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Determination of atrazine and degradation products in Luxembourgish drinking water: origin and fate of potential endocrine disrupting pesticides

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Abstract:	Several pesticides have been hypothesized to act as endocrine disrupting compounds, exhibiting hormonal activity and perturbing normal physiological functions. Among these, especially s-triazine-herbicides have received increased attention. Atrazine is, despite being banned in many countries, including the EU (2004), still the world's most widely used herbicide. Despite its discontinued use, considerable concentrations of atrazine and its degradation products, mainly desethylatrazine (DEA) and deisopropylatrazine (DIA), are still found in the environment, including drinking water sources. The aim of this investigation was to study concentrations

	<p>of especially s-triazine-herbicides and major degradation products in drinking water, including spring water, tap water, and bottled water in Luxembourg. Spring water (2007/2008/2009, n=69/69/69), tap water (2008/2009, n=19/26), and bottled water (2007/2008/2009, n=5/13/7) were sampled at locations in Luxembourg and investigated for pesticides by HPLC-ESI-MS-MS. Atrazine was the predominant triazine, detectable in many spring water locations, tap and bottled water, ranging from 0-57(mean 9)ng/L, 0-44(mean 4)ng/L, and 0-4(mean 1)ng/L, respectively. DEA and DIA in spring water ranged from: 0-120(mean 19)ng/L and 0-27(mean 3)ng/L, with higher concentrations from agricultural areas and low molar ratios of DEA:atrazine<0.5 and high ratios of atrazine:nitrate suggesting point-source contaminations. DEA and DIA in tap water were 0-62(mean 14)ng/L and 0-6(mean<1)ng/L and in bottled water 0-11(mean 2)ng/L and 0-7(mean 2 ng/L). Simazine and other triazines were detected in traces (<5 ng/L). Thus, the conducted monitoring suggested the presence of low concentrations of s-triazines in raw and finished water, presumably partly due to non-agricultural contamination; with concentrations being below thresholds advocated by EU directive 98/83/EC.</p>

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Endocrine Disrupting Pesticides in Water

Determination of atrazine and degradation products in Luxembourgish drinking water: origin and fate of potential endocrine disrupting pesticides

T. Bohn*, E. Cocco, L. Gourdol, C. Guignard, L. Hoffmann

Centre de Recherche Public – Gabriel Lippmann, Department Environment and Agro-Biotechnologies, 41, rue du Brill, L-4422 Belvaux, Luxembourg

***Correspondence :** Torsten Bohn, Centre de Recherche Public – Gabriel Lippmann

E-mail : bohn@lippmann.lu

Phone: (+352)470261480

Fax : (+352) 47 02 64

Abstract

Several pesticides have been hypothesized to act as endocrine disrupting compounds, exhibiting hormonal activity and perturbing normal physiological functions. Among these, especially s-triazine-herbicides have received increased attention. Atrazine is, despite being banned in many countries, including the EU (2004), still the world's most widely used herbicide. Despite its discontinued use, considerable concentrations of atrazine and its degradation products, mainly desethylatrazine (DEA) and deisopropylatrazine (DIA), are still found in the environment, including drinking water sources. The aim of this investigation was to study concentrations of especially s-triazine-herbicides and major degradation products in

drinking water, including spring water, tap water, and bottled water in Luxembourg. Spring water (2007/2008/2009, n=69/69/69), tap water (2008/2009, n=19/26), and bottled water (2007/2008/2009, n=5/13/7) were sampled at locations in Luxembourg and investigated for pesticides by LC-ESI-MS/MS. Atrazine was the predominant triazine, detectable in many spring water locations, tap and bottled water, ranging from 0-57 (mean 9) ng/L, 0-44 (mean 4) ng/L, and 0-4 (mean 1) ng/L, respectively. DEA and DIA in spring water ranged from: 0-120 (mean 19) ng/L and 0-27 (mean 3) ng/L, with higher concentrations from agricultural areas and low molar ratios of DEA:atrazine<0.5 and high ratios of atrazine:nitrate suggesting point-source contamination. Levels of DEA and DIA in tap water were 0-62 (mean 14) ng/L and 0-6(mean<1)ng/L and in bottled water 0-11 (mean 2) ng/L and 0-7 (mean 2 ng/L). Simazine and other triazines were detected in traces (<5 ng/L). Thus, the conducted monitoring suggested the presence of low concentrations of s-triazines in raw and finished water, presumably partly due to non-agricultural contamination; with concentrations being below thresholds advocated by EU Directive 98/83/EC.

Keywords : s-triazine herbicides, atrazine degradation products, tap and spring water, DEA: atrazine ratio, nitrate, 2,6-dichlorobenzamide.

Introduction

Endocrine disrupting compounds (EDC) are molecules exhibiting hormonal or antihormonal activities, thus interacting and potentially perturbing normal physiological functions. These include natural compounds, such as mycotoxins, e.g. zearalenone (Mantovani *et al.*, 2009; Giraud *et al.*, 2010) and soy isoflavonoids (Cederroth *et al.*, 2010), and man-made chemicals such as pharmaceuticals (Pailler *et al.*, 2009) and polychlorinated biphenyls (Lasserre *et al.*, 2009), and chemicals employed in agriculture. The latter group comprises a large number of

pesticides, including e.g. herbicides, fungicides, and insecticides. As these compounds have been released on purpose and are often in direct contact with the future food product, great prudence in their choice, way of application and concentration has to be maintained.

Nevertheless, with the increased research on EDC, including pesticides, several compounds have been hypothesized to impact the human hormonal system. Among pesticides, especially the s-triazine-herbicides such as terbutylazine, cyanazine, simazine and atrazine have achieved much attention in recent years, even though they have been used since the 1950ties (Ribaud and Bouzahr, 2010). Despite the strict classification of atrazine (2-chloro-4-ethylamino-6-isopropylamine-s-triazine) as an EDC has been discussed (Solomon, 2009), it has been suggested to inflict the central nervous system (Coban and Filipov, 2007; Rodriguez et al., 2005), the endocrine system (Rayner et al., 2004; Hayes et al., 2006) and the immune system (Rowe et al., 2008; Brodtkin et al., 2007), effecting the reproductive health of rats (Kniewald *et al.*, 2000), pigs (Betancourt *et al.*, 2006), and amphibians (Hayes et al., 2006), fostering feminization in the latter (Hayes et al., 2010). However, data on the impact on mammals and particularly humans is scant and effects remains unclear (Solomon, 2009).

Nevertheless, atrazine is still the most used herbicide in many countries and possibly worldwide (Thurman and Scribner, 2008). It is used for weed removal, especially against broadleaf and grassy weeds, employed in agricultural but also in urban areas such as in private gardens, on railway tracks, or other weed control programs. Atrazine possesses a high groundwater ubiquity score (GUS) of ca. 3.6 (Vogue *et al.*, 1994) and thus a high potential for groundwater contamination. In fact, the major way of human intake for this pesticide is via water (Bray et al., 2008). In the EU, usage of atrazine was abandoned in 2004 following general precautionary principles; however, significant concentrations can still be found in the

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3 76 environment such as in groundwater and surface water (Loos et al., 2010; Kuster et al., 2010;
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5 77 Planas et al., 2006; Administration de la Gestion de l'Eau, 2005), often in concentrations
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8 78 above the 0.1 µg/L limit set by the EU directive 98/83/EC. In addition to raw water, atrazine
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10 79 has been detected in finished (tap) water, up to 60 µg/L in the US (Wu *et al.*, 2010), while
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13 80 concentrations in bottled water are generally assumed to be much lower, due to filtration steps
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15 81 through activated carbon during production.
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20 83 Despite its persistence, atrazine has been shown to undergo degradation in the soil due to
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22 84 bacterial activities. These breakdown products include mainly the comparatively stable
23
24 85 desethylatrazine and deisopropylatrazine (Papiernik and Spalding, 1998; Jason et al., 2010),
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26 86 which have likewise been detected in groundwater (Shomar et al., 2006; Loos et al., 2010),
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28 87 and tap water (Gfrerer *et al.*, 2002), however, the s-triazine ring can be further hydroxylated
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30 88 (Krutz et al., 2010) and finally cleaved (Scribner *et al.*, 2005). However, whether and to what
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32 89 extent these breakdown products possess endocrine disrupting functions is not entirely clear,
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34 90 albeit it is being assumed for DEA and DIA (Ralston-Hooper *et al.*, 2009).
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40 92 While data on s-triazines, especially atrazine content in spring water and tap water has been
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42 93 reported for some European countries, such as for France (Brignon, 2007; Mirgain et al.,
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44 94 1993; Ministère de la Santé et des Solidarités, 2005) and Germany (Leistra and Boesten,
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46 95 1989; Tappe et al., 2002; Bundesministerium für Umwelt, 2005), no data has recently been
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48 96 published for Luxembourg. Thus, the aim of this study was to investigate pesticide, especially
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50 97 s-triazine contamination of individual spring water locations in Luxembourg, to verify
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52 98 whether contamination is related to agricultural activities, and to finally determine
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54 99 concentrations in finished drinking water (tap water) and bottled water in Luxembourg.
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101 **Material and Methods**

102 *Reagents and Standards*

103 Unless otherwise stated, all chemicals were of analytical grade or superior. All herbicide
104 standards including the s-triazine herbicides atrazine, simazine, terbuthylazine, sebuthylazine,
105 cyanazine, and hexazinon, and the atrazine degradation products desethylatrazine and
106 deisopropylatrazine, the urea herbicides isoproturon, chlortoluron, monolinuron,
107 methabenzthiazuron, metoxuron, diuron, linuron, and metobromuron, the chloroacetanilide
108 herbicides metazachlor and metolachlor, and 2,6-dichlorobenzamide (BAM, **Figure 1**), a
109 breakdown product of the herbicide dichlobenil, were obtained from LGC Promochem
110 (Molsheim, France). Methanol, acetone, and acetonitrile were procured from Biosolve
111 (Valkenswaard, The Netherlands).

113 *Sampling campaigns*

114 Several campaigns were carried out in the frame of this study:

- 115 a) Spring water: 69 springs in the Luxembourg City area from sandstone aquifer, the
116 major aquifer in Luxembourg (for a detailed description, see Gourdol et al., 2010),
117 were monitored between summer 2007 and spring 2009, with a total of 10 collection
118 campaigns (July/August 2007, November 2007, January 2008, March 2008, May
119 2008, June/July 2008, September 2008, October/November 2008, January 2009,
120 March 2009, **Figure 2**). All these springs are involved in the water drinking supply of
121 Luxembourg City and were revisited during the entire period to compare changes in
122 individual springs. These springs can be grouped into 9 distinct hydrogeological
123 zones whose recharge areas covered a range of different surface usages including
124 forest, cropland, urban areas and grassland (Figure 2). For each individual sampling,
125 ca. 2 x 500 mL of water were sampled in pre-washed glass bottles (analyses of

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3 126 pesticides) or polyethylene bottles (analysis of nitrate), and stored at 4°C until
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5 127 analysis (conducted within 48h of collection). During the sampling campaigns, spring
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7 128 discharges were also evaluated.
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10 129 b) Tap water: Luxembourgish tap water from various locations in Luxembourg (n=34,
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12 130 from n=28 different locations, analyzed in duplicate) in spring 2008 and spring 2009
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14 131 were analyzed (**Figure 3**). For this purpose, tap water from private households was
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16 132 collected after having let the water tap open for at least 2 minutes, and then approx.
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18 133 1.5 L were collected in glass bottles and stored at 4°C until analysis. The campaign
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20 134 focused on southern Luxembourg with its higher population and population density.
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23 135 c) Bottled water: A sampling of various bottled water varieties sold in Luxembourg in
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25 136 summer 2007, 2008 and 2009, with a focus on locally produced water samples (n=25
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27 137 in total, with n=15 from Luxembourg), analyzed in duplicate, was procured. Both
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29 138 sparkling (n=4) and plain water (n=21) was included, a total of 10 different brands
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31 139 was investigated. Samples were procured in local supermarkets (CACTUS S.A.,
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33 140 CORA), which constitute the most visited chains in Luxembourg.
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41 142 ***Pesticide extraction and concentration***

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43 143 The water pre-concentration was realized similar as described earlier (Trenholm *et al.*, 2006)
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45 144 using an automatic SPE workstation (Autotrace SPE workstation, Caliper, Hopkinton, MA,
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47 145 USA), and cartridges containing a vinylpyrrolidone-divinylbenzene copolymer (oasis HLB,
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49 146 200 mg, Waters, Elstree, UK). For this purpose, SPE cartridges were preconditioned with 10
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51 147 mL methanol followed by 10 mL of water. Then, 500 mL of water was loaded at 10 mL/min.
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53 148 Pesticides were then eluted 2 times with 5 mL of methanol. Samples were then evaporated to
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55 149 near dryness with a Speedvac concentrator (Heto, Denmark) and reconstituted in ca 0.5 mL of
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57 150 water:acetonitrile 80/20 (v/v) and stored at 4°C until analysis.
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152 **HPLC-MS-MS detection**

153 Detection and quantification was achieved by HPLC coupled to a tandem mass spectrometer

154 (LC-MS/MS, Dionex Ultimate 3000, Sunnyvale CA, USA, together with an AB Sciex API

155 3200 mass spectrometer, Concord, ON, Canada), using electrospray ionization (ESI) in

156 positive mode and multiple reaction monitoring (MRM), similar as described earlier (Baker *et*

157 *al.*, 2000; Panuwet *et al.*, 2008). An Acclaim 120 C-18 column (3 μm , 2.1 x 100 mm, Dionex)

158 was used at 40°C with a mobile phase consisting of water containing 0.1% formic acid (FA)

159 (solvent A) and acetonitrile containing 0.1% FA (solvent B). The gradient started at 20% of B

160 for 3 min and was changed to 40% B until 12 min, to 90% B until 15 min, to 100% B until

161 15.5 min, remaining at 100% until 18 min, decreased to 20% B at 18.5 min and stayed at 20%

162 B until 20 min. Flow rate was 0.25 mL/min, injection volume was 25 μL . Quantification was

163 achieved by using external standards and external calibration curves, typically in the range of

164 1 ng/L, and deemed acceptable if R exceeded 0.98. For the MS-MS parameters, vaporizer

165 temperature was set to 600°C. The capillary voltage was 5 kV. Nebulizer and turbo gas were

166 set at 55 and 50 psig, curtain and collision gas at 15 and 5 psig, respectively. Collision offset

167 voltages were individually optimized for each component (**Table 1**). The retention time,

168 parent mass, and quantifier ion for each pesticide are displayed in Table 1.

170 **Nitrate**

171 Nitrate was assessed from water originating from Luxembourg-City springs in order to assess

172 agricultural usage of the land (i.e. coming from fertilizer usage) and to compare results with

173 pesticide concentrations. Nitrate in spring water was measured by ion chromatography,

174 following the manufacturer's suggestion (Dionex Corporation, 1991). In short, samples were

175 filtered through an IC acrodisc Gelman LC13 0.45 μm filter (Pall Gelman, Dreieich,

Germany) and separated by ion chromatography employing a DX 500 (Dionex) and a AS12A-4 mm column, using a micromembrane suppressor (ASRS-Ultra, Dionex) and a gradient eluent of 2.7 mM Na₂CO₃/3.0 mM NaHCO₃. Quantification was achieved using external standards.

Data analyses

Unless otherwise reported, all values represent mean ± standard deviation (SD). For correlation between land usage and concentration of pesticides, Pearson correlation coefficients were used for evaluating the relation between nitrate and pesticide concentration, relation of atrazine and degradation products (using their molar ratios), and pesticide and nitrate concentration and land use. For the latter purpose, the mean total discharge of the respective pesticides (and nitrate) from springs of the same geographical area was calculated and plotted against the respective land usage in %. For all correlations, both slope and intercept were considered. A P-value<0.05 (2-sided) was considered statistically significant.

Results

Spring water locations

The most common pesticide derived compound was 2,6-dichlorobenzamide, a degradation product of dichlobenil (**Table 2**), and detectable in the majority of springs. Atrazine and/or degradation products (DEA and DIA) were also detected in most springs, and concentrations were similar during the entire 2-year period and the 10 campaigns (**Figure 4**), with no detectable global trend. The major molecule of the s-triazine herbicides and their degradation products found was DEA, followed by atrazine and DIA (Table 2). Simazine was detected in traces while other s-triazine-herbicides, including cyanazine, sebuthylazine, and hexaxinon

were not detected in any of the samples. Only 4 springs were completely free of detectable pesticides.

The range of land usage varied from 1.7 to 38.8 cropland (average 16.1% (Ministère de l'Environnement Luxembourg, 1999)), and from 3.2 to 26.6% (mean 15.4%) for urban usage, with a clear correlation between nitrate and cropland ($R=0.86$, $P<0.05$). A low but significant correlation between the usage of land as cropland and concentration of DEA was found ($R=0.44$, $p<0.001$), with lower positive correlations for atrazine and DIA. Interestingly, a high correlation between BAM and urban land use was found ($R=0.81$, $P<0.05$). When investigating the ratio between DEA and atrazine (expressed on a molar basis, **Figure 5**), a ratio that has been used to discriminate between point-source contamination and non-point source contamination (Scribner *et al.*, 2005), most springs plotted on a regression line with a mean slope of ca. 2.8 ($R=0.94$). However, a second group of measurements was determined with a slope of approximately 0.3 ($R=0.95$), belonging to a group of 6 springs located together, and associated with low cropland usage (3.4%), but a comparatively high urban coverage (16.8%). When plotting atrazine vs. nitrate, a similar pattern with the same 2 groups of springs was revealed, with a ratio of atrazine (pmol/L) : nitrate (mg/L) of >ca. 3.4, indicative for the 6 springs. BAM and nitrate were only very weakly correlated ($R=0.11$). Nitrate range was between 3.8 and 78.1 (mean 31.5) mg/L for all springs. In addition, a high ratio of DIA:DEA was reported to indicate that other DIA precursors such as cyanazine and simazine were present (Scribner *et al.*, 2005). In the present investigation, the DIA:DEA ratio was generally below 1, mostly below 0.5.

Tap water

Tap water was collected at a number of locations (n=15 villages or cities, with each 1-5 sampling locations) in Luxembourg. Again, DEA was the predominant s-triazine herbicide

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3 225 derivative, found at concentrations between 0–62 (mean: 14)ng/L, followed by atrazine with
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5 226 0–44 (mean: 4)ng/L and DIA, ranging from 0-6 ng/L, with a sum of atrazine, DEA and DIA
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8 227 of 0-111 (mean 18)ng/L (**Figure 6**). Only 2 out of 15 sampling locations were negatively
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10 228 tested for atrazine and degradation products. Tap water concentrations for southern
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12 229 Luxembourg (defined here as all locations and including those south of Luxembourg City)
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14 230 were lower in atrazine and degradation products DEA and DIA (13 ng/L) compared to those
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16 231 north of Luxembourg City (37 ng/L); however, results were not significantly different. BAM
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18 232 (5 from 15 sampling locations negative) was the only other herbicide detected, with
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20 233 concentrations of 0 –96 (mean: 13)ng/L, except for terbuthylazine, which was found in one
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22 234 occasion at ca. 1 ng/L.
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29 236 ***Bottled water***

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31 237 Due to the limited number of brands available in Luxembourg, 7 Luxembourgish water
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33 238 varieties, with (n=2) and without carbon dioxide, from 2 major companies and areas were
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35 239 investigated. In addition, 3 French brands commonly traded and consumed in Luxembourg,
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37 240 were investigated (**Table 3**). From the n=14 Luxembourgish samples investigated, only 1
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39 241 contained measurable amounts of atrazine (no degradation products), with a content of ca. 1.5
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41 242 ng/L. 2 of the French brands were also negative, but the third one (n=3 samples) contained
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43 243 measurable amounts of atrazine. Overall mean concentrations for atrazine were 1(range 0-
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45 244 4)ng/L, and degradation products DEA (2, range 0-11ng/L) and DIA (2, range 0-7ng/L). In
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47 245 addition to these, only BAM could be detected in 4 out of 25 samples (all originating from
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49 246 Luxembourg), with average concentrations of 2(range 0-14)ng/L.
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Discussion

In the present study, we have demonstrated that despite its ban in 2004, low but measurable concentrations of the s-triazine herbicides atrazine, and its degradation products DEA and DIA were detectable in the majority of spring waters from Luxembourg City, and at similar concentrations at most tap water locations, but were almost absent in bottled water from Luxembourg, being the first study reporting values for Luxembourg from unfinished and finished drinking water. It also is suggested that the ratio of atrazine:nitrate, in addition to the ratio of DEA:atrazine could be used to differentiate between non-point-source agricultural contamination of atrazine and non-agricultural contamination, also detected in the present study. Other s-triazine herbicide contamination was negligible by comparison.

Usage of atrazine had been officially declared illegal by individual states, as in Germany (1991) or France (2002), before being eventually banned EU-wide and thus also in Luxembourg in 2004. However, also in countries where atrazine was banned for a comparably long time, still significant concentrations of the parent compound and its main bacterial breakdown products, DEA and DIA can be detected, with concentrations of DEA typically surpassing that of atrazine. For example, in Bavaria, Germany, approx. 5% of sampling points had concentrations of $>0.1 \mu\text{g/L}$ atrazine and DEA, and in almost 50% of the sampling points either DEA, atrazine, or both could be detected in 1999, 8 years after the atrazine ban (Tappe *et al.*, 2002). In countries where atrazine is still in use, such as the US, concentrations are even higher, with atrazine peak concentrations in raw water of up to 230 $\mu\text{g/L}$ (Wu *et al.*, 2010). The long persistence of these compounds is related to the long half-life of atrazine and their de-alkylated metabolites DEA and DIA in deeper soil layers or water, with half-lives of atrazine and DEA around 28-178 days (Krutz *et al.*, 2010), with shorter half-lives in surface and especially adapted soils, i.e. soils that were regularly treated with s-triazines (Krutz *et al.*, 2010). As DEA is more polar and about 20 times more water soluble

(Scribner *et al.*, 2005), it was suggested to bind less strongly to the soil than atrazine, therefore resulting in higher concentration in groundwater, in which degradation is impeded due to lower bacteria counts that could result in degradation (Scribner *et al.*, 2005). DIA conversion rate from atrazine is low compared to DEA, explaining its lower abundance in the environment (Krutz *et al.*, 2010).

The spring water concentrations in the present study, ranging from 0-57 ng/L for atrazine, and 0-120 ng/L for its degradation product DEA were generally, but not always (7 cases >0.1 µg/L DEA) in agreement with EU directive 98/83/EC (even though defined for finished water), demanding a concentration of a single pesticide below 0.1 µg/L. The sum parameter of <0.5 µg/L required by the same EU directive was not violated. Older data from Luxembourgish groundwater from various aquifers (n=72 locations from various regions) from 2005 and older (Administration de la Gestion de l'Eau, 2005) indicated similar, even slightly higher concentrations for atrazine (range 0-550 ng/L, mean 16 ng/L) and DEA (range 0-158 ng/L, mean 16 ng/L), although DEA was not detected in the majority of groundwater sampling points by then (47 negative out of 72), nevertheless highlighting their long persistence.

The only other herbicide or derivative with notable concentrations detected was BAM, a degradation product of dichlobenil, with concentrations of up to 346 ng/L. Dichlobenil has been applied mainly for lawns and gardens. As it was also found to be persistent in the groundwater, it has meanwhile also been banned in EU countries, including Luxembourg in 2010 (EU Commission, 2008). Its endocrine disrupting potential is currently not understood, but it has been found to affect reproduction in animals (Cox, 1997), and constitutes the major groundwater contaminant in other EU countries (Holtze *et al.*, 2008). In the present investigation, BAM was found in 51 spring water samples (out of ca. 700) to surpass the 0.1 µg/L EU limit slightly. In addition, these high concentrations were only found in few

individual springs, which are usually not consumed unpooled. After pooling of various water sources, including additional water from northern Luxembourg as is typical for Luxembourg-City, concentrations might be more likely to fall below 0.1 µg/L.

While DEA, the major s-triazine degradation compound detected, can be formed from atrazine and propazine (a less frequently used herbicide in Europe), DIA can be formed from atrazine, simazine, or cyanazine. Thus, a high ratio (>0.6, (Scribner *et al.*, 2005) of DIA:DEA would indicate several parental sources of DIA. However, this ratio was almost never reached in the present investigation (only in 3 out of ca. 700 point measurements), indicating, in addition to the actual low measured concentrations, that simazine and cyanazine were much less frequently used herbicides compared to atrazine.

Another ratio that has been employed is DEA:atrazine, with a low ratio (ca. 0.5) suggesting point-source contamination, as otherwise, due to its increased formation in soil over time from atrazine (due to microbiological activity), DEA concentrations would tend to be higher. Thus, the DEA:atrazine ratio was used as an indicator of point-source contamination (Scribner *et al.*, 2005) perhaps suggesting non-agricultural usage of atrazine, even though additional methods, including several geostatistic approaches (Leterme *et al.*, 2006) have aimed to differentiate between point and non-point contamination. In the present study, we recognized that by using this cut-off ratio (0.5), 6 springs located together possessed such a remarkably lower ratio, indicating point-source contamination. In addition, the same springs showed also lower nitrate concentrations and a ratio of atrazine (pmol/L): nitrate (mg/L)>3.4, also suggesting that the atrazine source was not linked to general agricultural farming practices, and that the ratio of atrazine to nitrate could be an additional suitable marker to differentiate between non-point-source agricultural use and other applications. A correlation between nitrate and atrazine has already been noted earlier (Umweltbundesamt Oesterreich, 2005).

323 Interestingly, the poor correlation of BAM and nitrate and the high correlation between BAM
324 and urban land usage suggested that dichlobenil was not necessarily originating from
325 agricultural use. However, despite the indication of local atrazine contamination sources, the
326 positive correlation of crop land use and DEA concentration indicated that agriculture was, in
327 general, the major source of contamination for atrazine.

328 Correspondingly to the spring water contamination with atrazine, tap water also clearly
329 showed the presence of atrazine and its metabolites DEA and DIA. Tap water contamination
330 was also reported for other countries, including the US (up to 60 µg/L, Wu *et al.*, 2010),
331 China (Gfrerer *et al.*, 2002), and France, where atrazine and DEA surpassed in ca. 1% of the
332 investigated drinking water 0.1 µg/L (Ministère de la Santé et des Solidarités, 2005), in some
333 areas such as the Alsace, with even >13% (atrazine) and >17% (DEA,
334 <http://vorort.bund.net/suedlicher-oberrhein/wasser-oberrhein.html>). Concentrations in the
335 present study were all below this concentration; however, individual concentrations varied
336 considerably, with some places showing concentrations just below the EU requirement, with
337 up to 208 ng/L for the sum of detected pesticides including their degradation products and up
338 to 96 ng/L for individual pesticides or their derivatives, i.e. BAM, emphasizing the
339 importance of continuous efforts to further lower their concentration in drinking water and
340 monitor drinking water locally to observe changes of these contaminants over longer periods
341 of times. Processes that are used for preparing drinking (tap) water typically include
342 chlorination, flocculation, sedimentation, filtration, followed by a second disinfection stage
343 such as chlorination, UV, or ozonization. It has been shown in earlier reports that atrazine and
344 degradation products are quite resistant to these processes and are hardly removed (Benotti *et*
345 *al.*, 2009). In our study, when comparing mean spring water contamination (unfinished water)
346 of atrazine and degradation products (sum 31 ng/L) with mean tap water concentrations in
347 Luxembourg City (23 ng/L), only a slight reduction in s-triazine content could be assumed.

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3 348 However, the comparison is impeded by the fact that different locations were investigated,
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5 349 and that tap water for Luxembourg City is diluted with drinking water from northern
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7 350 Luxembourg.
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10 351 Bottled water exhibited the lowest concentrations compared to individual spring water
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12 352 locations and tap water, and does not seem to contribute considerably to intake of potential
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14 353 EDC. The one positive sample produced in Luxembourg contained very low amounts of s-
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16 354 triazine herbicides, below 1.5 ng/L, interestingly, only atrazine. The absence or low pesticide
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18 355 concentrations measured in those waters may be explained by the fact that they were obtained
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20 356 from deeper or more remote groundwater sources, and that bottled water is typically
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22 357 undergoing filtration through charcoal filters, a process which is known to bind and remove
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24 358 pesticides, including s-triazine herbicides (Wu *et al.*, 2010), a step which has not generally
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26 359 been put into practice for tap water, partly due to its comparatively high costs.
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29 360 When estimating a mean consumption of 2.1 L water per day and capita from various
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31 361 beverage sources (Walti *et al.*, 2005), with ca. 35% coming from tap water, ca. 15% bottled
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33 362 water, and the remainder from various other sources (tea, coffee, fruit juices, sodas) that are
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35 363 assumed to be low in s-triazine herbicides comparable to bottled water, and combine this
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37 364 consumption pattern with mean triazine and degradation product concentrations obtained in
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39 365 this study (Σ atrazine + DEA +DIA in tap and bottled water: 18 ng/L and ca. 2 ng/L,
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41 366 respectively), it can be estimated that via this route approx. 16 ng/day s-triazine herbicides are
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43 367 consumed in Luxembourg by its ca. 0.5 mio. inhabitants. Even though other food items may
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45 368 contribute to atrazine intake (United States Department of Public Health and Human Services,
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47 369 2003), water is assumed to be by far the most contributing source. An ADI (acceptable daily
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49 370 intake) of 5 $\mu\text{g/kg}$ body weight for atrazine has been suggested (National Registration
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51 371 Authority, 1997), thus the estimated intake as determined in this study would be far lower.
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3 372 In conclusion, the large majority of pesticides and their degradation products measured in
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5 373 water samples in Luxembourg were in accordance with European Regulations, emphasizing
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8 374 that these safeguards were generally observed. Given that the environment is most often
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10 375 polluted by several pollutants that could act in synergy, it would be prudent in the future to
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12 376 continue measuring pesticides, their degradation products, and their intake, which is
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15 377 especially important for pregnant women and infants, which are most prone to adverse effects
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17 378 such as those caused by endocrine disruptor activity.
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Figure Captions:

Figure 1: Pesticides and degradation products monitored during sampling campaigns in Luxembourg between summer 2007 and spring 2009.

Figure 2: Locations of spring water (n=69, circles in map) included for pesticide analyses from Luxembourg City between summer 2007 and spring 2009. All spring water locations used for drinking water supply in Luxembourg-City were included.

Figure 3: Locations of tap water sampling (n=28) for determination of pesticides in 2008 (stars) and 2009 (triangles) in Luxembourg.

Figure 4 : Concentrations of major herbicides and degradation products detected in spring water from Luxembourg City (n=69) between summer 2007 and spring 2009. Values reflect mean $\pm$ SE. BAM=2,6 dichlorobenzamide, DEA=desethylatrazine, DIA=deisopropylatrazine.

Figure 5: Relation between atrazine, degradation products desethylatrazine (DEA), deisopropylatrazine (DIA), and nitrate in Luxembourgish spring water (n=69) during the years 2007-2009 and 10 measurement campaigns.

Figure 6: Concentration of atrazine and degradation products desethylatrazine (DEA) and deisopropylatrazine (DIA) in tap water from various locations in Luxembourg, collected in spring 2008 and spring 2009. Concentrations shown are means of n=1-5 locations, depending on the number of tap water locations investigated per location.

Table 1

Retention time, parent mass, transition ions and collision energy and declustering potential for measuring pesticides following extraction of 500 mL of water samples for each pesticide and SRM conditions for the LC-MS-MS analyses in positive mode.

pesticide	retention time (min)	parent ion (m/z)	DP ^a (V)	transition 1 CE ^b (V)	transition 2 CE ^b in (V)
atrazine	12.39	216.1	49	174.2 (22)	104.0 (40)
desethylatrazine	4.71	188.1	42	146.1 (22)	104.1 (35)
deisopropylatrazine	2.60	174.1	70	68.0 (42)	104.0 (30)
simazine	9.01	202.1	50	104.1 (33)	124.1 (24)
terbuthylazine	14.76	230.1	52	174.1 (24)	104.0 (43)
sebuthylazine	14.41	230.1	52	174.1 (24)	104.0 (43)
cyanazine	9.67	241.1	50	214.1 (24)	104.1 (40)
hexaxinon	8.40	253.1	50	171.1 (50)	71.0 (50)
isoproturon	13.15	207.2	44	72.0 (40)	165.1 (19)
chlortoluron	11.95	213.1	38	72.0 (37)	140.0 (32)
monolinuron	12.91	215.0	38	125.9 (25)	99.0 (47)
methabenzthiazuron	11.50	222.1	39	165.1 (22)	150.1 (44)
metoxuron	8.04	229.1	46	72.0 (8)	229.1 (22)
diuron	13.20	233.1	38	72.0 (37)	233.1 (34)
linuron	14.87	249.0	35	160.0 (24)	132.9 (47)
metobromuron	13.77	259.0	36	170.1 (23)	90.9 (47)
metazachlor	14.12	278.1	29	134.1 (31)	210.1 (15)
metolachlor	15.59	284.2	39	252.1 (21)	176.2 (33)
2,6- dichlorobenzamide	3.30	190.0	44	109.0 (50)	172.9 (23)
atrazine-d5 (IS) ^c	12.26	221.2	40	179.2 (24)	-

^a DP, declustering potential

^b CE, collision energy

^c IS, internal standard (monitoring purpose)

Table 2.

Pesticides monitored in Luxembourgish spring water (n=69), number of findings above limit of detection* and concentrations between summer 2007 and spring 2009.

pesticide	locations with detectable concentration*	analyses with detectable concentrations	min conc.	max conc.	mean conc.
	(n)	(%)	(ng/L)	(ng/L)	(ng/L)
atrazine	58	78.3	0	57	9
desethylatrazine	59	82.6	0	120	19
deisopropylatrazine	47	49.6	0	27	3
simazine	27	24.9	0	5	1
terbuthylazine	1	0.2	0	1	0
sebuthylazine	0	0	0	0	0
cyanazine	0	0	0	0	0
hexaxinon	0	0	0	0	0
isoproturon	11	2.0	0	5	0
chlortoluron	11	10.9	0	6	0
monolinuron	1	0.2	0	4	0
methabenzthiazuron	0	0	0	0	0
metoxuron	0	0	0	0	0
diuron	7	4.5	0	11	0
linuron	0	0	0	0	0
metobromuron	0	0	0	0	0
metazachlor	0	0	0	0	0
metolachlor	0	0	0	0	0
2,6- dichlorobenzamide	58	79.9	0	346	30
Σ ATZ +DEA+DIA**	61	83.5	0	202	32
Σ all pesticides	65	89.4	0	352	63

* number of locations with at least one finding above the limit of detection (1 ng/L)

**ATZ= atrazine, DEA=desethylatrazine, DIA=deisopropylatrazine

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Table 3.

Investigation of several bottled water types frequently sold in Luxembourg, procured from local supermarkets during spring 2008 and spring 2009.

No.	Origin	n ⁺	Type	atrazine (ng/L)	DEA ^{&} (ng/L)	others (ng/L)
1	Luxembourg, north (Canton Echternach)	1	sparkling	n.d.*	n.d.	n.d.
2	Luxembourg, north, (Canton Echternach)	3	weakly sparkling	n.d.	n.d.	n.d.
3	Luxembourg, north, (Canton Echternach)	3	non-sparkling	n.d.	n.d.	11.9±2.4**
4	Luxembourg, west, (Canton Redingen)	4	non-sparkling	0.4±0.7 [§]	n.d.	3.3±3.7**
5	Luxembourg, west, (Canton Redingen)	1	non-sparkling	n.d.	n.d.	n.d.
6	Luxembourg, west, Canton Redingen	1	non-sparkling	n.d.	n.d.	n.d.
7	Luxembourg, west, (Canton Redingen)	2	non-sparkling	n.d.	n.d.	n.d.
8	France, (Haute-Savoie)	3	non-sparkling	n.d.	n.d.	n.d.
9	France, (Alsace-Lorraine)	2	non-sparkling	n.d.	n.d.	n.d.
10	France, (Auvergne)	5	non-sparkling	2.7±0.9	9.2±1.4	6.2±1.1*** 0.1±0.1**
mean s-triazines [#]				0.3	0.9	1.8

⁺ number of samples from each variety. Each purchased variety was investigated in duplicate

* n.d. = not detected

** 2,6,-dichlorobenzamide

*** DIA= deisopropylatrazine

[§] Concentration below detection limit results from a mean of 4 samples measured with only 1 being positive.

[&] DEA= desethylatrazine

[#] Mean of atrazine, DEA and DIA over the 10 investigated water brands, respectively

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