

SUPPORTING INFORMATION TO ARTICLE:

## Identification of transformation products of organic contaminants in natural waters by computer-aided prediction and high-resolution mass spectrometry

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## Sampling of surface water

Table S 1 Sampling dates of seven sampling points in Switzerland

| Sampling points     | Date of sampling | Number |
|---------------------|------------------|--------|
| Aabach Moenchaltorf | 5 May 2007       | 1      |
| Gossauer Bach       | 16 August 2007   | 2      |
| Chindsmuehlebach    | 29 August 2007   | 3      |
| Furtbach            | 29 June 2007     | 4      |
| Hauptkanal          | 6 May 2007       | 5      |
| La Petite Glane     | 6 May 2007       | 6      |
| Le Boiron           | 10 July 2007     | 7      |

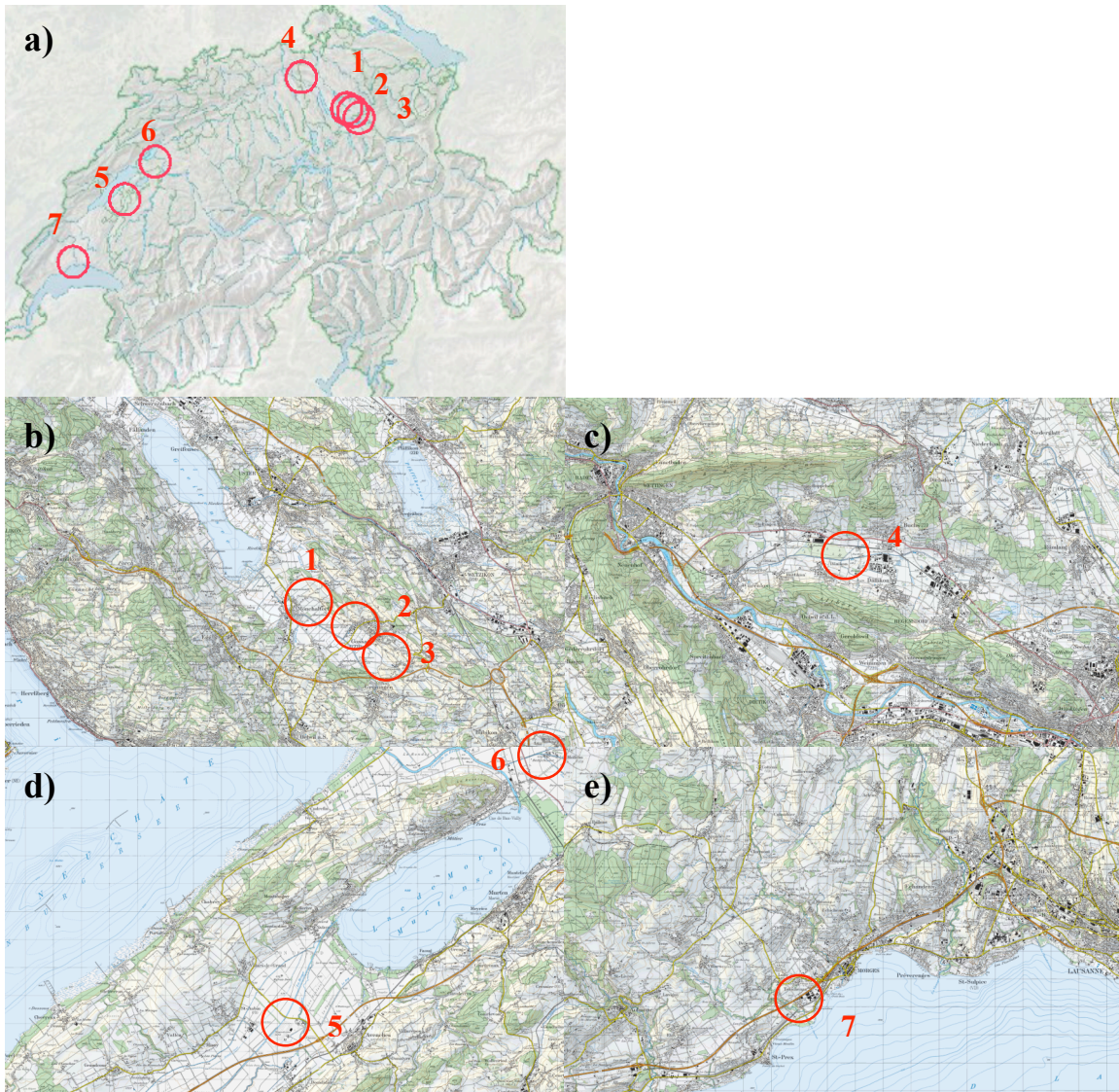


Figure S 1 Sampling points of seven different catchments in Switzerland; a) map of Switzerland with seven sampling points, b) catchment near lake Greifensee, c) catchment of Furtbach near river Glatt, d) catchment near lake Murtensee, and e) catchment near lake Geneva.

## ***Materials and reference standards***

The following reagents were used for sample preparation, LC-MS analysis, and reference standard preparation. Nanopure water was produced in-house by a Millipore instrument. Water and methanol (Acros Organics, Belgium) were HPLC grade. Ethanol p.a., ammonia and formic acid 98-100 % p.a. were purchased from Merck. LiOH·H<sub>2</sub>O > 99% was purchase from Fluka. Nitrogen gas with a purity of 99.999% was obtained from Carbagas. The reference standards for pesticides, biocides and pharmaceuticals including some transformation products (purity  $\geq$  97%) were purchased from the following providers: Sigma-Aldrich, Fluka, Acros Organics, Chemos GmbH, ReseaChem, Interchim and TRC Canada. Galaxolidone was a gift from Dr. Jean-Daniel Berset (Amt für Gewässerschutz und Abfallwirtschaft des Kantons Bern, Switzerland).

Standard stock solutions of 1 mg/ml were prepared by dissolving 20 mg standard in 20 ml of ethanol. Depending on their solubility, some compounds were dissolved in methanol, methanol/0.1 M HCl (v:v 2:3), isopropanol, or acetonitrile. Out of these stock solutions reference standard mixes with 205 standard compounds, including 64 transformation products for which standards were available, at 1  $\mu$ g/ml and 0.1  $\mu$ g/ml standard concentrations were prepared by dilution with ethanol. The standard mixes were stored in the dark at -20°C. To avoid substance precipitation, the mixes were ultrasonicated and let stand at room temperature for 2 hours before use.

## ***LC-MS/MS analysis***

The HPLC system consisted of a quaternary pump of type Rheos 2200 from Flux Instruments (Basel, Switzerland) and a HTS PAL autosampler of CTC Analytics AG (Zwingen, Switzerland). The analytical column was an XBridge C-18 column from Waters (2.1 mm x 50 mm with particle size of 3.5  $\mu\text{m}$ ) with a precolumn filled with the same material (2.1 mm x 10 mm). 20  $\mu\text{l}$  sample extracts were injected into the LC system. The mobile phase components were  $\text{H}_2\text{O}$  (water) and MeOH (methanol), both containing 0.1 vol% formic acid. The flow rate was 200  $\mu\text{l}/\text{min}$ . A two-step linear gradient was run from 90%  $\text{H}_2\text{O}$  to 50 %  $\text{H}_2\text{O}$  in 4 min, and to 5 %  $\text{H}_2\text{O}$  in another 13 min. These conditions were held for 6 min before column re-equilibration started with 10% MeOH and 90%  $\text{H}_2\text{O}$ . Detection of analytes was performed with an LTQ (Linear Trap Quadrupole) orbitrap mass spectrometer from Thermo Fisher Scientific Corporation with an ESI (electrospray ionization) interface in positive and negative mode. Parameters adjusted for the ion source ESI were sheath gas flow (0.6 l/min), auxiliary gas flow (1.5 l/min), and capillary temperature (300°C). Source voltage, source fragmentation, tube lens and capillary voltage were set to 5 kV, 10 V, 60 V and 25 V in positive mode, and values of -4 kV, -10 V, -60 V and -25 V in negative mode, respectively. A mass range from 50 - 2000 m/z was analyzed in full scan with a maximal ion count of 1,000,000. External mass calibration was used to ensure a mass accuracy of < 5 ppm over a mass range from 100 to 800 m/z. After transfer from the ion trap, fragmentation was achieved in the C-trap with nitrogen as collision gas. Product ions formed in the so called higher energy collision dissociation (HCD) are detected in the orbitrap. In a first step, high-resolution mass spectra (HR-MS) with a resolution of 60,000 and a mass accuracy of < 5 ppm were recorded to extract the target analytes for preliminary screening. For positive peak findings, data-dependent high-resolution product ion spectra (HR-MS/MS) at a resolution of 7,500 were produced for the five most intense ions using dynamic exclusion lists for a duration of 10 s. Data were analyzed with Xcalibur (Thermo Scientific, USA) in the Quan Browser for quantitative analysis with reference standards and in the Qual Browser for the identification procedure. For the prediction of expected MS/MS spectra, the spectral interpretation software MassFrontier 4.0 (Thermo Scientific, USA) was used.

## List of investigated micropollutants

52 parent compounds (24 pesticides, 7 biocides, 21 pharmaceuticals / personal care products) with high usage or sales data and complex chemical structures covering a broad physicochemical properties range were chosen for this study.

**Table S 2** Priority list of pesticides with CAS number, chemical structure, number of possible transformation products obtained from literature or predicted by UM-PPS, and usage class.

| Compound      | CAS number  | Chemical structure | Number of transformation products on target list   | Usage                         |
|---------------|-------------|--------------------|--|-------------------------------|
| 2,4-D         | 94-75-7     |                    | Literature: 18 UM-PPS: 12<br>Overlap: 6 Target: 24 | herbicide                     |
| Amitraz       | 33089-61-1  |                    | Literature: 8 UM-PPS: 10<br>Overlap: 2 Target: 16  | insecticide<br>(also biocide) |
| Asulam        | 3337-71-1   |                    | Literature: 3 UM-PPS: 10<br>Overlap: 2 Target: 11  | herbicide                     |
| Atrazine      | 1912-24-9   |                    | Literature: 17 UM-PPS: 10<br>Overlap: 6 Target: 21 | herbicide                     |
| Azoxystrobin  | 131860-33-8 |                    | Literature: 3 UM-PPS: 32<br>Overlap: 1 Target: 34  | fungicide                     |
| Bromoxynil    | 1689-84-5   |                    | Literature: 6 UM-PPS: 23<br>Overlap: 4 Target: 25  | herbicide                     |
| Chloridazon   | 1698-60-8   |                    | Literature: 6 UM-PPS: 14<br>Overlap: 0 Target: 20  | herbicide                     |
| Chlorthalonil | 1897-45-6   |                    | Literature: 13 UM-PPS: 29<br>Overlap: 5 Target: 37 | fungicide                     |
| Cymoxanil     | 57966-95-7  |                    | Literature: 9 UM-PPS: 20<br>Overlap: 1 Target: 28  | fungicide                     |
| Cyprodinil    | 121552-61-2 |                    | Literature: 6 UM-PPS: 26<br>Overlap: 2 Target: 30  | fungicide                     |
| Diazinon      | 333-41-5    |                    | Literature: 16 UM-PPS: 9<br>Overlap: 4 Target: 21  | insecticide<br>(also biocide) |
| Dicamba       | 1918-00-9   |                    | Literature: 5 UM-PPS: 18<br>Overlap: 2 Target: 21  | herbicide                     |
| Diuron        | 330-54-1    |                    | Literature: 9 UM-PPS: 32<br>Overlap: 5 Target: 36  | herbicide                     |
| Epoxiconazole | 133855-98-8 |                    | Literature: 5 UM-PPS: 42<br>Overlap: 1 Target: 46  | fungicide                     |
| Ethofumesate  | 26225-79-6  |                    | Literature: 3 UM-PPS: 14<br>Overlap: 2 Target: 15  | herbicide                     |

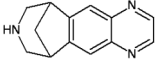
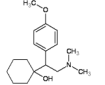
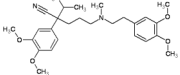
|              |            |  |  |                           |
|--------------|------------|--|--|---------------------------|
| Fluazinam    | 79622-59-6 |  | Literature: 5 UM-PPS: 71<br>Overlap: 3 Target: 73  | fungicide and insecticide |
| Isoproturon  | 34123-59-6 |  | Literature: 16 UM-PPS: 26<br>Overlap: 6 Target: 36 | herbicide                 |
| Mecoprop-P   | 93-65-2    |  | Literature: 2 UM-PPS: 16<br>Overlap: 1 Target: 17  | herbicide                 |
| Metamitron   | 41394-05-2 |  | Literature: 4 UM-PPS: 3<br>Overlap: 0 Target: 7    | herbicide                 |
| Metolachlor  | 51218-45-2 |  | Literature: 16 UM-PPS: 47<br>Overlap: 4 Target: 59 | herbicide                 |
| Metribuzin   | 21087-64-9 |  | Literature: 5 UM-PPS: 7<br>Overlap: 1 Target: 11   | herbicide                 |
| Phenmedipham | 13684-63-4 |  | Literature: 5 UM-PPS: 57<br>Overlap: 1 Target: 61  | herbicide                 |
| Prochloraz   | 67747-09-5 |  | Literature: 9 UM-PPS: 34<br>Overlap: 2 Target: 41  | fungicide                 |
| Sulcotrione  | 99105-77-8 |  | Literature: 5 UM-PPS: 53<br>Overlap: 2 Target: 56  | herbicide                 |

**Table S 3 Priority list of biocides with CAS number, chemical structure, number of possible transformation products obtained from literature or predicted by UM-PPS, and usage class.**

| Compound      | CAS number | Chemical structure | Number of transformation products on target list   | Usage                       |
|---------------|------------|--------------------|--|-----------------------------|
| Carbendazim   | 10605-21-7 |                    | Literature: 4 UM-PPS: 4<br>Overlap: 1 Target: 7    | fungicide (also pesticide)  |
| DEET          | 134-62-3   |                    | Literature: 16 UM-PPS: 23<br>Overlap: 7 Target: 32 | insect and acarid repellent |
| Dichlofluanid | 1085-98-9  |                    | Literature: 13 UM-PPS: 16<br>Overlap: 0 Target: 29 | fungicide                   |
| Irgarol       | 28159-98-0 |                    | Literature: 3 UM-PPS: 22<br>Overlap: 1 Target: 24  | herbicide                   |
| Propiconazole | 60207-90-1 |                    | Literature: 6 UM-PPS: 35<br>Overlap: 3 Target: 38  | fungicide (also pesticide)  |
| Terbutryn     | 888-50-0   |                    | Literature: 6 UM-PPS: 16<br>Overlap: 4 Target: 18  | herbicide (also pesticide)  |
| Triclosan     | 3380-34-5  |                    | Literature: 6 UM-PPS: 32<br>Overlap: 0 Target: 38  | antimicrobial agent         |

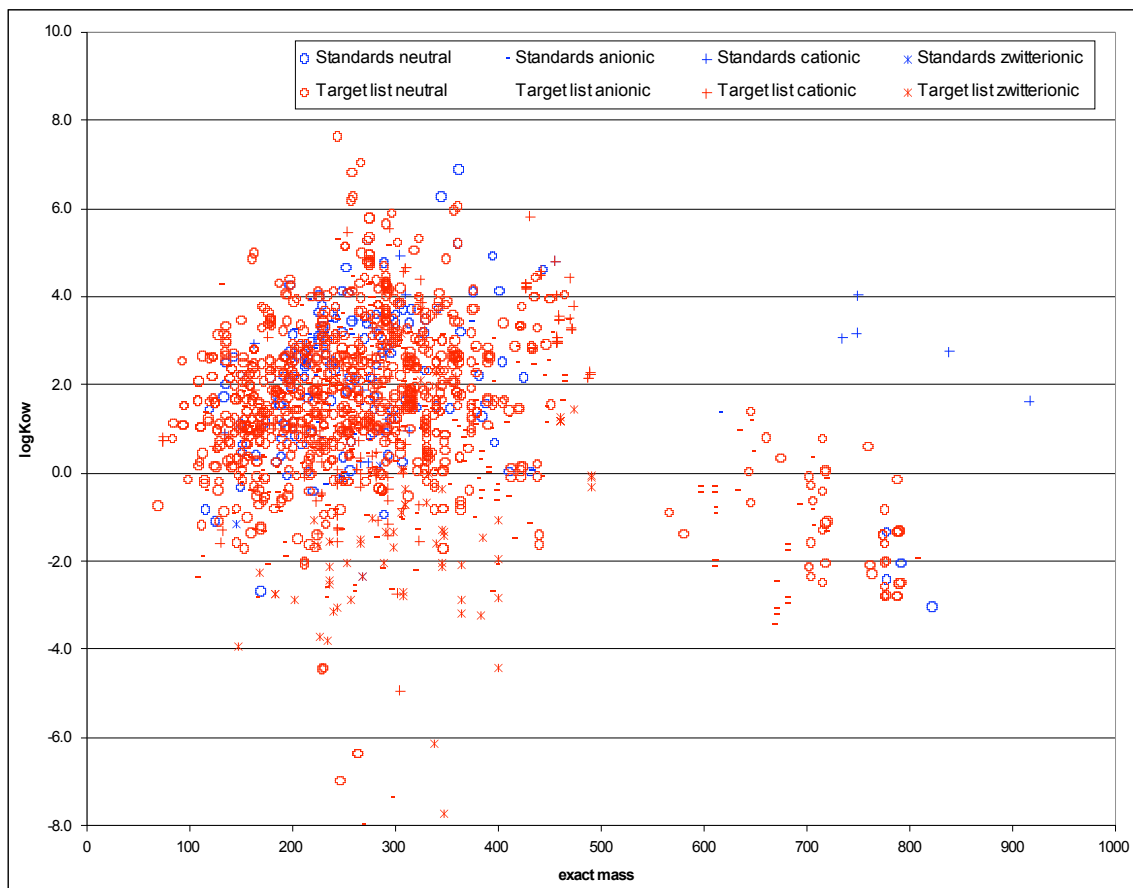
**Table S 4 Priority list of pharmaceuticals / personal care products with CAS number, chemical structure, number of possible transformation products obtained from literature or predicted by UM-PPS, and usage class.**

| Compound         | CAS number  | Chemical structure | number of metabolites / transformation products on target list | Usage                                 |
|------------------|-------------|--------------------|--|---------------------------------------|
| Aminopyrine      | 58-15-1     |                    | Literature: 9 UM-PPS: 13<br>Overlap: 2 Target: 20              | Analgetic                             |
| Amoxicillin      | 26787-78-0  |                    | Literature: 3 UM-PPS: 59<br>Overlap: 1 Target: 61              | Antibiotic                            |
| Atenolol         | 29122-68-7  |                    | Literature: 10 UM-PPS: 19<br>Overlap: 5 Target: 24             | beta-blocker                          |
| Bezafibrate      | 41859-67-0  |                    | Literature: 8 UM-PPS: 39<br>Overlap: 2 Target: 45              | Lipid regulator                       |
| Carbamazepine    | 298-46-4    |                    | Literature: 13 UM-PPS: 34<br>Overlap: 3 Target: 44             | Anti-convulsant                       |
| Ciprofloxacin    | 85721-33-1  |                    | Literature: 4 UM-PPS: 30<br>Overlap: 0 Target: 34              | Antibiotic                            |
| Diclofenac       | 15307-86-5  |                    | Literature: 23 UM-PPS: 53<br>Overlap: 1 Target: 75             | Anti-inflammatory agent               |
| Exemestane       | 107868-30-4 |                    | Literature: 1 UM-PPS: 51<br>Overlap: 0 Target: 52              | steroidal aromatase inhibitor         |
| Fenofibrate      | 49562-28-9  |                    | Literature: 7 UM-PPS: 14<br>Overlap: 2 Target: 19              | Lipid regulator                       |
| Fluoxetine       | 54910-89-3  |                    | Literature: 3 UM-PPS: 29<br>Overlap: 1 Target: 31              | Anti-depressant                       |
| Galaxolide       | 1222-05-5   |                    | Literature: 4 UM-PPS: 36<br>Overlap: 0 Target: 40              | Musk fragrant                         |
| Ibuprofen        | 15687-27-1  |                    | Literature: 18 UM-PPS: 16<br>Overlap: 0 Target: 34             | Analgetic and anti-inflammatory agent |
| Iopromide        | 73334-07-3  |                    | Literature: 4 UM-PPS: 106<br>Overlap: 2 Target: 108            | X-ray contrast agent                  |
| Mefenamic acid   | 61-68-7     |                    | Literature: 4 UM-PPS: 75<br>Overlap: 0 Target: 79              | Anti-inflammatory agent               |
| Methylphenidate  | 113-45-1    |                    | Literature: 3 UM-PPS: 25<br>Overlap: 1 Target: 27              | Stimulant                             |
| Naproxen         | 22204-53-1  |                    | Literature: 2 UM-PPS: 9<br>Overlap: 0 Target: 11               | Anti-inflammatory agent               |
| Sulfamethoxazole | 723-46-6    |                    | Literature: 7 UM-PPS: 10<br>Overlap: 0 Target: 17              | Antibiotic                            |
| Trimethoprim     | 738-70-5    |                    | Literature: 7 UM-PPS: 15<br>Overlap: 2 Target: 20              | Antibiotic                            |

|             |             |   |   |   |
|-------------|-------------|---|---|---|
| Varenicline | 249296-44-4 |  | Literature: 4 UM-PPS: 15<br>Overlap: 0 Target: 19 | nicotinic<br>receptor partial<br>antagonist |
| Venlafaxine | 93413-62-8  |  | Literature: 3 UM-PPS: 18<br>Overlap: 2 Target: 19 | Anti-depressant                             |
| Verapamil   | 52-53-9     |  | Literature: 4 UM-PPS: 83<br>Overlap: 4 Target: 83 | calcium channel<br>blocker                  |

## Comparison of physico-chemical properties between target list transformation products and reference standards

Physico-chemical properties including the molecular size,  $\log K_{ow}$ , and speciation at pH7 of the proposed 1794 transformation products are compared with the reference standard set consisting of 205 compounds (Figure S 2).



**Figure S 2 Comparison of the standard set (205 compounds, in blue) with all transformation products on the target list (1794, in red) with respect to molecular size (exact mass),  $\log K_{ow}$ , and speciation at pH7 (neutral, anionic, cationic, and zwitterionic).**

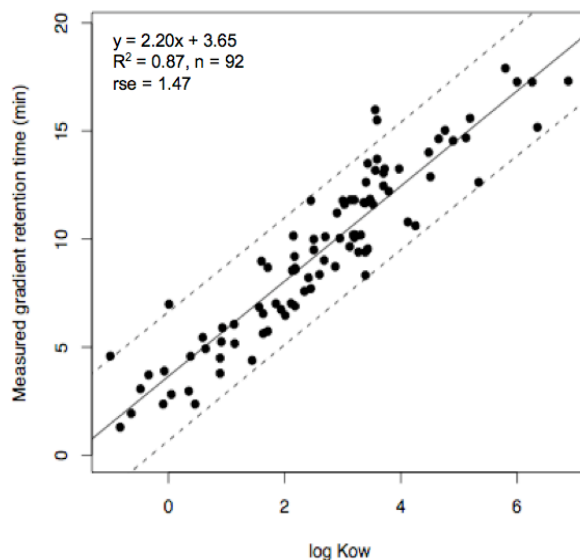
About 1650 out of 1794 transformation products lie within a  $\log K_{ow}$  range of -2.0 to 6.0 and within a mass range of 100 to 900 g/mol. Based on experience with the reference standard set, these compounds are deemed analyzable.

## Checking the plausibility of measured retention times

### Deriving an upper limit for plausible retention times

This section explains in more detail how an upper plausibility limit for retention times of both neutral and ionic TPs was derived. No lower limit could be set because (i) little is known about the retention of ionic species in HPLC systems other than that they will maximally show a similar, but most likely a lower retention time than the corresponding neutral species, and (ii) the extent of speciation in a MeOH:H<sub>2</sub>O gradient cannot be predicted with great accuracy.

The upper limit against which measured TP retention times are to be compared is based on an empirical linear correlation between gradient retention time and octanol-water partition coefficient ( $\log K_{ow}$ ) and was defined as the upper limit of the 95% prediction interval of this correlation. The correlation with  $\log K_{ow}$  represents a reasonable basis for examining the plausibility of measured gradient retention times because (i) analysis of the governing interactions had shown that isocratic retention on C18 columns with MeOH as organic modifier were reasonably well correlated with  $\log K_{ow}$  (1), (ii) assuming linear and constant solvent strength for a given organic modifier in HPLC, gradient retention times can be shown to be linearly related to partitioning under isocratic conditions at the start of the gradient (2), and (iii) experimental or predicted data for  $\log K_{ow}$  are readily accessible. The correlation was trained for a set of 92 parent compound reference standards that are predominantly (> 75%) neutral at an elution pH of 3 according to predictions with Chem-Axon (3) (for a list see below). For most of the standards, experimental  $\log K_{ow}$  values were available to establish the correlation. If not, their  $\log K_{ow}$  was predicted using Kow-Win (4). The resulting linear correlation between measured gradient retention times and  $\log K_{ow}$  for the 92 reference standards as well as its 95% prediction interval is shown in Figure S3 including the underlying data and the statistics of the linear fit.



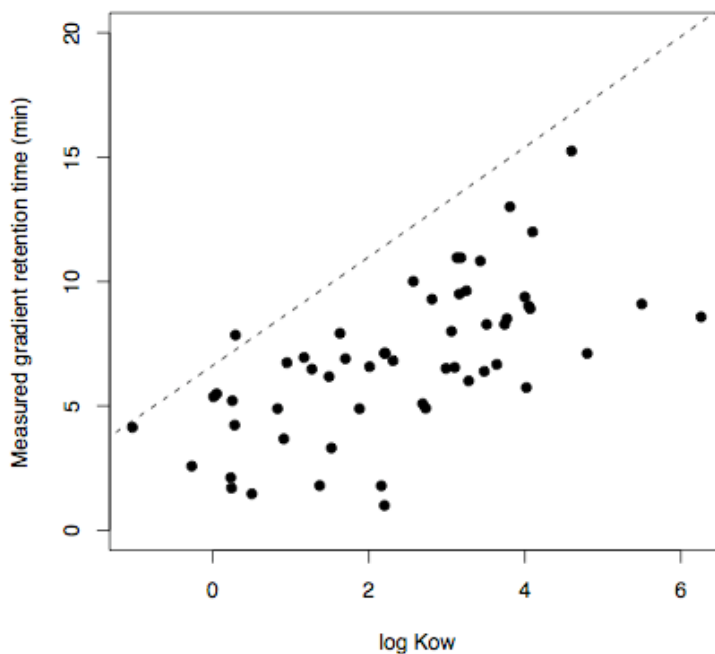
**Figure S 3** Linear correlation between gradient retention times and  $\log K_{ow}$  for 92 reference standards (all parent compounds, including pesticides, pharmaceuticals, and biocides) that are predominantly neutral at the elution pH of 3. The dashed lines delineate the 95% prediction interval. rse: residual standard error.

The 92 parent compounds used for deriving the linear correlation between retention time and  $\log K_{ow}$  were 1,2-Benzisothiazolin-3-one (CAS 2634-33-5), 2-Hydroxy-4-methoxybenzophenone (CAS 131-57-7), 2-Methyl-4-isothiazolin-3-one (CAS 2682-20-4), 2-n-Octyl-4-isothiazolin-3-one (CAS 26530-20-1), 4,5-Dichloro-2-n-octyl-isothiazol-3(2H)-one (CAS 64359-81-5), 5-Chloro-2-methylisothiazolin-3-one (CAS 26172-55-4), 5-Methyl-benzotriazole (CAS 136-85-6), Acetochlor (CAS 34256-82-1), Alachlor (CAS 15972-60-8), Aldicarb (CAS 116-06-3), Atrazine (CAS 1912-24-9), Azoxystrobin (CAS 131860-33-8), Bentazone (CAS 25057-89-0), Benzothiazole (CAS 95-16-9), Benzotriazole (CAS 95-14-7), Bezafibrate (CAS 41859-67-0), Bifenox (CAS 42576-02-3), Bromacil (CAS 314-40-9), Bromoxynil (CAS 1689-84-5), Bronopol (CAS 52-51-7), Carbamazepine (CAS 298-46-4), Carbetamide (CAS 16118-49-3), Chloridazone (CAS 1698-60-8), Chlortoluron (CAS 15545-48-9), Clomazone (CAS 81777-89-1), Caffeine (CAS 58-08-2), Cymoxanil (CAS 57966-95-7), Cypermethrin (CAS 52315-07-8), Cyproconazole (CAS 94361-06-5), Desmedipham (CAS 13684-56-5), Diclofenac (CAS 15307-86-5), Diflufenican (CAS 83164-33-4), Diglyme (CAS 111-96-6), Dimethachlor (CAS 50563-36-5), Dimethenamid (CAS 87674-68-8), Dinoseb (CAS 88-85-7), Diuron (CAS 330-54-1), Epoxyconazole (CAS 133855-98-8), Ethofumesate (CAS 26225-79-6), Exemestane (CAS 107868-30-4), Fenofibrate (CAS 49562-28-9), Fluazinam (CAS 79622-59-6), Fludioxonil (CAS 131341-86-1), Flufenacet (CAS 142459-58-3), Flusilazole (CAS 85509-19-9), Galaxolide (CAS 1222-05-5), Hexazinone (CAS 51235-04-2), Ibuprofen (CAS 15687-27-1), Ioxynil (CAS 1689-83-4), Isoproturon (CAS 34123-59-6), Ketoprofen (CAS 22071-15-4), Kresoxim-methyl (CAS 143390-89-0), Linuron (CAS 330-55-2), Mefenamic acid (CAS 61-68-7), Metalaxyl (CAS 57837-19-1), Metazachlor (CAS 57837-19-1), Metolachlor (CAS 51218-45-2), Monuron (CAS 150-68-5), N,N-diethyl-3-methylbenzamide (CAS 134-62-3), Napropamid (CAS 15299-99-7), Naproxen (CAS 22204-53-1), Nicosulfuron (CAS 111991-09-4), N-Methylacetanilide (CAS 579-10-2), Octocrylene (CAS 6197-30-4), Octyl-4-methoxycinnamate (CAS 83834-59-7), Orbencarb (CAS 34622-58-7), Paracetamol (CAS 103-90-2), Pethoxamid (CAS 106700-29-2), Phenazone (CAS 60-80-0), Phenmedipham (CAS 13684-63-4), Pinoxaden (CAS 243973-20-8), Primidone (CAS 125-33-7), Propachlor (CAS 1918-16-7), Propiconazole (CAS 60207-90-1), Prosulfocarb (CAS 52888-80-9), Sebuthylazine (CAS 7286-69-3), Simazine (CAS 122-34-9), Sucralose (CAS 56038-13-2), Sulfadiazine (CAS 68-35-9), Sulfadimethoxine (CAS 122-11-2), Sulfamethazine (CAS 57-68-1), Sulfamethoxazole (CAS 723-46-6), Sulfapyridin (CAS 144-83-2), Sulfathiazole (CAS 72-14-0), Tebuconazole (CAS 107534-96-3), Tebutam (CAS 35256-85-0), Terbutylazin (CAS 5915-41-3), Thifensulfuron-methyl (CAS 79277-27-3), Tonalid (CAS 21145-77-7), Triclosan (CAS 3380-34-5), Trifluraline (CAS 1582-09-8), and Trinexapac-ethyl (CAS 95266-40-3).

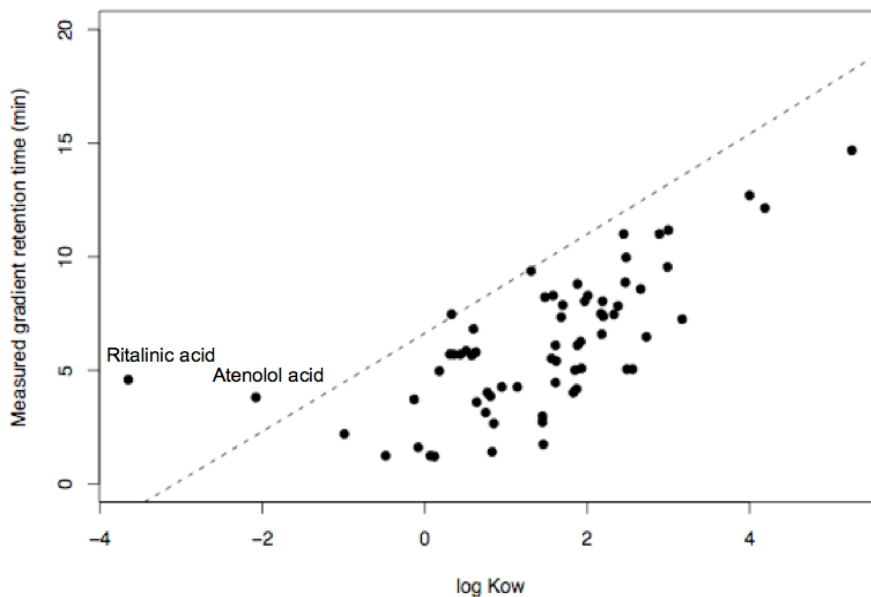
Since, as mentioned above, ionic species can be assumed to be similarly or less retained than their corresponding neutral species, the upper limit of the 95% prediction interval of the correlation between  $\log K_{ow}$  and retention times for neutral reference standards is expected to serve as a reasonable upper limit for checking the plausibility of measured times of both neutral and hence also ionic TPs. This is validated in two steps. First, the upper limit is compared against a set of 55 predominantly ionic reference standards that are all parent compounds (Figure S4). Second, it is compared against a set of 66 both ionic and neutral TPs for which reference standards were available (Figure S5). Since experimental  $\log K_{ow}$  values for TPs are typically not available, the procedure to estimate their  $\log K_{ow}$  values as used in Figure S5 was optimized to profit from experimentally measured  $\log K_{ow}$  values for the parent compound ( $\log K_{ow,exp}^{PC}$ ), when available. At the basis for the read-across lies the assumption that fragment methods such as EPI Suite KowWin are prone to systematic errors, especially for complex molecular structures, because correction factors for certain structural configurations might be missing. Since the parent compound and the transformation product, however, share most of their molecular structure and only differ in the functional groups that were transformed, this mistake is likely to be similar for parent compound and transformation product. Therefore, the mistake can be tentatively quantified by comparing KowWin predictions for the parent compound ( $\log K_{ow,EPI}^{PC}$ ) with its experimental  $\log K_{ow,exp}^{PC}$  (eq. 1) and by using the difference to correct the predicted  $\log K_{ow}$  value for the transformation product ( $\log K_{ow,EPI}^{TP}$ ) to yield a final estimate ( $\log K_{ow,est}^{TP}$ ) (eq. 2).

$$\Delta \log K_{ow} = \log K_{ow,exp}^{PC} - \log K_{ow,EPI}^{PC} \quad \text{eq. 1}$$

$$\log K_{ow,est}^{TP} = \log K_{ow,EPI}^{TP} + \Delta \log K_{ow} \quad \text{eq. 2}$$



**Figure S 4 Comparison of measured gradient retention times with upper plausibility limit (dashed line) for 55 reference standards (all parent compounds, including pesticides, pharmaceuticals, and biocides) that are predominantly ionic at the elution pH of 3.**



**Figure S 5 Comparison of measured gradient retention times with upper plausibility limit (dashed line) for 66 TPs for which reference standards were available and that are both predominantly neutral or ionic at the elution pH of 3.**

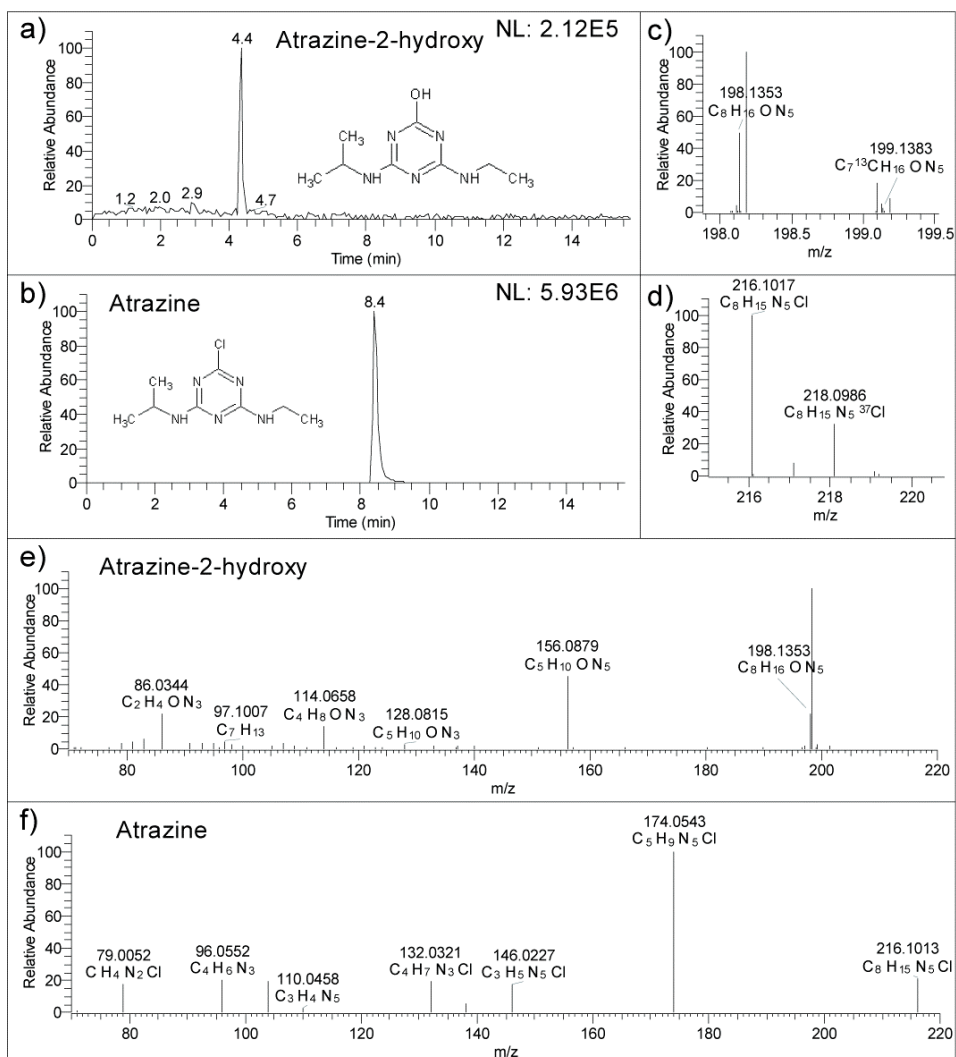
Figures S4 and S5 show that in the case of the ionic parent compounds 54 out of 55 compounds, and in the case of TPs 63 out of 66 compounds lie below the plausibility limit for measured retention times. A more detailed analysis of the two major outliers in Figure S5 (ritalinic acid and atenolol acid) showed that they are due to a bad performance of KowWin for these two compounds rather than due to problems with the suggested upper plausibility limit. The false negative outcome for these two compounds is most likely due to an amino acid correction used for these compounds in KowWin, which might not be justified. Indeed, other  $\log K_{ow}$  prediction software (e.g., ALOGPS 2.1 program of VCCLAB, Virtual Computational Chemistry Laboratory, <http://www.vcclab.org/lab/alogsps/start.html>) indicated higher  $\log K_{ow}$  values for these two compounds, which would make them fall well below the plausibility limit.

In summary, using the suggested upper plausibility limit for measured retention times produces less than 5% false negatives for ionic and neutral parent compounds and TPs. At the same time, the application of this plausibility limit within the identification procedure reduced the number of plausible TPs by 30% from 640 to 438 TPs. This demonstrates its usefulness as an efficient funneling criterion to reduce the number of TPs to be carried through to the next steps of analysis within the identification procedure.

### ***Chromatograms and spectra of found transformation products***

After application of the funneling procedure 19 transformation products were identified and found in surface water. In the following, chromatograms and spectra of these transformation products (for azoxystrobin acid see paper Figure 3) are described in detail.

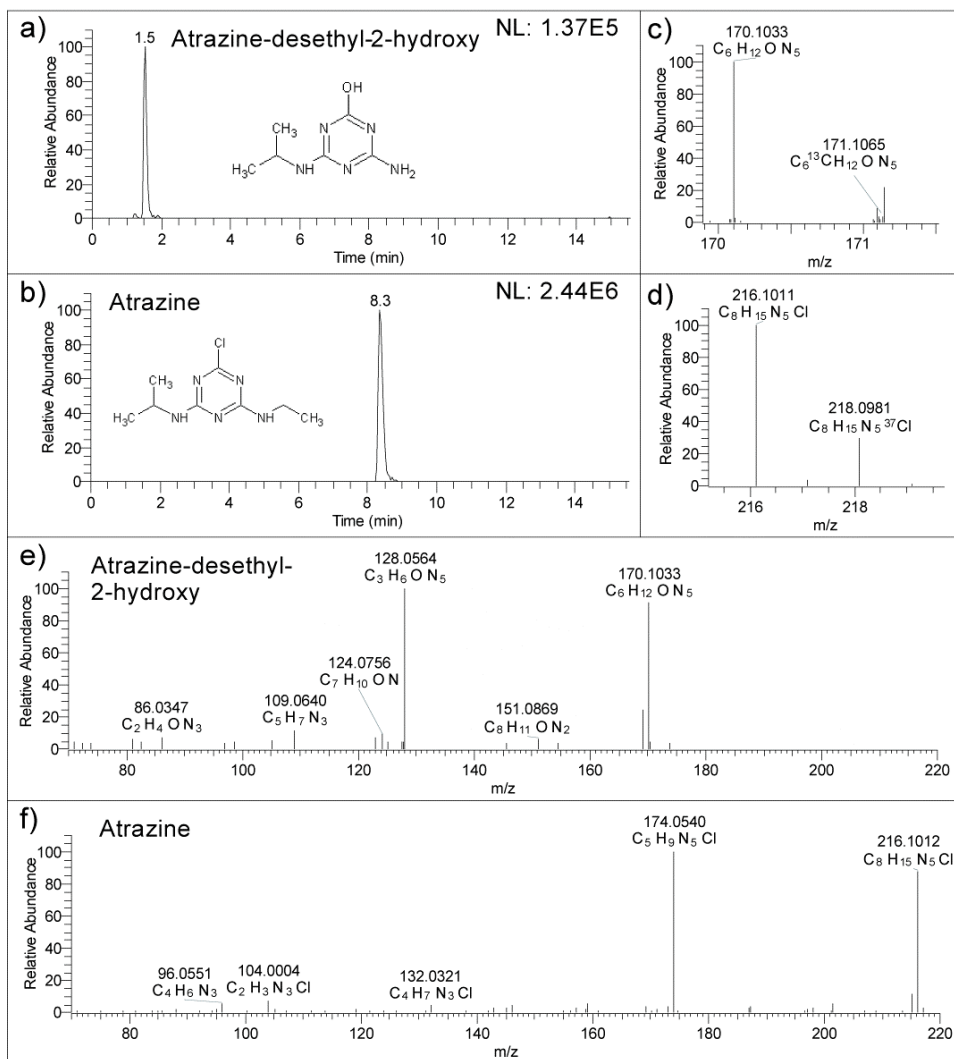
Atrazine-2-hydroxy, CAS no. 2163-68-0, sample *La Petite Glane*



**Figure S 6** Structure of the transformation product atrazine-2-hydroxy and its parent compound atrazine and the corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). All spectra are shown for the sample *La Petite Glane*.

The proposed pesticide transformation product atrazine-2-hydroxy (transformation product of atrazine) has the elemental composition  $C_8H_{15}N_5O_1$  ( $[M+H]^+ = 198.1349$ ). The  $^{13}C$  isotope pattern is in agreement with 8 carbon atoms ( $\sim 10\%$ ). The retention time of 4.4 min for atrazine-2-hydroxy is in agreement with its higher polarity than atrazine ( $\log K_{ow,est}$  2.1) and its predicted retention time ( $t_{est}^{TP}$  6.7 min), while atrazine ( $\log K_{ow,est}$  2.8) has a retention time of 8.4 min and a characteristic  $^{37}Cl$  isotope signal. Atrazine-2-hydroxy was ultimately identified based on the fragment 156.0879 ( $C_5H_{10}N_5O_1$ ), which was predicted by MassFrontier. This fragment is also in analogy to the most intense fragment for atrazine (174.0543  $C_5H_9N_5Cl_1$ ). The formation of atrazine-2-hydroxy by photolytic processes (e.g. 5) and by biological processes (e.g. 6) has been shown. The occurrence and fate of atrazine-2-hydroxy in surface water has been described previously (e.g. 7-9).

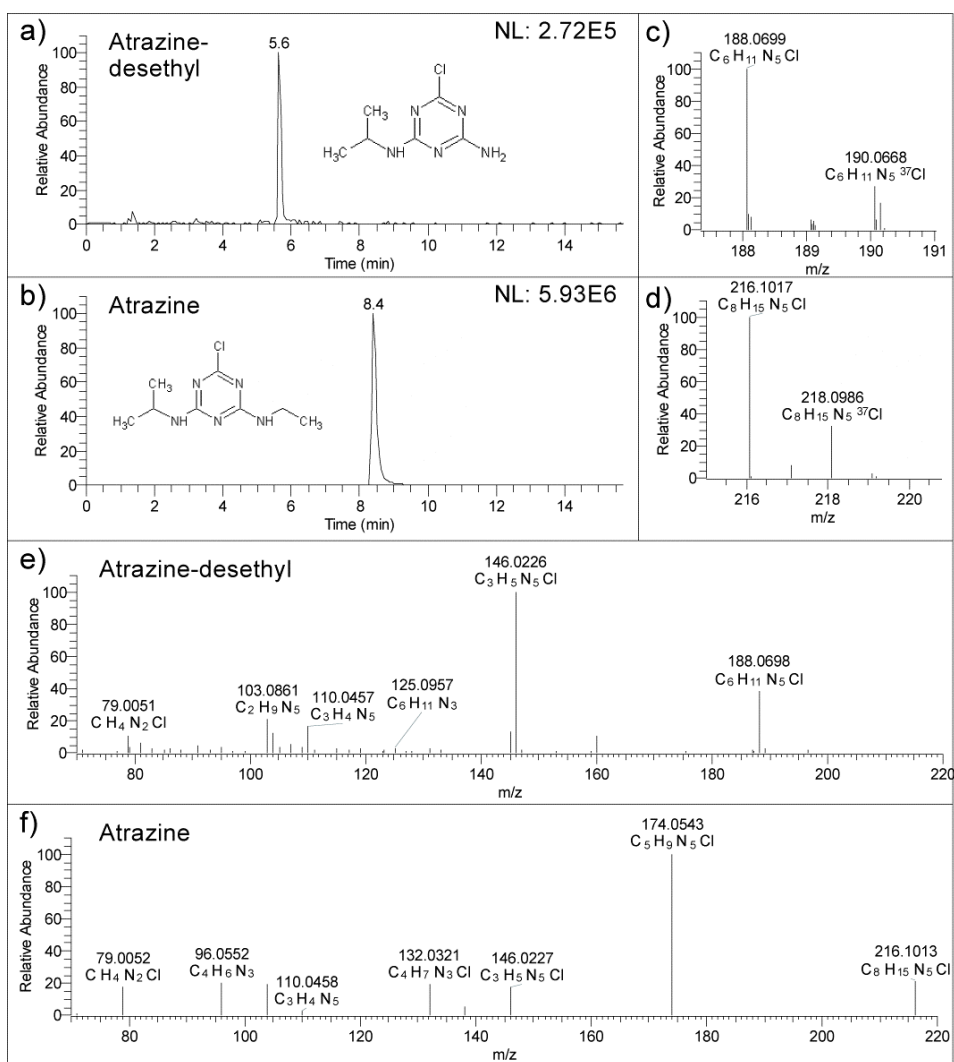
Atrazine-desethyl-2-hydroxy, CAS no. 19988-24-0, sample *Le Boiron*



**Figure S 7** Structure of the transformation product atrazine-2-hydroxy-desethyl and its parent compound atrazine and the corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). All spectra are shown for the sample *Le Boiron*.

The proposed pesticide transformation product atrazine-desethyl-2-hydroxy (transformation product of atrazine) has the elemental composition  $C_6H_{11}N_5O_1$  ( $[M+H]^+ = 170.1036$ ). The  $^{13}C$  isotope pattern is in agreement with 6 carbon atoms ( $\sim 10\%$ ). The retention time of 1.5 min for atrazine-desethyl-2-hydroxy is rather low and can only be explained with an increased polarity ( $\log K_{ow,est} = 1.1$ ), and partly different speciation (zwitterionic). Its predicted retention time  $t_{est}^{TP}$  is 4.4 min. Atrazine-desethyl-2-hydroxy was ultimately identified based on the MS/MS spectra with the fragments 128.0564 ( $C_3H_6N_5O_1$ ), 109.0640 ( $C_5H_7N_3$ ), 86.0347 ( $C_2H_4N_3O_1$ ) which were also predicted by MassFrontier. The occurrence and fate of atrazine-desethyl-2-hydroxy in surface water has been described previously (e.g. 7,9).

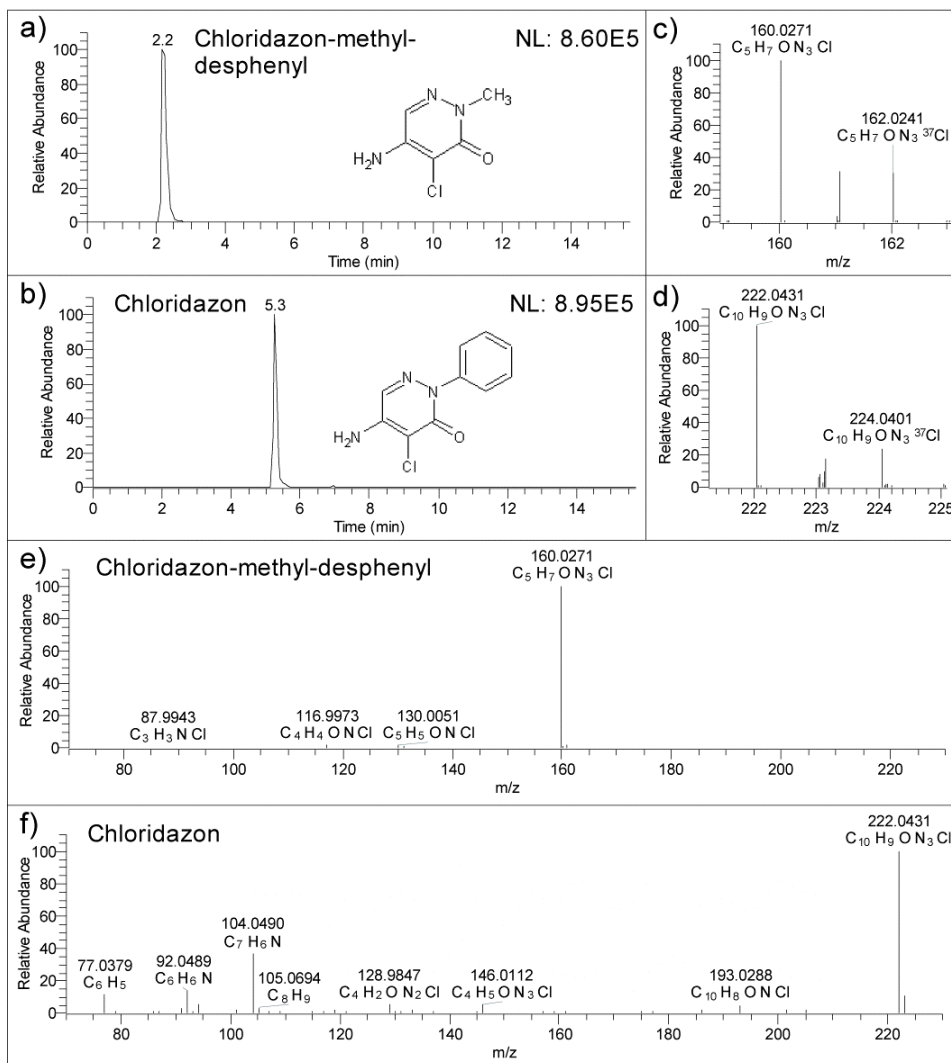
Atrazine-desethyl, CAS no. 6190-65-4, sample *La Petite Glane*



**Figure S 8** Structure of the transformation product atrazine-desethyl and its parent compound atrazine and the corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). All spectra are shown for the sample *La Petite Glane*.

The proposed pesticide transformation product atrazine-desethyl (transformation product of atrazine) has the elemental composition  $C_6H_{10}Cl_1N_5$  ( $[M+H]^+=188.0698$ ). The  $^{37}Cl$  isotope pattern is in agreement with 1 chlorine atom ( $\sim 35\%$ ). The retention time of 5.6 min for atrazine-desethyl is in agreement with its polarity ( $\log K_{ow,est}$  1.8) and similar to the predicted retention time ( $t_{est}^{TP}$  6.0 min). Atrazine-desethyl was ultimately identified based on the agreement of three fragments with the parent compound atrazine and the characteristic fragment 146.0226 ( $C_3H_5N_5Cl_1$ ). The occurrence and fate of atrazine-desethyl in surface water has been described previously (e.g. 7,9).

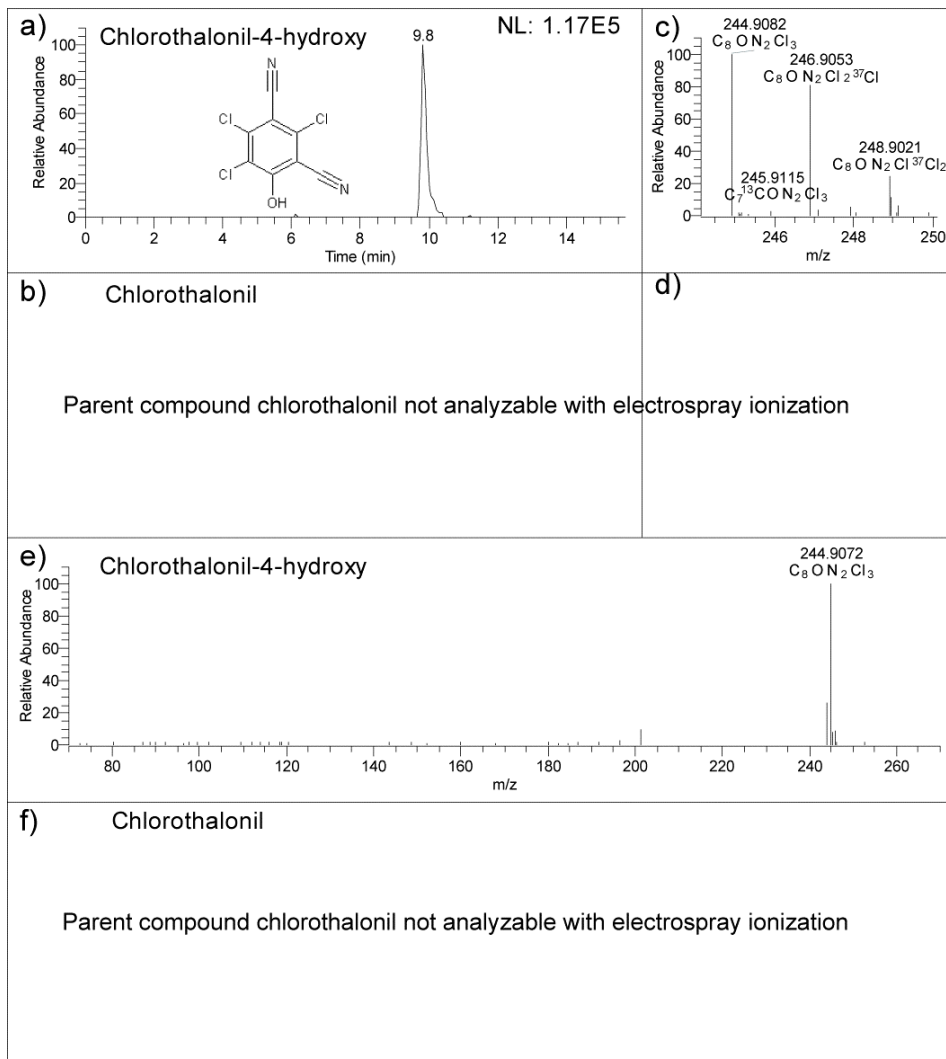
Chloridazon-methyl-desphenyl, CAS no. 17254-80-7, sample *La Petite Glane*



**Figure S 9 Structure of the transformation product chloridazon-methyl-desphenyl and its parent compound chloridazon and the corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). All spectra are shown for the sample *La Petite Glane*.**

The proposed pesticide transformation product chloridazon-methyl-desphenyl (transformation product of chloridazon) has the elemental composition C<sub>5</sub>H<sub>6</sub>Cl<sub>1</sub>N<sub>3</sub>O<sub>1</sub> ([M+H]<sup>+</sup>=160.0272). The <sup>37</sup>Cl isotope pattern is in agreement with 1 chlorine atom (~35%). The retention time of 2.2 min for chloridazon-methyl-desphenyl is in agreement with its high polarity (log *K*<sub>ow,est</sub> -1.4, *t*<sup>TP</sup><sub>est</sub> 2.0 min), in contrast to the parent compound chloridazon with a retention time of 5.3 min (log *K*<sub>ow,est</sub> 0.8) and a characteristic <sup>37</sup>Cl isotope signal. Chloridazon-methyl-desphenyl was ultimately identified based on the fragments 130.0051 (C<sub>5</sub>H<sub>5</sub>N<sub>1</sub>O<sub>1</sub>Cl<sub>1</sub>), 116.9973 (C<sub>4</sub>H<sub>4</sub>N<sub>1</sub>O<sub>1</sub>Cl<sub>1</sub>), and 87.9943 (C<sub>3</sub>H<sub>3</sub>N<sub>1</sub>Cl<sub>1</sub>). The occurrence and fate of chloridazon-methyl-desphenyl in surface water has been described previously (10). The formation of chloridazon-methyl-desphenyl takes place in soil (7,11).

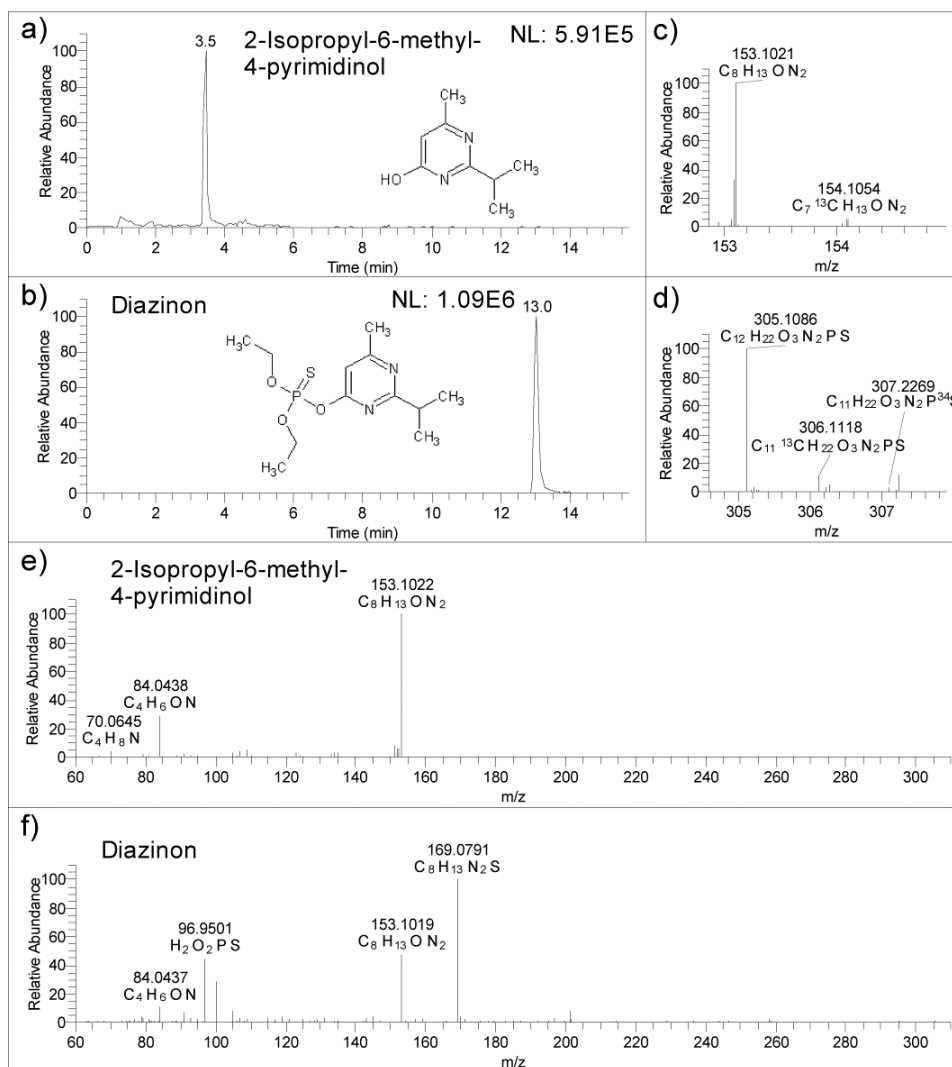
Chlorothalonil-4-hydroxy, CAS no. 28343-61-5, sample *Hauptkanal*



**Figure S 10 Structure of the transformation product chlorothalonil-4-hydroxy and its parent compound chlorothalonil and the corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). All spectra are shown for the sample *Hauptkanal*.**

The proposed pesticide transformation product chlorothalonil-4-hydroxy (transformation product of chlorothalonil) has the elemental composition C<sub>8</sub>H<sub>1</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>1</sub> and is detected in negative mode ([M-H]<sup>-</sup>=244.9082). The <sup>37</sup>Cl isotope pattern is in agreement with 3 chlorine atoms. The retention time of 9.8 min for chlorothalonil-4-hydroxy is in agreement with its polarity (log *K*<sub>ow,est</sub> 3.1) and its predicted retention time (*t*<sub>est</sub><sup>TP</sup> 9.1 min). The parent compound chlorothalonil lacks functional groups that can add or eliminate a hydrogen atom and is therefore not ionizable by electrospray ionization (ESI). Chlorothalonil-4-hydroxy was identified based on the plausibility of its retention time and its Cl isotope pattern. Given its molecular formula only a few chemical structures are possible, with the most probable structure being chlorothalonil-4-hydroxy. The occurrence and fate of chlorothalonil-4-hydroxy in surface water has been described previously (11-15).

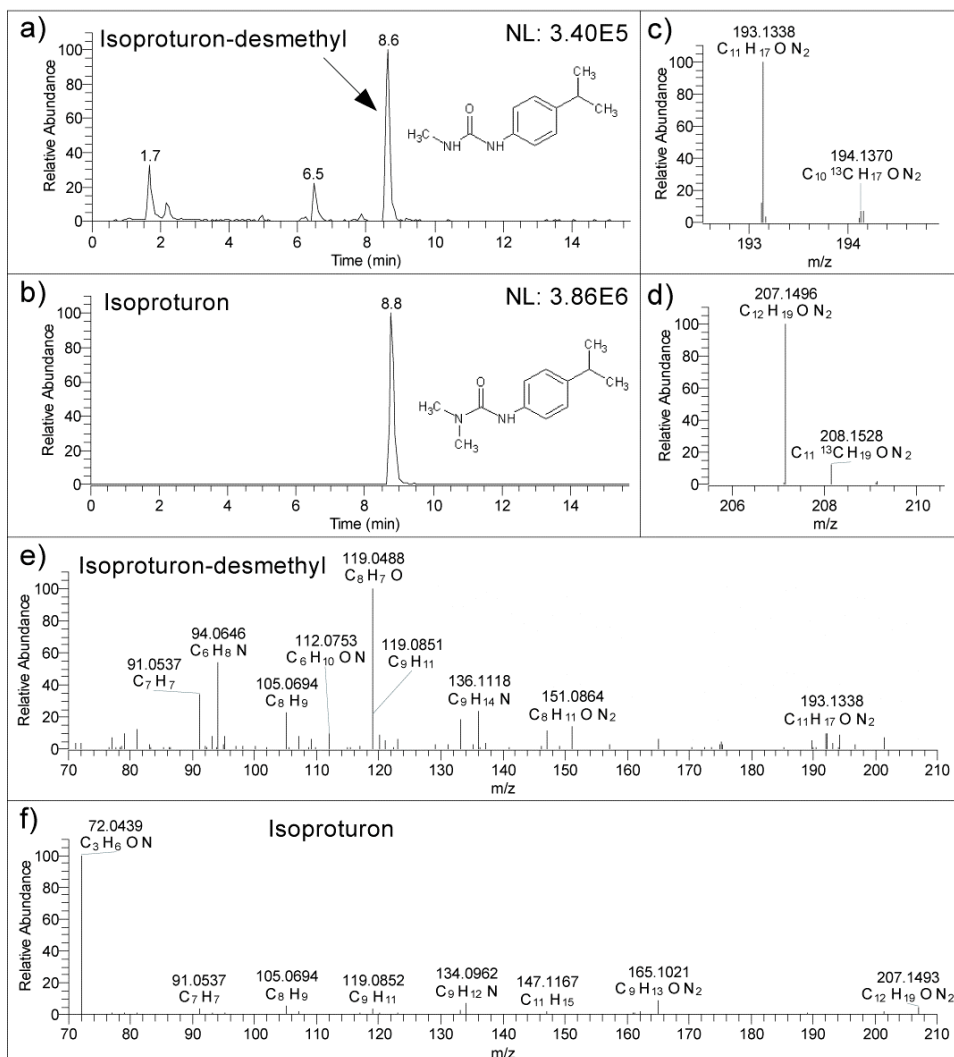
2-Isopropyl-6-methyl-4-pyrimidinol, CAS no. 2814-220-2, sample *Aabach Moenchaltorf*



**Figure S 11 Structure of the transformation product 2-isopropyl-6-methyl-4-pyrimidinol and its parent compound diazinon and the corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). All spectra are shown for the sample *Aabach Moenchaltorf*.**

The proposed pesticide transformation product 2-isopropyl-6-methyl-4-pyrimidinol (transformation product of diazinon) has the elemental composition C<sub>8</sub>H<sub>12</sub>N<sub>2</sub>O<sub>1</sub> ([M+H]<sup>+</sup>=153.1022. The <sup>13</sup>C isotope pattern is in agreement with eight carbon atoms. The retention time of 3.5 min was below the predicted retention time (log *K*<sub>ow, est</sub> 2.21, *t*<sup>TP</sup><sub>est</sub> 8.4 min), which is probably due to an overestimated log *K*<sub>ow, est</sub> by KowWin. 2-isopropyl-6-methyl-4-pyrimidinol was ultimately identified based on the fragments 84.0438 (C<sub>4</sub>H<sub>6</sub>O<sub>1</sub>N<sub>1</sub>) and 70.0645 (C<sub>4</sub>H<sub>8</sub>N<sub>1</sub>), which have been reported previously (16). The occurrence of 2-isopropyl-6-methyl-4-pyrimidinol has been studied in sewage sludge and sludge-fertilized soil samples (17) and its fate and formation has been described previously (7, 11,13,14).

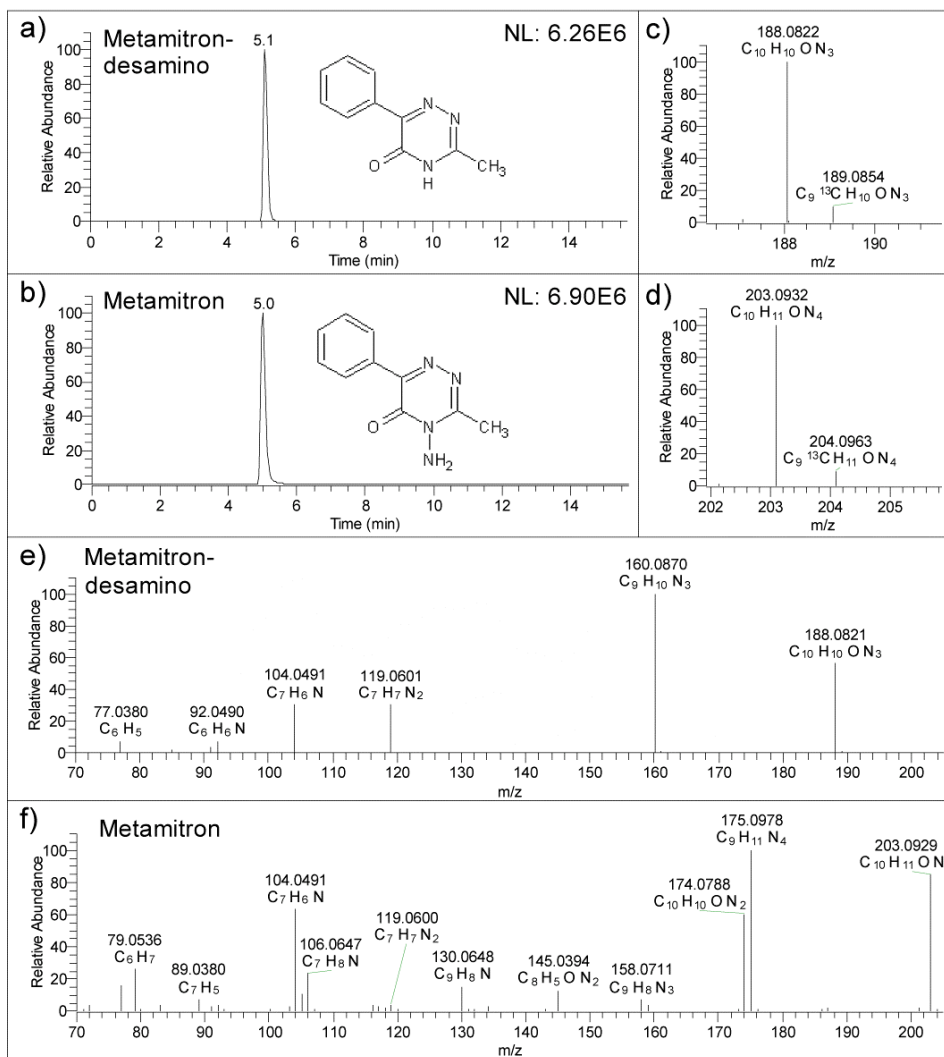
Isoproturon-desmethyl, CAS no. 34123-57-4, sample *La Petite Glane*



**Figure S 12 Structure of the transformation product isoproturon-desmethyl and its parent compound isoproturon and the corresponding chromatograms (a and b HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). All spectra are shown for the sample *La Petite Glane*.**

The proposed pesticide transformation product isoproturon-desmethyl (transformation product of isoproturon) has the elemental composition  $C_{11}H_{16}N_2O_1$  ( $[M+H]^+ = 193.1335$ ). The  $^{13}C$  isotope pattern is in agreement with 11 carbon atoms ( $\sim 10\%$ ). The retention time of 8.6 min for isoproturon-desmethyl agrees with its polarity ( $\log K_{ow,est}$  2.6,  $t_{est}^{TP}$  8.3 min). Isoproturon with a similar polarity ( $\log K_{ow,est}$  2.8) has a retention time of 8.8 min. Isoproturon-desmethyl was ultimately identified based on the agreement of fragments with the parent compound. The missing fragment 72.0439 ( $C_3H_6N_1O_1$ ) in the MS/MS spectra of isoproturon-desmethyl is an indication for the deprotonation at the urea moiety. The formation of isoproturon-desmethyl has been described previously (7,11,14,18,19). It has also been found to occur in surface water (20).

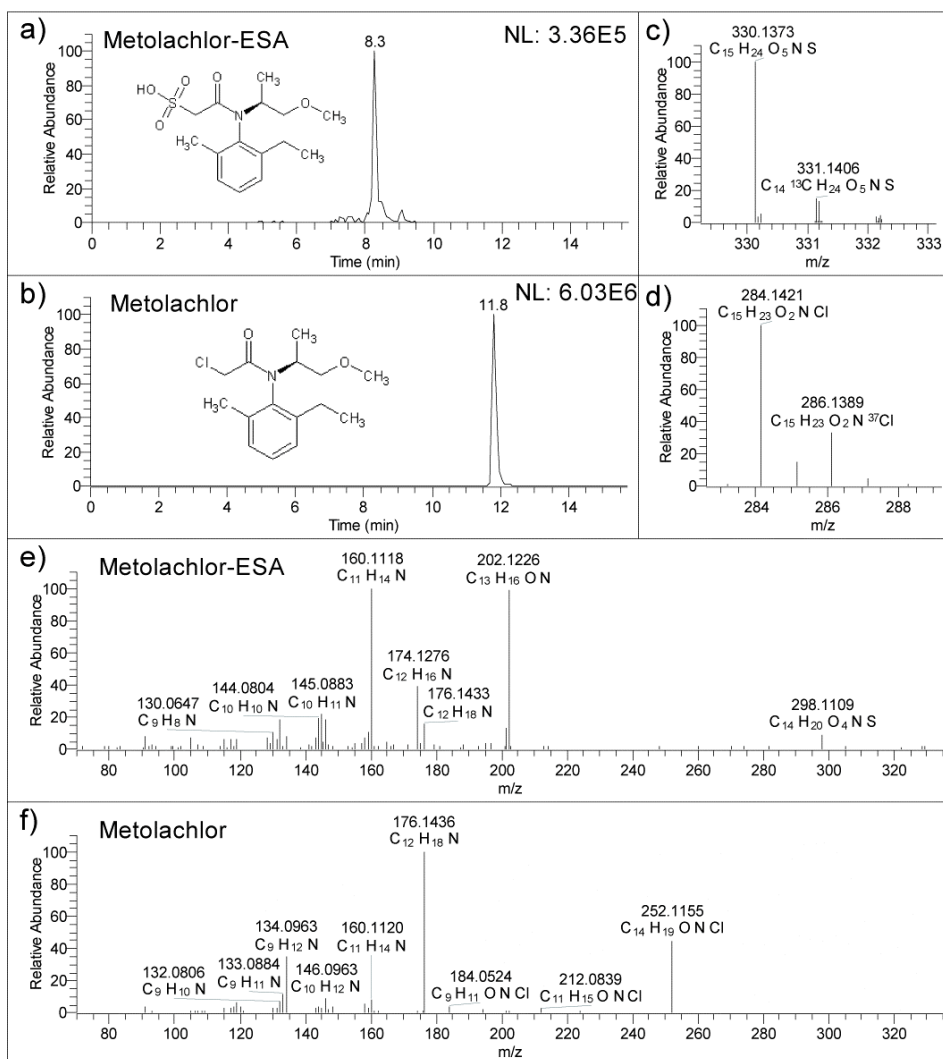
Metamitron-desamino, CAS no. 36993-94-9, sample *La Petite Glane*



**Figure S 13** Structure of the transformation product metamitron-desamino and its parent compound metamitron and the corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). All spectra are shown for the sample *La Petite Glane*.

The proposed pesticide transformation product metamitron-desamino (transformation product of metamitron) has the elemental composition  $C_{10}H_9N_3O_1$  ( $[M+H]^+ = 188.0818$ ). The  $^{13}C$  isotope pattern is in agreement with 10 carbon atoms ( $\sim 10\%$ ). The retention time of 5.1 min for metamitron-desamino ( $\log K_{ow,est} 2.5$ ,  $t_{est}^{TP} 7.7$  min) is very similar to its parent compound metamitron (neutral,  $\log K_{ow,est} 1.4$ ). Metamitron-desamino was ultimately identified based on the agreement of the fragment 160.0870 ( $C_9H_{10}N_3$ ) with the fragment 175.0978 ( $C_9H_{11}N_4$ ) of the parent compound in addition to other identical fragments. The occurrence of metamitron-desamino in soil has been described previously (7,21,22). Some studies report the formation of metamitron-desamino by photolysis (23-25). However, no studies have been found that describe its occurrence in surface water.

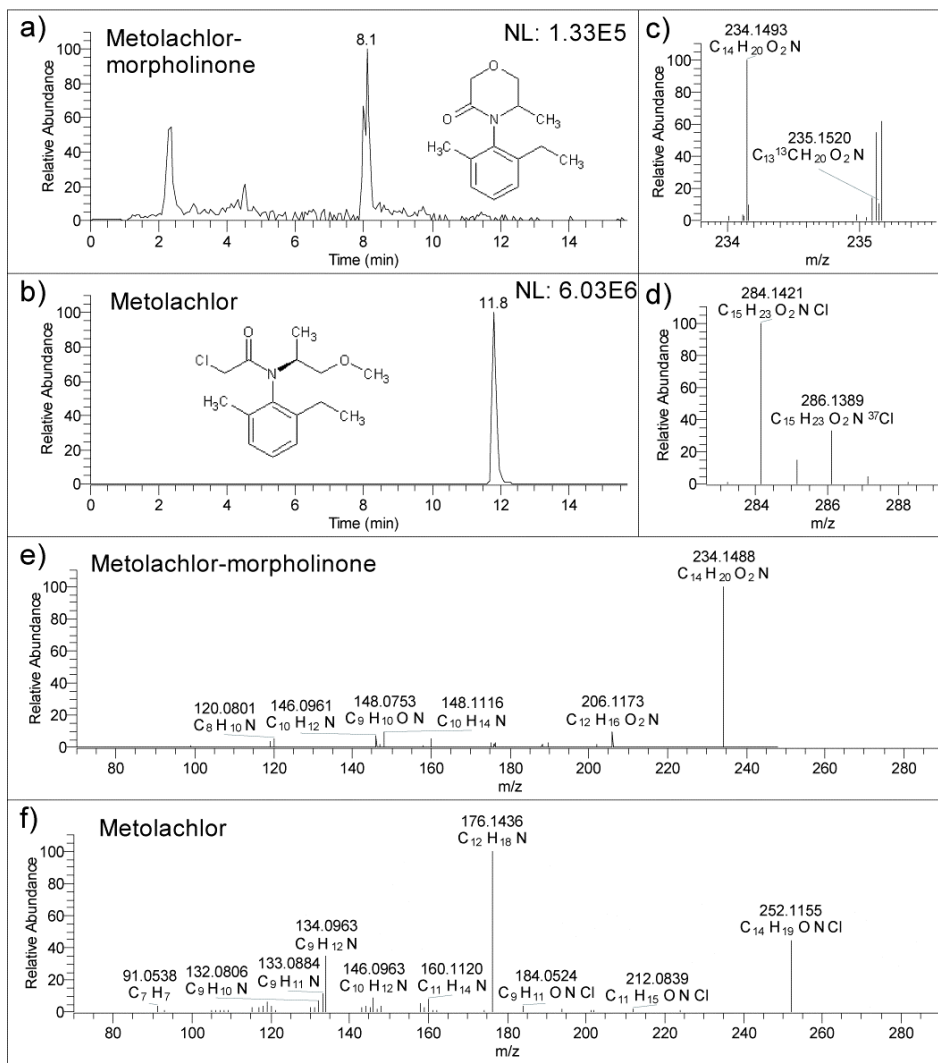
Metolachlor-ESA, CAS no. 171118-09-5, sample *La Petite Glane*



**Figure S 14 Structure of the transformation product metolachlor-ESA and its parent compound metolachlor and the corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). All spectra are shown for the sample *La Petite Glane*.**

The proposed pesticide transformation product metolachlor-ESA (transformation product of metolachlor) has the elemental composition  $C_{15}H_{23}N_1O_5S_1$  ( $[M+H]^+=330.1370$ ). The  $^{13}C$  isotope pattern is in agreement with 15 carbon atoms (~15%). The retention time of 8.3 min for the anionic metolachlor-ESA ( $\log K_{ow,est}$  1.7) is comparable to its predicted retention time  $t_{est}^{TP}$  of 7.1 min. Metolachlor (neutral,  $\log K_{ow,est}$  3.2) has a retention time of 11.6 min and a  $^{37}Cl$  isotope pattern (~35%). Metolachlor-ESA with its sulfonic acid moiety was also detectable in negative mode. It was ultimately identified based on the characteristic fragments 202.1226 ( $C_{13}H_{16}N_1O_1$ ), 176.1433 ( $C_{12}H_{18}N_1$ ), 174.1276 ( $C_{12}H_{16}N_1$ ), and 160.1118 ( $C_{11}H_{14}N_1$ ), which are all predicted by MassFrontier. The occurrence and fate of metolachlor-ESA in surface water has been described previously (7,26-29).

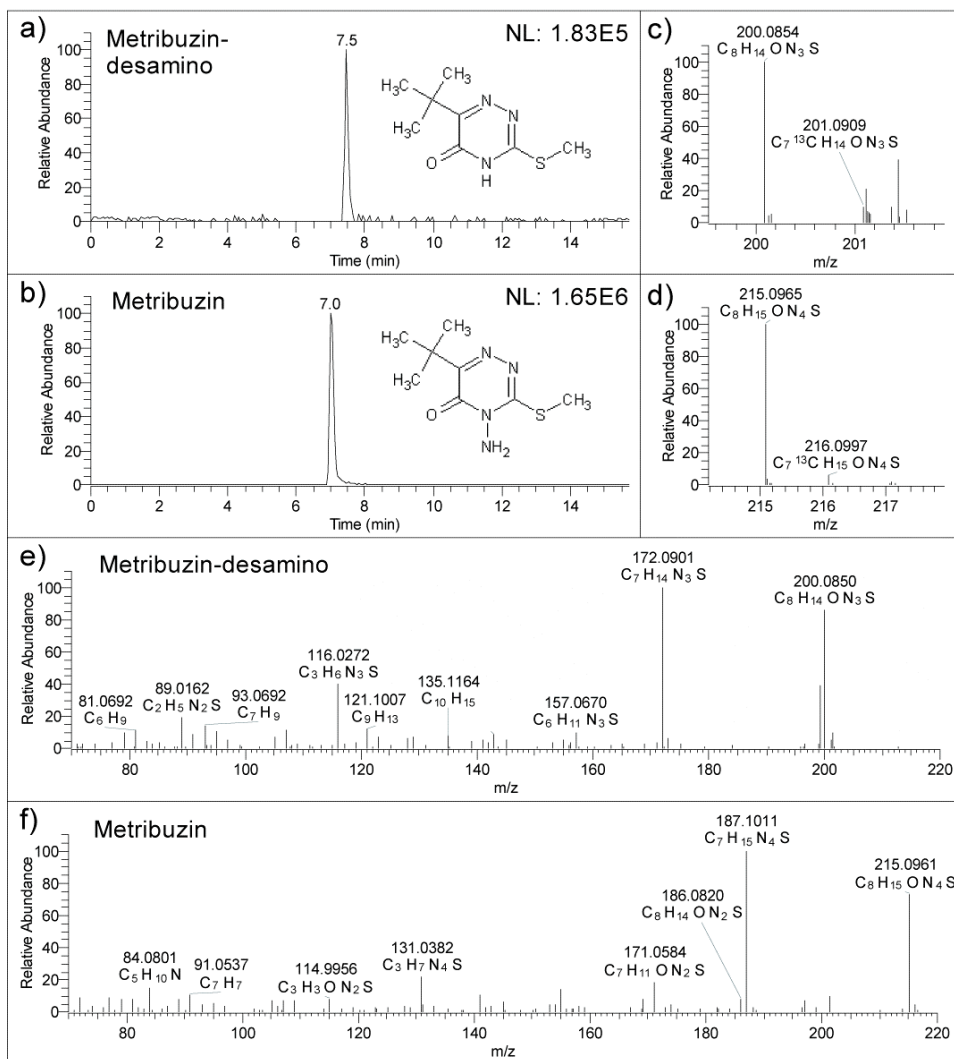
Metolachlor-morpholinone, CAS no. 120375-14-6, sample *La Petite Glane*



**Figure S 15** Structure of the transformation product metolachlor-morpholinone and its parent compound metolachlor and the corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). All spectra are shown for the sample *La Petite Glane*.

The proposed pesticide transformation product metolachlor-morpholinone (transformation products of the herbicide metolachlor) has the elemental composition  $C_{14}H_{19}N_1O_2$ . The  $^{13}C$  isotope pattern is in agreement with 14 carbon atoms (15%). The retention time of 8.1 min seems reasonable for the more polar metolachlor-morpholinone ( $\log K_{ow}$  2.54,  $t_{est}^{TP}$  10.2 min). Metolachlor-morpholinone was ultimately identified based on its MS/MS spectrum with the fragments 206.1173 ( $C_{12}H_{16}N_1O_2$ ), 148.1116 ( $C_{10}H_{14}N_1$ ), 146.0964 ( $C_{10}H_{12}N_1$ ), and 120.0801 ( $C_8H_{10}N_1$ ), which were all predicted by MassFrontier. The occurrence of metolachlor-morpholinone in natural water (26) and the formation in a photodegradation study have been described previously (27).

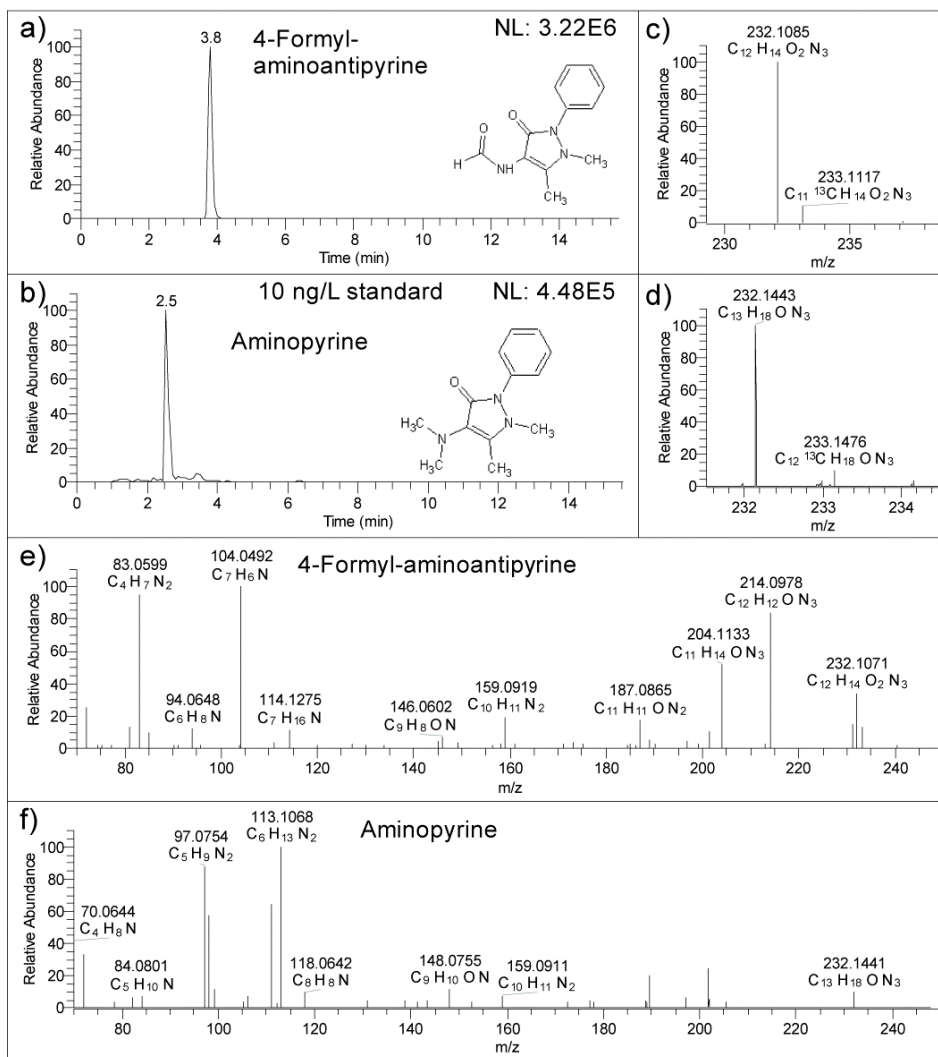
Metribuzin-desamino, CAS no. 35045-02-4, sample *Hauptkanal*



**Figure S 16 Structure of the transformation product metribuzin-desamino and its parent compound metribuzin and the corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). All spectra are shown for the sample *Hauptkanal***

The proposed pesticide transformation product metribuzin-desamino (transformation product of metribuzin) has the elemental composition C<sub>8</sub>H<sub>13</sub>N<sub>3</sub>O<sub>1</sub>S<sub>1</sub> ([M+H]<sup>+</sup>=200.0852). The <sup>13</sup>C isotope pattern is in agreement with 8 carbon atoms (~10%). The retention time of 7.5 min for metribuzin-desamino (log *K*<sub>ow,est</sub> 1.5, *t*<sup>TP</sup><sub>est</sub> 9.2 min) is similar to its parent compound metribuzin (log *K*<sub>ow,est</sub> 1.5) with a retention time of 7.0 min. Metribuzin-desamino was ultimately identified based on various fragments with corresponding molecular formulas. Metribuzin-desamino has been described previously (7,11). Its formation in soil was investigated (30,31), but there is no study about its formation or occurrence in surface water.

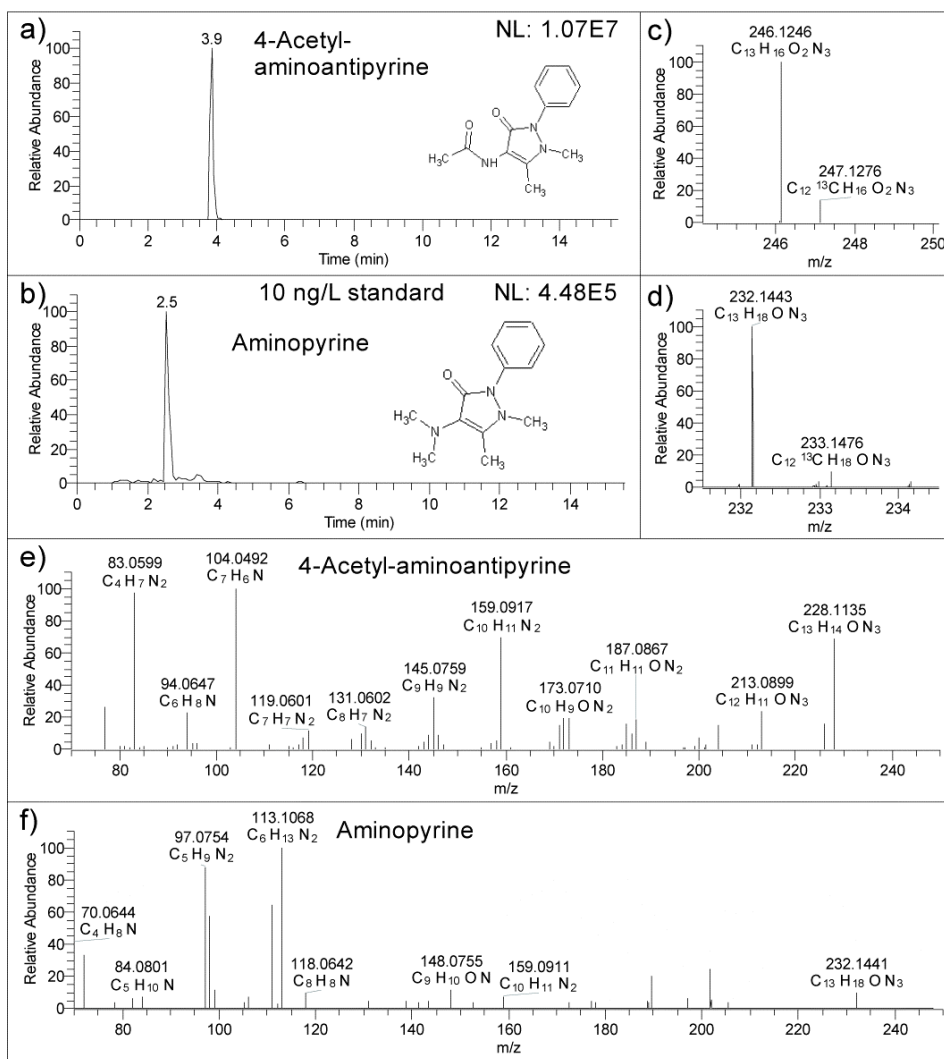
4-Formyl-aminoantipyrine, CAS no. 1672-58-8, sample *Furtbach*



**Figure S 17** Structure of the transformation product 4-formyl-aminoantipyrine and its parent compound aminopyrine and the corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). The spectra of 4-formyl-aminoantipyrine are shown for the sample *Furtbach*, the spectra of aminopyrine for a 10ng/l reference standard.

The proposed pharmaceutical transformation product 4-formyl-aminoantipyrine (known human metabolite of metamizole and aminopyrine) has the elemental composition  $C_{12}H_{13}N_3O_2$  ( $[M+H]^+=232.1081$ ). The  $^{13}C$  isotope pattern is in agreement with 12 carbon atoms (~15%). 4-Formyl-aminoantipyrine has a retention time of 3.7 min ( $\log K_{ow,est}$  0.5,  $t_{est}^{TP}$  5.5 min). Aminopyrine was not found in the samples (spectra shown for the reference standard). 4-Formyl-aminoantipyrine was ultimately identified based on its MS/MS spectrum with the characteristic fragments 214.0978 ( $C_{12}H_{12}N_3O_1$ ) and 204.1133 ( $C_{11}H_{14}N_3O_1$ ). Its occurrence in wastewater has been reported (32,33).

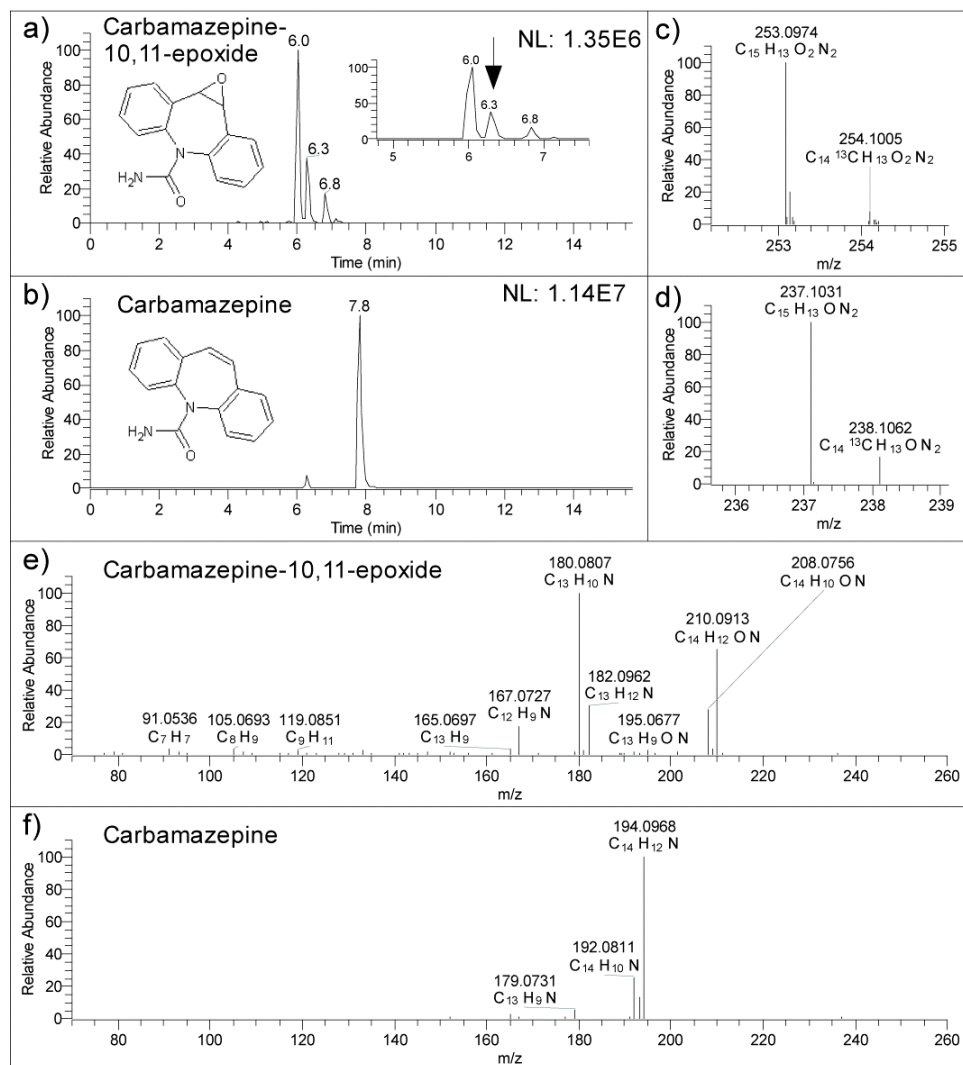
4-Acetyl-aminoantipyrine, CAS no. 83-15-8, sample *Furtbach*



**Figure S 18** Structure of the transformation product 4-acetyl-aminoantipyrine and its parent compound aminopyrine and the corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). The spectra of 4-acetyl-aminoantipyrine are shown for the sample *Furtbach*, the spectra of aminopyrine for a 10ng/l reference standard.

The proposed pharmaceutical transformation product 4-acetyl-aminoantipyrine (known human metabolite of metamizole and aminopyrine) has the elemental composition  $C_{13}H_{15}N_3O_2$  ( $[M+H]^+=246.1237$ ). The  $^{13}C$  isotope pattern is in agreement with 13 carbon atoms ( $\sim 15\%$ ). The retention time of 3.7 min of 4-acetyl-aminoantipyrine is in agreement with its polarity ( $\log K_{ow,est} -0.1$ ) and predicted retention time  $t_{est}^{TP}$  of 4.1 min. 4-Acetyl-aminoantipyrine was ultimately identified based on the agreement of six fragments, which were also predicted by MassFrontier. The occurrence of 4-acetyl-aminoantipyrine in influent and effluent of sewage treatment plants has been described previously (32). There are also some studies reporting high ng/l concentrations in surface water (34-36).

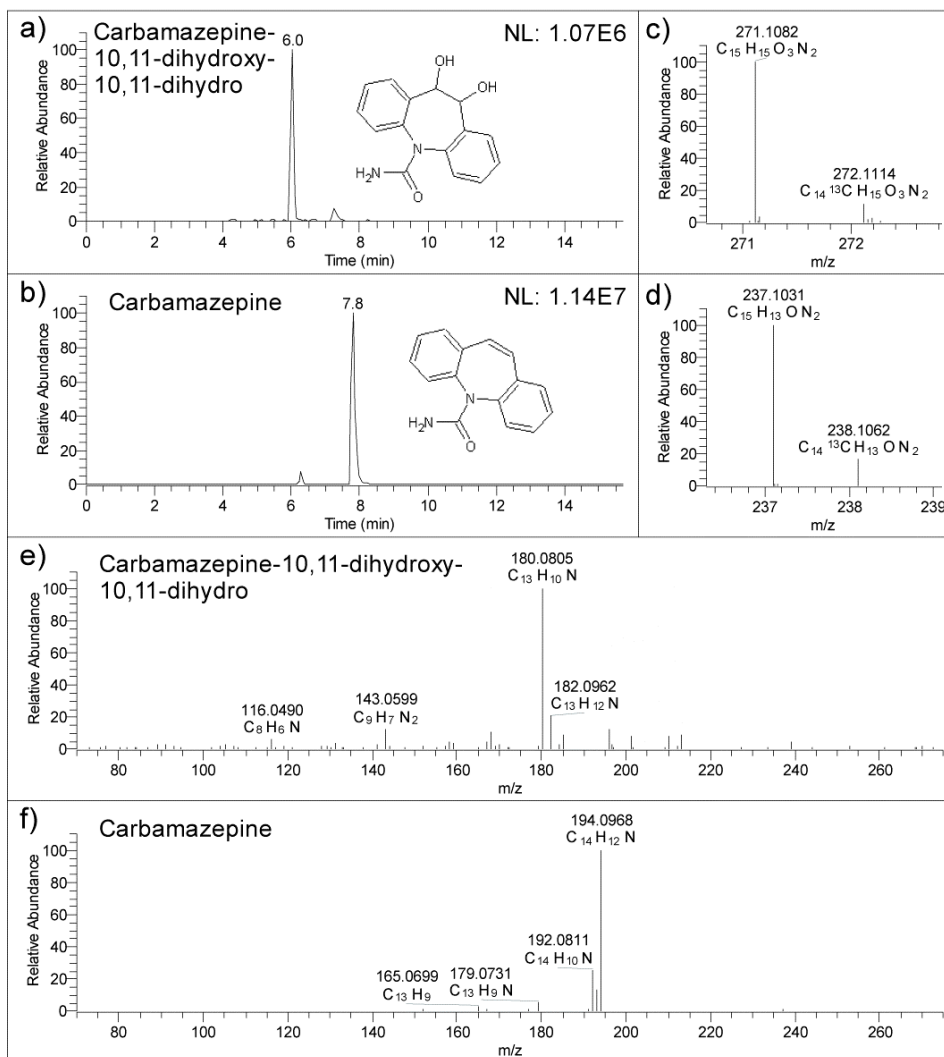
Carbamazepine-10,11-epoxide, CAS no. 36507-30-9, sample *Furtbach*



**Figure S 19** Structure of the transformation product carbamazepine-10,11-epoxid and its parent compound carbamazepine and the corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). All spectra are shown for the sample *Furtbach*.

The proposed pharmaceutical transformation product carbamazepine-10,11-epoxide (known human metabolite of carbamazepine) has the elemental composition  $C_{15}H_{12}N_2O_2$  ( $[M+H]^+ = 253.0972$ ). The  $^{13}C$  isotope pattern is in agreement with 12 carbon atoms ( $\sim 15\%$ ). The retention time of 6.3 min seems reasonable for the more polar transformation product ( $\log K_{ow,est} -1.0$ ,  $t_{est}^{TP} 4.8$  min), compared to the parent compound carbamazepine with a retention time of 7.8 min ( $\log K_{ow,est} 2.3$ ). Carbamazepine-10,11-epoxide was ultimately identified based on the agreement of retention time and MS/MS spectra with the reference standard. Characteristic fragments such as  $C_{13}H_{10}N_1$  ( $[M]^+ = 180.0808$ ) and  $C_{14}H_{12}O_1N_1$  ( $[M]^+ = 210.0911$ ) have been observed. The occurrence and fate of carbamazepine-10,11-epoxide in influent and effluent of sewage treatment plants has been described previously (37-39). It has been proposed to be a fungal transformation product (40).

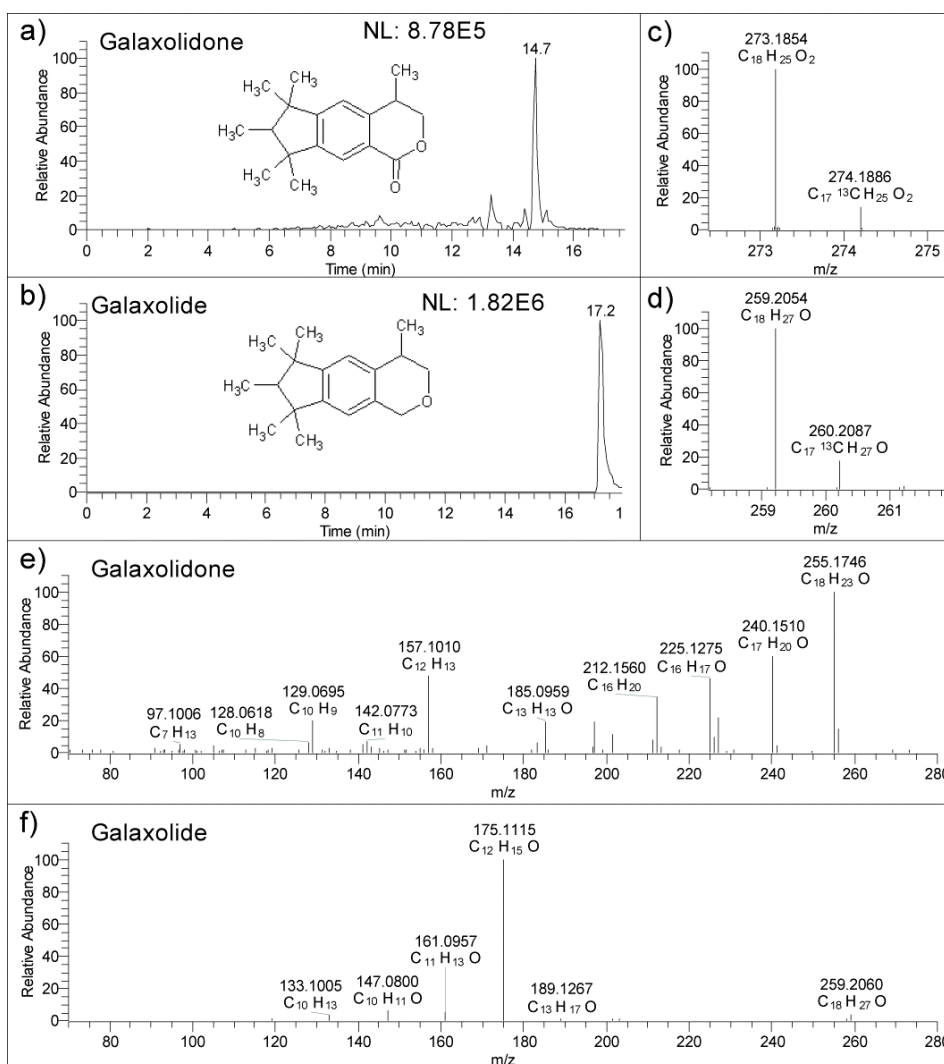
Carbamazepine-10,11-dihydroxy-10,11-dihydro, CAS no. 35079-97-1, sample *Furtbach*



**Figure S 20 Structure of the transformation product carbamazepine-10,11-dihydroxy-10,11-dihydro and its parent compound carbamazepine and the corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). All spectra are shown for the sample *Furtbach*.**

The proposed pharmaceutical transformation product carbamazepine-10,11-dihydroxy-10,11-dihydro (known human metabolite of carbamazepine) has the elemental composition  $C_{15}H_{14}N_2O_3$  ( $[M+H]^+ = 271.1077$ ). The  $^{13}C$  isotope pattern is in agreement with 15 carbon atoms ( $\sim 15\%$ ). The retention time of 6.0 min is in agreement with the predicted retention time  $t_{est}^{TP}$  of 5.2 min and its polarity ( $\log K_{ow,est}$  1.2). Carbamazepine-10,11-dihydroxy-10,11-dihydro was ultimately identified based on the characteristic fragments 182.0962 ( $C_{13}H_{12}N_1$ ) and 180.0805 ( $C_{13}H_{10}N_1$ ). The occurrence of carbamazepine-10,11-dihydroxy-10,11-dihydro in influent and effluent of sewage treatment plants has been described previously (37,39,41).

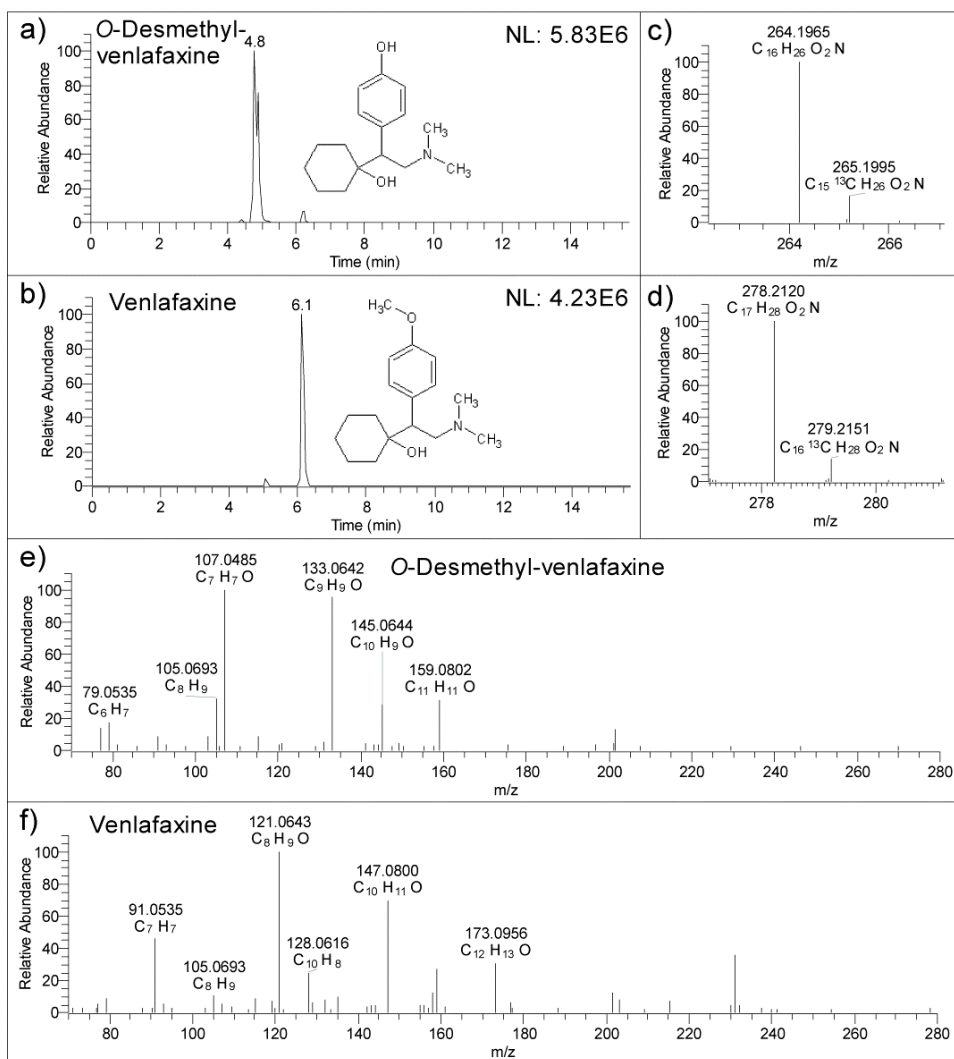
Galaxolidone, CAS no. 507442-49-1, sample *Gossau*



**Figure S 21 Structure of the transformation product galaxolidone and its parent compound galaxolide and the corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). All spectra are shown for the sample *Gossau*.**

The proposed pharmaceutical transformation product galaxolidone (industrial by-product and transformation product of galaxolide) has the elemental composition  $C_{18}H_{24}O_2$  ( $[M+H]^+ = 273.1849$ ). The  $^{13}C$  isotope pattern is in agreement with 18 carbon atoms ( $\sim 20\%$ ). The retention time of 14.7 min for galaxolidone ( $\log K_{ow,est}$  5.3) is comparable to the parent compound galaxolide ( $\log K_{ow,est}$  6.3) with a retention time of 17.2 min. It is also in agreement with the predicted retention time  $t_{est}^{TP}$  of 15.0 min. Galaxolidone was ultimately identified based on various fragments such as the demethylated fragments 240.1510 ( $C_{17}H_{20}O_1$ ), 225.1275 ( $C_{16}H_{17}O_1$ ), and 185.0959 ( $C_{13}H_{13}O_1$ ) after loss of  $H_2O$  (255.1748,  $C_{18}H_{23}O_1$ ). The occurrence of galaxolidone in influent and effluent of sewage treatment plants has been described previously (42,43).

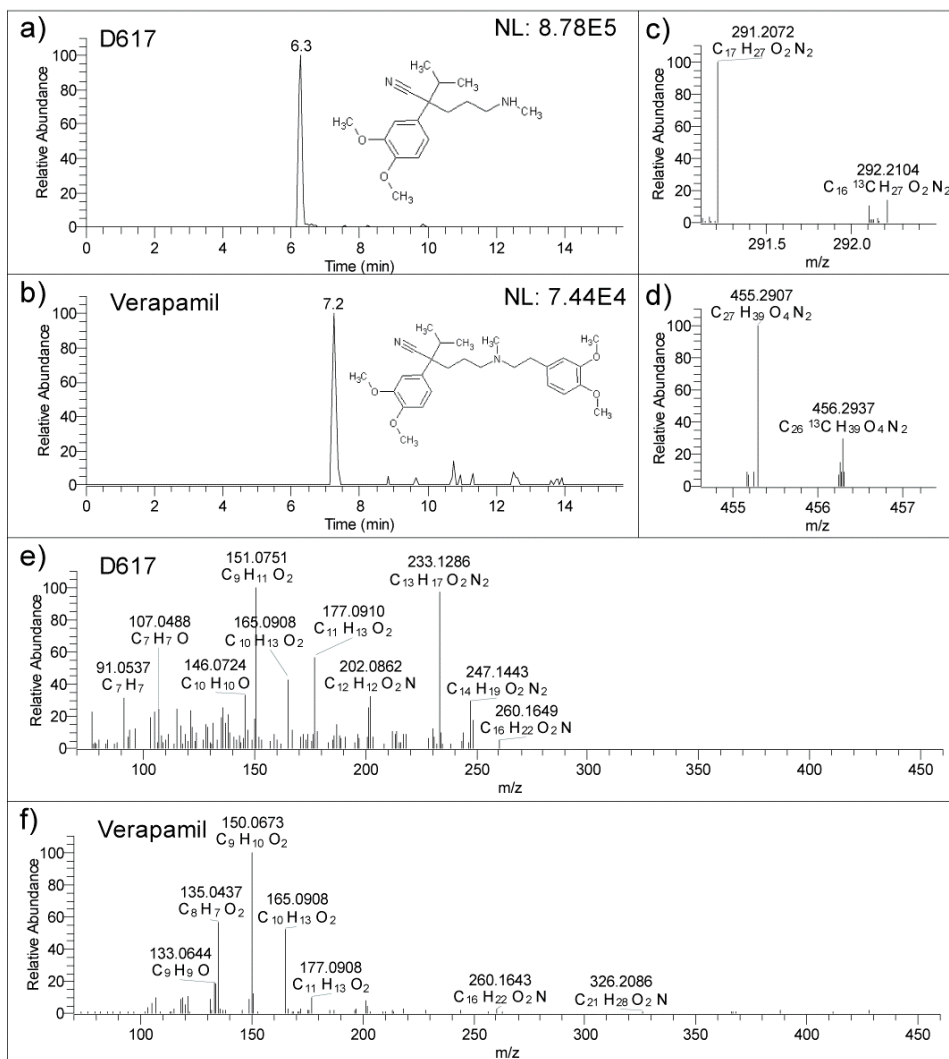
O-Desmethyl-venlafaxine, CAS no. 93413-62-8, sample *Furtbach*



**Figure S 22 Structure of the transformation product O-desmethyl-venlafaxine and its parent compound venlafaxine and the corresponding chromatograms (a and b), HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). All spectra are shown for the sample *Furtbach*.**

The proposed pharmaceutical transformation product O-desmethyl-venlafaxine (known human metabolite and transformation product of venlafaxine) has the elemental composition  $C_{16}H_{25}N_1O_2$  ( $[M+H]^+ = 264.1958$ ). The  $^{13}C$  isotope pattern is in agreement with 16 carbon atoms ( $\sim 15\%$ ). The retention time of 4.8 min for O-desmethyl-venlafaxine ( $\log K_{ow,est} 2.7$ ) is similar to the predicted retention time  $t_{est}^{TP}$  of 5.9 min. The parent compound venlafaxine ( $\log K_{ow,est} 3.3$ ) has a retention time of 6.2 min. O-desmethyl-venlafaxine was ultimately identified based on its MS/MS spectrum. Its occurrence in influent and effluent of sewage treatment plants and surface water has been described previously (44).

D617, CAS no. 34245-14-2, sample *Furtbach*



**Figure S 23** Structure of the transformation product D617 and its parent compound verapamil and the corresponding chromatograms (a and b HR-MS spectra of the molecular ions, including isotopic pattern (c and d) and the HR-MS/MS spectra (e and f). All spectra are shown for the sample *Furtbach*.

The proposed pharmaceutical transformation product D617 (known human metabolite and transformation product of verapamil) has the elemental composition C<sub>17</sub>H<sub>26</sub>N<sub>2</sub>O<sub>2</sub> ([M+H]<sup>+</sup>=291.2067). The <sup>13</sup>C isotope pattern is in agreement with 17 carbon atoms (~15%). The retention time for D617 (log *K*<sub>ow,est</sub> 3.0) is 6.3 min, and it has a predicted retention time *t*<sup>TP</sup><sub>est</sub> of 4.2 min. Its parent compound verapamil (log *K*<sub>ow,est</sub> 4.8) has a retention time 7.2 min. D617 was ultimately identified based on the fragments 247.1443 (C<sub>14</sub>H<sub>19</sub>N<sub>2</sub>O<sub>2</sub>), 233.1286 (C<sub>13</sub>H<sub>17</sub>N<sub>2</sub>O<sub>2</sub>), and 151.0751 (C<sub>9</sub>H<sub>11</sub>O<sub>2</sub>), which were all also predicted by MassFrontier. D617 is a known human metabolite (45), but has also been reported as a fungal transformation product (46) and as an aerobic biodegradation product (47). So far, it has not been found in surface water.

## ***Performance of funneling procedure***

Despite the 19 positive findings, there remains the question whether the funneling procedure missed any transformation products. Where reference standards for transformation products on the target list were available to us, the samples were screened for these and compared to the outcome after the funneling procedure to search for false negatives. Nine compounds were found in at least one of the investigated samples with the help of reference standards, but were deselected during the funneling procedure. During the retention time correlation step, two compounds, ritalinic acid (CAS 19395-41-6, transformation product / metabolite of methylphenidate) and atenolol acid (CAS , transformation product / metabolite of atenolol), were excluded due to an unlikely log  $K_{ow}$  predicted by KowWin as described in more detail in the chapter “Performance of retention time correlation for transformation products” (p. S13). The cut-off criterion of  $\geq 10^5$  was the reason for the other eight transformation products atrazine-desisopropyl (CAS 1007-28-9, transformation product of atrazine), ethofumesate-2-oxo (CAS 26244-33-7, transformation product of ethofumesate), metolachlor-OXA (CAS 152019-73-3, transformation product of metolachlor), N-acetyl-sulfamethoxazole (CAS 21312-10-7, transformation product / metabolite of sulfamethoxazole), chloridazon-desphenyl (CAS 6339-19-1, transformation product of chloridazon), 2,4-dichlorophenol (CAS 120-83-2, transformation product of 2,4-D), 4-chloro-2-methylphenol (CAS 1570-64-5, transformation product of mecoprop), and diuron-desmethyl (CAS 3567-62-2, transformation product of diuron) to not be considered further in our procedure.

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