L}^{-1}$ ) in water. Elements shown are nitrogen (as CN), phosphorus and bromine, as obtained by the NanoSIMS 50. Brightness indicates higher concentration. Raster size  $40 \mu\text{m}$ , acquisition time 30 min. GW: gut wall, L: lumen with food particles, MV: microvilli layer.

**Table 2**

Mean normalized intensity values (Br/C)  $\pm$  standard deviation in the gut wall and the microvilli layer for the different exposure groups after 48 h of exposure to deltamethrin.

|                                       | Normalized intensity values gut wall <sup>a</sup> | Normalized intensity values microvilli layer <sup>a</sup> |
|---------------------------------------|---------------------------------------------------|-----------------------------------------------------------|
| Control                               | $0.04 \pm 0.17^A$                                 | $0.03 \pm 0.14^A$                                         |
| $50 \mu\text{g L}^{-1}$ deltamethrin  | $0.20 \pm 0.26^B$                                 | $0.15 \pm 0.21^C$                                         |
| $200 \mu\text{g L}^{-1}$ deltamethrin | $0.62 \pm 0.66^D$                                 | $0.58 \pm 0.46^D$                                         |

<sup>a</sup> Values across rows or column not sharing the same superscript are statistically significant different ( $P < 0.05$ ).

more time-consuming, and the limitation that only parts of the samples can be used, namely the ones containing vitreous ice. According to Peteranderl and Lechene (2004), biological tissues are typically composed of approximately 60% carbon and 11% nitrogen. Although nitrogen has to be detected as cyanide ( $\text{CN}^-$ ) as it does not produce secondary ions, it is possible to show the general cell morphology by imaging the CN-distribution (because of different C and N concentrations in different cell compartments that induce different brightness). Phosphorus as part of nucleic acids can indicate the position of DNA and RNA, although it was not possible to locate nuclei due to their phosphorus content in this study. Schultz and Kennedy (1976) researched the fine structure of the digestive system of water fleas (*Daphnia pulex*) with electron microscopy and found that their nuclei showed little or no heterochromatin, which could also be a reason for the low phosphorus signal in this study.

## 5. Conclusion

This study demonstrates that NanoSIMS 50 allowed for determining the uptake of a Br containing pesticide following exposure inside cell tissues, and furthermore its possibility to show, in conjunction with established toxicity tests, the relation between toxic effects of a pesticide such as deltamethrin and its visualization in vivo. These results highlight that this method can be an interesting analytical method in biology and ecotoxicology for investigating the uptake of xenobiotics into living organisms.

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