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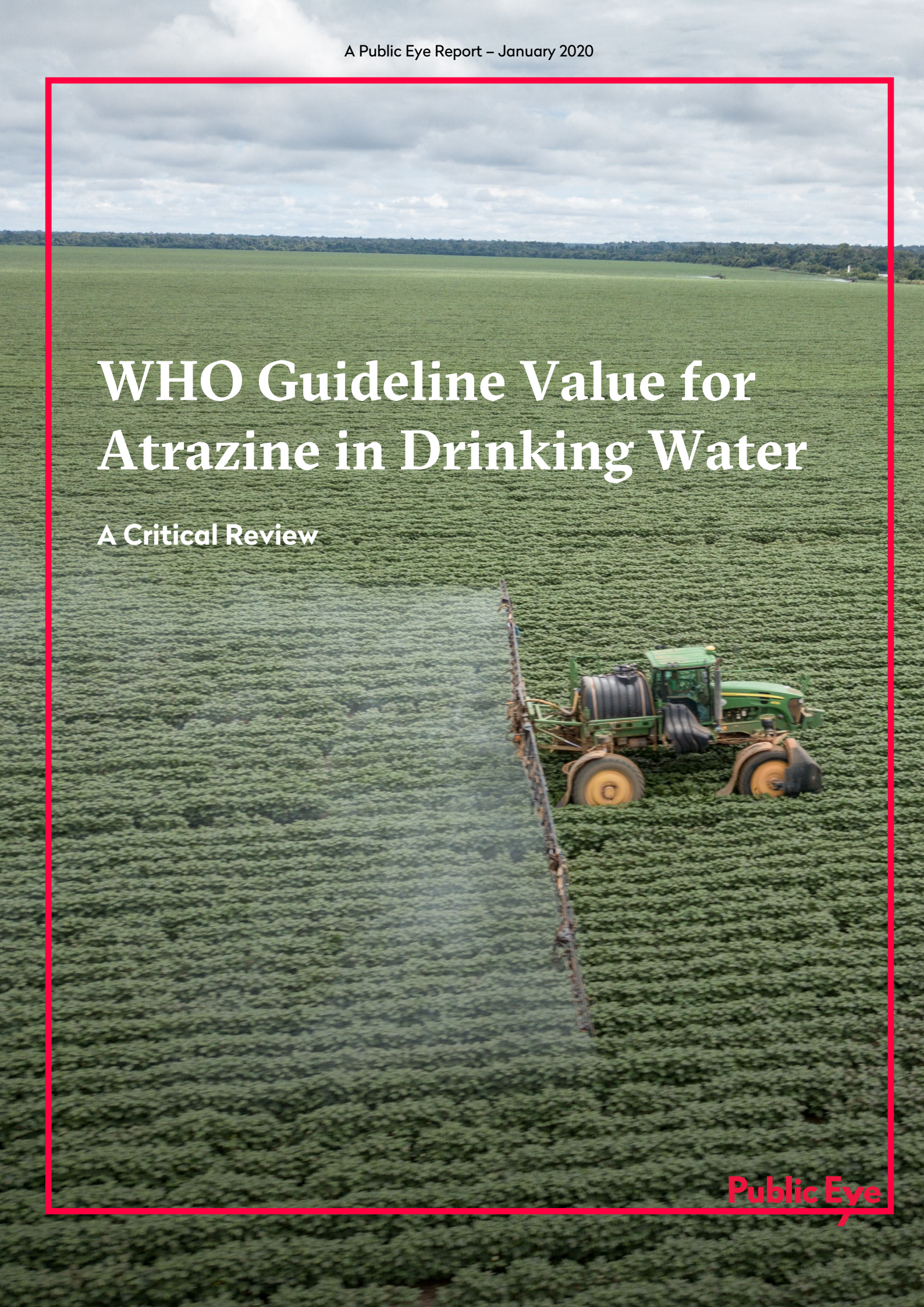
WHO Guideline Value for Atrazine in Drinking Water

A Critical Review



Public Eye

IMPRINT WHO Guideline Value for Atrazine in Drinking Water: A Critical Review. A Public Eye Report, January 2020, 40 pages | **Author** Peter Clausing | **Contributors** Laurent Gaberell, Jennifer Sass | **Cover picture** © Fábio Erdos

PUBLIC EYE Avenue Charles-Dickens 4, CH-1006 Lausanne, tél. +41 (0)21 620 03 03
fax +41 (0) 21 620 03 00, www.publiceye.ch, contact@publiceye.ch

1 Introduction

Atrazine is one of the most heavily used herbicides in the world. In 2017, the global production was approximately 85,000 metric tons of active ingredient, according to the Phillips McDougall AgrAspire database (Phillips McDougall 2018). It degrades very slowly in the environment. For instance, it is stable in water for more than 100 days, and therefore has been listed for many years as a water contaminant by the World Health Organization (WHO 2011a). While the previous WHO guideline value (or acceptable limit) for drinking water was 2 µg/liter[1] (WHO 2003), this limit was raised to 100 µg/liter (WHO 2011a) seven years ago. In contrast to the risk-based approach taken by WHO in tolerating higher concentrations in drinking water, the European Union (EU) applies a "parametric limit" of 0.1 µg/liter in drinking water for all pesticides.[2] Consequently, in October 2003, atrazine was banned in the EU because its residues in water samples frequently exceeded this limit of 0.1 µg/liter. More specifically, the European Commission concluded that "available monitoring data were insufficient to demonstrate that in large areas concentrations of the active substance and its breakdown products will not exceed 0.1 µg/l in groundwater" (European Commission 2003). This is in remarkable contrast to WHO that claimed that atrazine residues in ground water "are commonly well below 0.1 µg/l" (WHO 2017, p. 319). In this context it should be noted that no monitoring system comparable to that of the EU exists in most of the countries in Africa, Southeast Asia and South America.

To understand the rationale used by WHO for the drastic increase in its guideline value from 2 to 100 µg/liter we will briefly look at how such limits are established. The point of departure are toxicological studies performed in laboratory animals (tests in two different species of laboratory mammals are required for long-term effects like carcinogenicity and birth defects). They typically consist of a control group and at least three dose groups treated with the test chemical (in our case atrazine), i.e. low, mid- and high dose groups. Ideally, the doses in such a study have been selected in such a way that the low dose group will not show any effect, whereas the mid-dose group will show some effects and the high-dose group will show clear signs of toxicity. In this situation the dose administered to the low-dose group becomes the no-observed-adverse-effect-level (NOAEL). Different types of studies (long-term/carcinogenicity studies, reproductive toxicity studies etc.) in different species are performed for each compound. At the end, the lowest NOAEL is used to calculate the acceptable daily intake (ADI) in humans. The ADI is the total amount of a chemical which, according to the authorities, can be consumed daily with the expectation that health will not be harmed. The WHO Drinking-Water Guidelines Program uses the term 'tolerable daily intake' (TDI) which is synonymous with the term ADI (WHO 2011b).

The standard approach is to divide the NOAEL by 100 to calculate the ADI. This approach combines a factor 10 allowance for intra-species variation in sensitivity with a factor 10 allowance for

interspecies variation. If irreversible damage by the chemical (carcinogenicity, mutagenicity, reproductive toxicity, endocrine disruption) cannot be ruled out, additional measures may be taken, ranging from a complete ban of the chemical (in a certain geographical region like the EU) to an additional safety factor allowance. Conclusions about potential irreversible damage can be drawn from animal experiments as well as from studies in humans (epidemiological studies). Once the ADI has been established, the guideline value can be calculated (WHO 2011b) as follows:

Guideline value = ADI (mg/kg) x body weight (kg) x P (drinking water contribution), divided by C (amount of water consumed).

The default body weight in the WHO guideline (WHO 2011b) is 60 kg for adults and 10 kg for children (toddlers require additional considerations). The default amount of water consumed (C) is 2 liters/day for adults and 1 liter/day for children.

P (drinking water contribution) means the estimated percentage of the chemical consumed in water as compared to other sources of exposure, mainly residues in food. When an estimate for P is not available, a default percentage (used in the calculation as a decimal fraction) is allocated. This default percentage differs for atrazine between the earlier evaluation (WHO 2003) which used a P-value of 10% and the most recent evaluation which applied a default value of 20% (WHO 2011a). In a later document WHO explained that the value of 20% was introduced because the previous allocation of 10% "was found to be excessively conservative" (WHO 2017, p. 163).

The earlier guideline value of 2 µg/liter (WHO 2003) was based on a NOAEL of 0.5 mg/kg body weight using a safety factor of 1,000 (100 for inter- and intra-species variation and 10 to reflect potential carcinogenicity), and a factor P for drinking water contribution of 10%. The NOAEL was derived from a carcinogenicity study with Sprague-Dawley rats which showed an increased incidence of mammary tumors beginning at a concentration of 70 mg/kg diet (corresponding to 3.5 mg/kg body weight). The NOAEL was at 10 mg/kg diet (corresponding to 0.5 mg/kg body weight). Applying the uncertainty factor allowance of 1,000, the resulting ADI was 0.0005 mg/kg. In later years both JMPR and the International Agency for Research on Cancer (IARC) assessed that these mammary tumors were specific to the Sprague-Dawley rat strain.

There seems to be consensus that atrazine's mechanism for causing mammary tumors in rats is of a neuroendocrine nature (Simpkins et al. 2011). In the Sprague-Dawley rat strain, but not in the Fischer 344 strain, this involves increased prolactin blood levels, increased estrus cycle length and persistent diestrus, resulting in

early reproductive senescence associated with an increased incidence of mammary tumors. More recently, strain-specific differences of the epigenetic profiles of mammary gland tissue have been reported for four different rat strains, which may help to explain their diverging susceptibility with regard to hormone-induced mammary cancer in Sprague-Dawley versus Fischer 344 rats (Luzhna et al. 2015).

The revised atrazine drinking-water guideline prepared for the Third Edition of the WHO Guidelines for Drinking-water Quality (WHO 2011a) followed the assessment of the Joint Meeting on Pesticide Residues (JMPR), an expert group administered jointly by the Food and Agriculture Organization of the United Nations (FAO) and WHO. JMPR re-evaluated atrazine during its session of 18-27 September 2007. Two years later the results of this session were published. JMPR concluded that there is "no relevant carcinogenicity" in laboratory animals, and that epidemiological studies "do not support a causal association between exposure to atrazine and cancer in humans" (JMPR 2009).

The new guideline value for drinking water of 100 µg/liter atrazine (WHO 2011a) used the ADI of 0.02 mg/kg body weight derived by JMPR (2009) from the NOAEL of 1.8 mg/kg body weight in a 6-month rat study showing endocrine effects, i.e. on the luteinizing hormone, and an uncertainty factor allowance of 100. The additional factor 10 allowance for potential carcinogenicity was abandoned. In addition they used a drinking water contribution factor of 20% rather than the 10% of 2003. In this way WHO ended up with a

guideline value of 100 µg/liter, 50-times higher than the previous one.

In the following, the determination of JMPR's ADI of 0.02 mg/kg body weight (JMPR 2009) is scrutinized taking into account more recent literature as well as an evaluation performed by the U.S. Environmental Protection Agency (EPA 2011) and its Scientific Advisory Board (SAP 2011). This report will show that contrary to JMPR assessments (2009), there are serious reasons to consider atrazine as a (possible?) carcinogen beyond the issue of mammary tumors in Sprague-Dawley rats (see discussion above). Projecting the precautionary principle as stipulated in the EU pesticide legislation (EU 2009), and recommended in an advisory legal opinion of the Inter-American Court of Human Rights (IACHR 2017), the safety factor allowance of 100 currently used by WHO appears insufficient and needs reconsideration. Furthermore, WHO itself states: "Situations in which the nature or severity of effect might warrant an additional uncertainty factor include studies in which the endpoint is malformation of a fetus or in which the endpoint determining the NOAEL is directly related to possible carcinogenicity." Because children are considered to be particularly vulnerable to atrazine, based on evidence for endocrine disrupting properties, a default intake of 1 liter of water per 10 kg body weight (WHO 2017, p. 164) should be taken into account. Finally, the NOAEL of 1.8 mg/kg used by JMPR (2009) is challenged, based on high-quality studies showing effects at even lower doses.

2 Carcinogenicity

Carcinogenicity as a potentially irreversible and fatal health damage is a matter of particular concern which can lead to a complete ban of a pesticide (in certain geographical regions) or – as described above – to the use of additional safety factor allowances when calculating the ADI or TDI. Three lines of evidence are typically taken into consideration for the assessment of a carcinogenic hazard: studies in experimental animals, epidemiological studies in (unintentionally exposed) humans and mechanistic considerations (i.e. how the compound under consideration could elicit carcinogenic effects).

ANIMAL STUDIES

In this section the evaluations by JMPR and IARC are discussed. In its Monograph No. 53 IARC classified atrazine as a category 2B carcinogen – possibly carcinogenic in humans (IARC 1991). Eight years later this classification was abandoned and IARC (1999) concluded that atrazine is "not classifiable as to its carcinogenicity to humans (Group 3)".

In its session of 2007 JMPR reviewed 3 mouse and 10 rat carcinogenicity studies, although only 5 of the 10 rat studies were full carcinogenicity studies using both males and females (JMPR 2009). JMPR stated that it was "focusing on the issues of carcinogenicity" of atrazine (JMPR 2009, p. 38). However, in its review of evidence from animal studies, JMPR focused almost exclusively on one tumor type – the phenomenon of an increased incidence of mammary tumors in the Sprague-Dawley rat strain. With its statement "focusing on the issues of carcinogenicity", JMPR gives the impression of having performed a comprehensive assessment of carcinogenicity. Therefore, a major problem with JMPR's report is the lack of data presentation concerning tumors other than the mammary tumors seen in rat studies. Further, in the 3 mouse studies, details on tumor findings are completely lacking: JMPR provided only blanket statements that no carcinogenicity was observed in these studies. Due to this lack of transparency, it was impossible to assess the validity of these generalized statements.

With regard to the 4 rat carcinogenicity studies considered by JMPR, the following problems were identified:

- For the Mayhew (1986)[3] study an increase in testicular interstitial cell tumors at the top dose (1,000 ppm) was mentioned, but dismissed because it was "within the range of spontaneous occurrence" and "in part" due to the increased survival at this dose group (JMPR 2009, p.54). While no further details were given in the JMPR report, IARC, presumably referring to the same study, stated that "the incidence fell within the historical range for controls at that test laboratory (0-12%)" (IARC 1999, p. 82). Concerning these historical control data (HCD), Stevens et al. (1998) revealed that this range referred to four earlier studies. This represents a relatively small database[4] and information is lacking as to whether these data were from studies conducted with the same strain of rats and within the last five years – important criteria for the validity of HCD (OECD 2012). In addition, it should be noted that OECD Guidance 116 discourages the use of the (simple) range and, instead recommends the use of interquartile ranges for comparing current study data with

HCD. In the same paragraph of this guidance document, it is emphasized that "the concurrent control group is always the most important consideration in the testing for increased tumour rates", and "that historical control data should only be used if the concurrent control data are appreciably 'out of line' with recent previous studies" (OECD 2012, p.135). No assessment was provided as to whether the control incidence of testicular interstitial cell tumors was considered "appreciably out of line". Taking into account past abuses of HCD by other authorities (cf. Clausing et al. 2018), more transparency is required to make JMPR's and IARC's assessments convincing.

- The Pettersen & Turner (1995)[5] rat study was "conducted to determine the effects of atrazine on the mammary and pituitary glands" (JMPR 2009, p.59). However, the outcome concerning effects on the pituitary gland is not mentioned at all in the JMPR report. It is not clear whether there were no effects on the pituitary gland or whether such effects were not evaluated.
- In the Thakur (1992b)[6] study, Fischer 344 rats were used. These rats do not feature the early reproductive senescence that makes Sprague-Dawley rats unique and, therefore, unsuitable for extrapolating the mammary tumor effects seen in this strain of rats to humans. In this study, a dose-dependent, though weak increase in the incidence of mammary tumors in female rats was acknowledged, i.e. 7, 8, 12, 17 and 13% incidence at 0, 10, 70, 200 and 400 ppm, respectively, with 60 animals per group (JMPR 2009, p.63). Our own calculations[7] revealed that this increase was statistically significant using the Jonckheere-Terpstra Test (p=0.0473, one-sided exact test), a test particularly suited to analyzing umbrella-type dose-responses. The more commonly used Cochran-Armitage Trend test (recommended by OECD 2012) had an error probability of p=0.0972 (one-sided exact test).

In its overall conclusion IARC stated "that the mammary tumours associated with exposure to atrazine involve a non-DNA-reactive, hormonally mediated mechanism" and that the increase in incidence of mammary gland tumors seen in Sprague-Dawley rats is not relevant for humans (IARC 1999, p. 99). Likewise, JMPR (2009, p. 116) stated that there is "no relevant carcinogenicity".

It is recognized that the mechanism for the formation of mammary gland tumors in Sprague Dawley rats is unique and not representative of humans. But, the justification for dismissing the two other tumor findings mentioned above (testicular interstitial cell tumors, mammary tumors in Fischer 344 rats) needs further scrutiny. Because the detailed data have not been fully disclosed, it is not possible to do this in the present report.

EPIDEMIOLOGICAL STUDIES

JMPR (2009) referred to the assessment performed by IARC (1999) and evaluated a number of further investigations published after IARC's monograph.

IARC (1999) evaluated seven publications with case-control studies (published between 1985 and 1993) and one cohort study (published in 1996). A meta-analysis of three of the case-control studies performed in the U.S. identified a significant association between the use of triazine herbicides (including atrazine) and Non-Hodgkin's lymphoma (Hoar Zahm et al. 1993). In an Italian study, borderline significance was established for an association between triazine exposure and ovarian tumors (Donna et al. 1984). No other significant associations were reported in these publications. IARC concluded that there is inadequate evidence of carcinogenicity in humans (IARC 1999, p.99), in line with hazard category 3 assigned by IARC. The difference compared with "evidence suggesting lack of carcinogenicity in humans", hazard category 4, should be noted (IARC 2015).

JMPR (2009) referred to the review of epidemiological studies performed by IARC (1999) and assessed 13 epidemiological publications which were not considered by IARC – 10 of them because they were published after IARC's monograph of 1999, two of them presumably because they were review papers and did not present original work (Neuberger 1996; Sathiakumar and Delzell 1997), and one (Mills 1998) for unknown reasons. JMPR's overall conclusion was that "epidemiological studies do not support a causal association between exposure to atrazine and cancer in humans" (JMPR 2009, p.117). Nevertheless, in six of these publications, JMPR described significant and borderline significant associations between atrazine, or other triazines, and tumors. Acknowledging significant associations with tumors and then concluding a lack of support for an association

between exposure and increased tumor incidences is a contradiction.

Two years later the U.S. EPA prepared a detailed review based on 40 studies (EPA 2011), which was subsequently scrutinized by EPA's Scientific Advisory Committee (SAP 2011). SAP criticized the EPA for insufficiently considering that "epidemiology data failed to provide compelling evidence that atrazine is not carcinogenic (SAP 2011, p.15, emphasis added).

Here, thyroid cancer, ovarian cancer, Non-Hodgkin's lymphoma (NHL) and hairy-cell leukemia will be discussed, tumors for which SAP – in contrast to EPA – concluded that there is "suggestive evidence" for an association with atrazine exposure.

One of the publications assessed by EPA was not available for IARC (1999) and JMPR (2009), because it was published only in 2011. This was an analysis of data from the Agricultural Health Study (AHS), a large, ongoing cohort study in the U.S. (Beane Freeman et al. 2011). AHS data are considered of high quality, because, in contrast to case-control studies, this cohort study avoids recall bias.[8]

THYROID CANCER

Beane Freeman et al. (2011), a paper not available at the time of the JMPR review, analyzed the AHS data for an association between atrazine exposure and various tumor types. To our knowledge, it is the only study that has ever looked at thyroid cancers in relation to atrazine exposure. The authors identified a 4.84-fold risk (95% CI 1.31, 17.93) of thyroid cancer for the most highly exposed quartile of pesticide applicators participating in this study. The observed association remained essentially unchanged when the authors controlled for body mass index – a known risk factor for thyroid cancer. Further, across all four exposure groups, there was a linear trend with an error probability of 8%, which can be considered borderline significance.

In spite of the strength of the AHS database, the "lack of a clear exposure-response trend" and an alleged lack of a known biological mechanism led EPA to conclude that there is only "some" evidence of a positive link between atrazine and thyroid cancer (EPA 2011, p.52). EPA summarized its assessment by stating that "evidence is insufficient to determine the nature of the association between thyroid cancer and atrazine" (EPA 2011, p.70). Contrary to EPA, the Scientific Advisory Panel proposed to allocate the category "suggestive evidence of carcinogenic potential" (SAP 2011, p.71), because it considered the association observed between atrazine and thyroid cancer in the Beane Freeman et al. (2011) study as strong.

No longer can EPA's view be upheld that atrazine does not "act as a tumor promotor in experimental systems of the thyroid" and that a clear mode of action is lacking (EPA 2011, p. 69). As early as 2006 it was documented that the G protein-coupled receptor 30 (GPR30)[9], a receptor located in cell membranes, mediates non-genomic actions of estradiol and known endocrine disruptors like bisphenol A and genistein (Thomas & Dong 2006). For genistein and 4-hydroxytamoxifen it was shown that the 2 compounds are capable of eliciting cascades of biochemical reactions in thyroid carcinoma cells which may induce progression to thyroid cancer (Vivacqua et al. 2006). Later it was demonstrated that atrazine also binds to GPR30, thereby exerting estrogen-like activity in ovarian and breast cancer cell lines and in cancer-associated fibroblasts (Albanito et al. 2015). This may explain why atrazine is able to up-regulate aromatase activity in cancer cells (Sanderson et al. 2001), although it neither binds to the (classical) nuclear estrogen receptor, nor activates this receptor (Connor et al. 1996; Tennant et al. 1994).

The interaction between triazines and GPR30 is very complex. The cell milieu may affect this interaction (Florian et al. 2016). The involvement of different signal transduction pathways may occur, activated by one and the same compound through the same receptor, but with variable outcomes depending on the exposure level and the cell-context (Chevalier et al. 2016; Lappano et al. 2016).

These recent publications provide evidence that atrazine has tumor-promoting properties in human cancer cell lines exerted through the GPR30 receptor and that other compounds (genistein and 4-hydroxytamoxifen) activating the same receptor have tumor-promoting effects in human thyroid cancer cell lines. Thus, it can be concluded that atrazine could be capable of acting as a tumor promotor in the thyroid.

The fact that no tumor-promoting effect of atrazine was seen in vivo, i.e. in a rat study using N-bis(2-hydroxypropyl)nitrosamine for tumor initiation (Son et al. 2003), could reflect the inappropriateness of this

model rather than invalidating the *in vitro* findings described above, when the complexity of the interactions between triazines and GPR30 is taken into account. The insight into the role of GRP30 in eliciting estrogenic activities reinforces the concerns expressed by SAP about EPA's exclusive focus "on a single mechanism of action, the neuroendocrine pathway and suppression of the LH surge" (SAP 2011, p. 15). This narrow focus may have impeded an appropriate hazard evaluation and risk assessment.

OVARIAN CANCER

An association between atrazine exposure and ovarian cancer was demonstrated in a study from Italy (Donna et al. 1989). However, in this study the odds ratio (OR, the statistical measure for such an association) was not adjusted for exposure to other herbicides. In addition, the finding was only significant when a 90% CI was used instead of 95%[10] (OR 2.7, CI 1.0-6.9 for definitely exposed cases). This study was taken into consideration by JMPR (2009).

An analysis of the AHS database (Alavanja et al. 2005), not considered by JMPR but assessed by EPA (2011), supported the finding by Donna et al. (1989). Alavanja and co-authors (2005) showed a significantly increased risk of 2.97 (95% CI 1.28–5.85) when comparing female pesticide applicators of Iowa and North Carolina. A population-based case-control study in California (Young et al. 2005) did not yield significant associations between atrazine and ovarian cancer. While this study was considered imprecise and flawed by EPA (EPA 2011, p.50), JMPR emphasized that "no evidence of a dose-response relationship for triazines and ovarian cancer was found" (JMPR 2009, p.108). In the analysis of the AHS database, Beane Freeman et al. (2011) identified a non-significant but elevated risk of ovarian cancer (OR 2.91, CI 0.56-13.60; Beane Freeman et al. 2011). The wide confidence interval could be interpreted as a lack of statistical power rather than a lack of association, because, according to EPA (2011), this analysis was based on only 9 ovarian cancer cases, only 4 of whom reported ever having used atrazine.

Overall EPA assessed that the evidence for an association between atrazine and ovarian cancer is "weakly suggestive" (EPA 2011, p.68). SAP disagreed and proposed to use the category "suggestive evidence of carcinogenic potential" with regard to ovarian cancer (SAP 2011, p.71).[11]

Emphasizing atrazine's neuro-endocrine mode of action by suppressing the lutenizing hormone surge, EPA pointed out that "no known alternative mode of action" existed at the time of their evaluation (EPA 2011, p.69). As explained in the paragraph on thyroid cancer, this view is no longer valid. Albanito et al. (2015) demonstrated the activation of estrogenic pathways via GPR30 by atrazine in two ovarian cancer cell lines leading to their proliferation in a dose-dependent manner.

NON-HODGKIN'S LYMPHOMA (NHL)

NHL is the tumor type most extensively assessed in epidemiological studies of triazines in general and of atrazine in particular. IARC (1999) reviewed four publications containing results on NHL. While all four reported a slightly increased risk, only one of these increases was statistically significant (OR 1.4, 95% CI 1.1-1.8) (Hoar et al. 1986). JMPR (2009) briefly referred to existing reviews (Neuberger 1996; Sathiakumar & Delzell 1997; IARC 1999). In addition, results obtained from a plant manufacturing triazines (MacLennan et al. 2003) were mentioned, as well as those from an analysis of the AHS database (Rusiecki et al. 2004). Although providing brief summaries of these studies, JMPR did not make a specific assessment concerning NHL.

In contrast, EPA (2011) provided a detailed analysis, summarized in Table 1. But although 4 of the 7 studies showed statistical significance, EPA concluded that "overall, the database lacks evidence of an association between atrazine and triazine exposure and ... NHL". The major reason for this conclusion was the contradiction between two analyses of the AHS database. Rusiecki et al. (2004) identified a (non-significant) trend of an increasing risk of developing NHL with increasing exposure to atrazine. This trend disappeared in an updated analysis with twice as many cases of NHL (Beane Freeman et al. 2011).

SAP stated that "although evidence from the AHS cohort does not suggest a causal association between atrazine and NHL, the studies conducted in the Midwest U.S. and in France provide positive evidence" (SAP 2011, p. 63)[12]. Based on this, SAP disagreed with EPA and concluded that there is suggestive evidence for a causal association between atrazine and non-Hodgkin's lymphoma.

HAIRY CELL LEUKEMIA

Hairy cell leukemia (a rare subtype of NHL) was not taken into consideration by IARC or JMPR, although one of the two French publications describing an association with triazines was published before 1999 (Clavel et al. 1996). In this hospital-based case-control study, covering the period 1980-1990 and recruiting participants from 18 different hospitals, an OR of 1.9 (95% CI 1.0-3.5; 23 cases, 25 controls) was observed for the combination of possible and definite exposure. For definite exposure, only the OR was 2.4 (95% CI 1.2-4.8; 20 cases, 18 controls). In a more recent hospital-based case-control study (conducted in six French medical centers between 2000 and 2004), a significant OR of 1.4 (95% CI 1.4-19.3; 4 cases, 17 controls) was observed for triazine use (Orsi et al. 2009). It should be noted that no other study investigating a possible association between atrazine/triazines and hairy cell leukemia has been published so far. In other words, these findings are to be considered undisputed.

EPA assessed: "The hospital-based case control studies performed in France during two separate study periods present some evidence of a positive link, however the potential for systematic error in the conduct of these studies including selection bias due to the method of control identification, weakens the strength of this evidence in the overall assessment of the epidemiologic database", and concluded that "overall, the database lacks evidence of an association between atrazine and triazine exposure and these lymphoma and leukemia sub-types, including NHL" (EPA 2011, p. 59, emphasis added). This evaluation was supported by an industry sponsored study claiming that "the use of hospital controls is of particular concern as they could be a potential source of selection and reporting bias" (Bofetta et al. 2013).

Contrary to this, SAP stated that "both studies adequately described their control selection process and provide evidence against selection bias", and added that "Although systematic bias in the control-selection process can never be entirely ruled out in any case-control study, it does not appear to be a source of major bias in these studies" (SAP 2011, p.63).

Consequently, SAP disagreed with the EPA that evidence of an association between triazines exposure and hairy-cell leukemia is lacking, and concluded that the two French studies provide suggestive evidence for a causal association between triazines and hairy-cell leukemia" (SAP 2011, p.63, see footnote 4).

In criticizing SAP's conclusion, Bofetta et al. (2013, p.122) opined that an association between atrazine (or triazine) exposure and hairy-cell leukemia was not supported by the "current understanding of the biology" of that tumor. In particular, they referred to publications by Blomberry et al. (2012), Ewalt et al. (2011) and Tiacci et al. (2011) that identified a linkage between the *BRAF* mutation and hairy cell mutation. But the statement by Bofetta and co-authors (2013) is misleading. The publications they cited describe the discovery that a mutation in the *BRAF* gene activates hairy cell leukemia, but they say nothing about how this mutation was acquired. It is general consensus that somatic mutations (such as the *BRAF* mutation) are one of the mechanisms of how chemicals, including pesticides, can cause cancer. In fact, while the positive association of hairy-cell leukemia with "farming activities" was repeatedly established, the etiology of hairy-cell leukemia remains largely unknown Monnereau et al. (2014). According to these authors, available knowledge strengthens the hypothesis that occupational pesticide exposure may be involved in the etiology. The findings by Clavel et al. (1996) and Orsi et al. (2009) are part of this knowledge. It is wrong to refer to Blomberry et al. (2012), Ewalt et al. (2011) and Tiacci et al. (2011) in claiming that hairy-cell leukemia is not supported by the "current understanding of the biology" of this neoplasm, as Bofetta et al. (2013) did. Instead, although the etiology of hairy-cell leukemia is still unknown, a possible mechanism for hairy-cell leukemia could be genotoxic damage to somatic cells. This possibility will be discussed in the next section (Genotoxicity).

MECHANISTIC EVIDENCE FOR CARCINOGENICITY

Besides the GPR30-mediated effects mentioned above, a number of other mechanisms are considered to be involved in carcinogenesis (Smith et al. 2016). Among them are genotoxicity (and events causing genomic instability), oxidative stress and immunomodulation. The first two are interrelated, because oxidative stress is a major factor for damage to the DNA. Genotoxicity can result in uncontrolled replication of cells and, thereby, growth of tissue (neoplasia) due to a disturbance of the genetic program controlling the replication of cells. Oxidative stress (a

biochemical intracellular imbalance) is caused by an excess of highly reactive molecules, so-called reactive oxygen species, which can trigger tumor development, either through the genetic damage they cause or due to direct influences in their role as signaling molecules, e.g. on cell proliferation (Ziech et al.2010, Nowsheen et al. 2012).

GENOTOXICITY

In its assessment, JMPR concluded that atrazine is "unlikely to be genotoxic *in vivo*"[13] (JMPR 2009, p.116). To support this assessment, JMPR listed the results of 20 in vivo tests, 15 of which were negative, while 4 exhibited a genotoxic response. Furthermore, the result of one was designated "equivocal", although a dose-dependent statistically significant effect was observed (Tennant et al. 2001). This publication was the last one considered by JMPR. A literature search revealed that 11 further papers have been published, one of them prior to the publication of the JMPR report. The results are presented in Table 2. In total, 14 of the 16 in vivo tests described in these 11 papers demonstrated genotoxic effects. In some of these tests, genotoxicity was detected at concentrations around WHO's previous guideline value for drinking water of 2 ppb atrazine. When testing atrazine as the active ingredient, two tests in goldfish were negative (Cavas 2011), in contrast to positive findings in five tests performed in fruit flies, zebrafish and mice (Torres et al. 1992, Zhu et al. 2010, Adeyemi et al. 2015, Wirbisky et al. 2016, Gao et al. 2016). Moreover, genotoxic effects were always found when atrazine-based formulations were tested (Cavas 2011, Nwani et al. 2011, Campos-Pereira et al. 2012, Nwani et al. 2014, Goncalves et al. 2017). Importantly, the only epidemiological study available demonstrated a positive association between urinary levels of atrazine and chromosomal damage in peripheral lymphocytes of children (Ruiz-Guzman et al. 2017). It should be noted that simultaneous exposure to both the active ingredient atrazine and its formulants can happen when the exposure is through the consumption of contaminated water.

Based on the studies summarized in Table 2, JMPR's conclusion that atrazine is "unlikely to be genotoxic *in vivo*" is questionable and needs re-consideration.

OXIDATIVE STRESS

Reactive oxygen species overwhelming the organism's antioxidant capabilities results in oxidative stress, which is considered one of the mechanisms of carcinogenesis. Growing evidence shows that atrazine can cause oxidative stress not only in fish (e.g. Xing et al. 2012, Blahova et al. 2013, Wang et al. 2013), but also in laboratory rodents.

In mice statistically significant changes of oxidative stress biomarkers were seen subsequent to treatment with 200 mg/kg body weight, injected intraperitoneally every other day for a total of 4 injections (Jin et al. 2014), and after oral administration of 100, 200 and 400 mg/kg body weight for 21 days (Gao et al. 2016).

Rats were tested using oral administration of technical grade atrazine (between 97 and 99% purity) as well as atrazine-based formulations. Atrazine (active ingredient) elicited oxidative stress after repeated oral administration at doses between 25 and 300 mg/kg body weight (Singh et al. 2008, 2011; Bhatti et al. 2011; Pogrmic-Majkic et al. 2012; Song et al. 2014; Abass et al. 2016, Zhao et al. 2014). For atrazine-based formulations, significant changes of oxidative stress biomarkers were shown at 120 and 400 mg/kg (Adesiyan et al 2011; Campos-Pereira et al. 2012), but not at 12.5 mg/kg (Abarikwu et al. 2014).

ALTERATIONS OF THE IMMUNE SYSTEM

Two different immunological processes are involved in tumorigenesis (Smith et al. 2016). Immunosuppression can prevent the immune system from eliminating neoplastic cells, which may then replicate and progress into tumors. On the other hand, chronic inflammation is considered to be involved in multiple aspects of cancer development and tumor progression, and was described as "an enabling hallmark of cancer" (Hanahan and Weinberg 2011, cited in Smith et al. 2016) with strong links to oxidative stress and genetic instability. In a joint WHO/UNEP publication, the authors assessed that atrazine "has convincing effects on the immune system" (Bergman et al. 2013, p. 169). JMPR evaluated that "modulation of the immune system occurs after exposure to atrazine, albeit at doses greater than those known to disrupt neuroendocrine function and suppress LH and prolactin release" (JMPR 2009, p. 103). JMPR failed to discuss immunomodulation as a potential mechanism of carcinogenicity.

The U.S. EPA acknowledged that the available studies suggest that atrazine can affect the immune system and that the mechanism of this

immunotoxicity and its possible relevance for human health effects is not thoroughly understood yet (EPA 2010). It can be assumed that, for this reason, EPA did not comment on the observed immunotoxicity as a possible mechanism of carcinogenicity in the context of the neuroendocrine nature of atrazine's mode of action for mammary tumors. Available studies revealed two types of immunotoxic effects – an indirect effect through atrazine's interference with the hypothalamic-pituitary-adrenal (HPA) axis and a direct interaction of atrazine and/or its metabolites with immune cells. Similar to JMPR (2009), EPA assessed that data indicating atrazine-induced immunotoxicity are not "a more sensitive endpoint than the atrazine-induced effects on neuroendocrine function".

More recently, a number of further studies in mice have been published demonstrating immunosuppressive effects of atrazine on lymphocytes as well as natural killer cells at oral doses as low as 25 mg/kg body weight (Zhang et al. 2011, Zhao et al. 2013, Chen et al. 2013). In vitro studies have helped to understand the mechanism of atrazine's immunosuppressive effects (Chen et al. 2015, Lee et al. 2016) and described that the observed effects were more pronounced in cells from male animals (Thueson et al. 2015), similar to the results seen earlier in studies with rats (Rooney et al. 2003) and mice (Rowe et al. 2006).

3
Endocrine disruption

The endocrine disrupting effects of atrazine on the HPA axis are well-known. They have been summarized by EPA (2010), where the cascade of key events was described as follows:

Hypothalamic changes resulting in an increased release of corticotropin-releasing hormone (CRH), resulting in an increased release of adrenocorticotropin hormone (ACTH) from the pituitary. This, in turn, leads to an increased production of coticosterone and progesterone by the adrenals. Finally, a decrease in the release of the gonadotropin-realeasing hormone (GnRH) from the hypothalamus occurs, which can be the result of one or all HPA changes described above.

It should be noted that other interferences with the endocrine system (via the GPR30-receptor, reviewed in Section 2.2) are also known.

JMPR did not recognize endocrine disruption as a specific hazard. In its list of "Critical endpoints for setting guidance values for exposure to atrazine" neuroendocrine action is mentioned in the category of "Other toxicological studies" JMPR (2009, p. 117). Nevertheless, JMPR used a 6-month study in Sprague-Dawley rats with an endocrine endpoint (estrous cycle alteration, suppression of the LH surge) as the study of reference for determining the lowest NOAEL to derive the ADI. This NOAEL was at a dietary concentration of 25 ppm corresponding to a dose of 1.8 mg/kg body weight (JMPR 2009, p. 91). As described in more detail below (Section 6, Low Dose Effects) disruption of the estrous cycle was also seen in pigs at doses as low as 1 mg/kg body weight (Gojmerac et al. 1999), whereas the lowest effect level in the rat study mentioned above was 3.65 mg/kg body weight (resulting from a dietary concentration of 50 ppm).

The European Commission had already listed atrazine as an endocrine disruptive compound of high concern several years earlier (European Commission 2000) and banned atrazine in 2003 because of the inability to keep levels in groundwater below the maximum residue limit of 0.1 µg/liter (European Commission 2003). Likewise, U.S. EPA stated in its Atrazine Chemical Summary, "Studies thus far suggest that atrazine is an endocrine disruptor" (EPA 2007, p.1).

A large body of literature exists concerning the effects of atrazine on the hypothalamo-pituitary-gonadal axis. Its effects on gonadal development in fish, amphibians and/or reptiles were briefly reviewed by Kortenkamp et al. (2011) and extensively reviewed by Rohr and McCoy (2010). In their review it was shown that in fish and amphibians atrazine affected one or more endpoints of gonadal morphology in 7 of 10 studies, altered sex hormone levels in 6 of 7 studies, and spermatogenesis in 2 of 2 studies. In addition, size, motor activity, immune function, olfaction and certain types of behavior were affected. Some of the effects were seen at concentrations between 2 and 0.1 µg/liter water. While such concentrations are environmentally highly relevant, extrapolation to the human situation is difficult where exposure results from the consumption of contaminated water or food and from occupational activities. Atrazine's endocrine disrupting properties were demonstrated across all five taxonomic classes of

vertebrates (Hayes et al. 2011), and, therefore, must be considered a very robust effect.

The most recent and comprehensive review of reproductive dysfunction caused by atrazine's interference with the hypothalamo-pituitary-gonadal axis was prepared by Wirbisky and Freeman (2015). These authors summarized the results of more than 130 publications, analyzing the evidence separately for males and females in non-mammalian and mammalian species, including the cellular and genetic mechanisms underlying the effects observed. In addition, they evaluated the evidence from 10 epidemiological publications. One recurring conclusion throughout this review was that inconsistent findings could be due to their ultimate dependence on treatment levels and/or duration. This has been thoroughly demonstrated for atrazine's effect on testicular steroidogenesis. While treatment of peripubertal rats for 1-3 days stimulated testosterone synthesis, testicular steroidogenesis was inhibited after treatment for 27 days (Pogrmic-Majkic et al. 2016). Such seemingly contradictory results were explained by Pogrmic-Majkic et al. (2016) with different signaling pathways that regulate steroidogenesis in opposing directions depending on dose and duration of treatment. With regard to atrazine's disruption of the ovarian function, Wirbisky and Freeman (2015) discuss the existence of two complementary mechanisms, i.e. the elevation of progesterone and a decrease in the expression of luteinizing hormone receptors in granulosa cells. It should be noted that the FIFRA Scientific Advisory Panel, during its July 26-28, 2011 meeting, expressed criticism that the "EPA Issue Paper[14] focused almost exclusively on a single mechanism of action, the neuroendocrine pathway and suppression of the LH surge, which largely has relevance only to some reproductive outcomes and a few other potential outcomes" (SAP 2011).

The potential human health consequences of the atrazine effects on the hypothalamo-pituitary-gonadal axis shown in laboratory studies were also demonstrated in three epidemiological studies.

Swan et al. (2003) analyzed the metabolite atrazine mercapturate in urine as a biomarker and categorized the participants in this study into "cases" (poor semen quality) and "controls" (normal semen quality). For those with no detectable atrazine mercapturate in urine, 17 of 41 subjects represented "cases", compared to 9 out of 10 cases for participants with detectable atrazine mercapturate levels, yielding a statistically significant odds ratio of 11.3 (95% CI: 1.3-98.9).

Cragin et al. (2011) performed a population-based case control study comparing menstrual cycle irregularities and hormone levels (luteinizing hormone, estradiol, progesterone) for 18-40 year old women residing in communities with extensive atrazine use (counties in Illinois, U.S., estimated tap water level: 0.7 ppb) versus sparse use (counties in Vermont, U.S., estimated tap water level: 0.4 ppb). It should be noted that, according to the authors, the year of data collection (2005) was a year of untypically low use of atrazine in the Illinois area as compared to the years before and after. While statistical significance was rarely reached in the various comparisons, levels for all hormones were consistently lower in Illinois women. Menstrual cycle length irregularities were significantly associated with residence in Illinois (OR=4.69; 95% CI: 1.58-13.95) and the consumption of more than 2 cups (474 ml) of unfiltered water per day (OR=5.73; 95% CI: 1.58-20.77). Although the results were considered preliminary, the authors concluded that exposure to atrazine in municipal drinking water at levels below the U.S. EPA maximum contamination limit for chronic exposure of 3 ppb "is associated with menstrual cycle length irregularity, reduced reproductive hormone levels and longer follicular phase in women" (Cragin et al. 2011, p. 1300).

Namulanda et al. (2017) investigated the relationship between the occurrence of early menarche (before 11.5 years of age) and in utero exposure to atrazine as assessed by diaminochlorotriazine (DACT) concentrations in maternal gestational urine samples. While no statistical significance was found in the total cohort for an association between DACT levels in maternal urine and the occurrence of early menarche, the association was statistically significant in the subset of girls with complete confounder information (OR=1.86; 95% CI: 1.03-3.38). The observation of an early menarche seems to contradict the delayed pubertal development seen in rats (cf. Rayner et al. 2004). However, the consideration by Wirbisky and Freeman (2015) should be kept in mind that inconsistent findings could be due to their ultimate dependence on treatment levels and/or duration. While the effects in laboratory rats were only seen at doses of 30 mg/kg body weight or higher (Ashby et al. 2002, Laws et al. 2003, Rayner et al. 2004), exposure

in humans (Namulanda et al. 2017) was definitely lower.

Collectively, these findings underscore the difficulties of defining a "safe dose" of atrazine for humans. Like other endocrine disrupting compounds, a number of atrazine effects seem to lack the traditional monotonic dose-response. In accordance with the application of the precautionary principle, this justifies the use of endocrine-disruption as a hazard-based cut-off criterion for atrazine, or at least the use of an additional uncertainty factor.

4 Reproductive toxicity

JMPR (2009) assessed atrazine's potential for reproductive toxicity based exclusively on regulatory studies submitted by industry. It acknowledged "reduced body-weight gain in pups at parentally toxic doses" (lowest relevant NOAEL 3.6 mg/kg) and "increased resorptions and incomplete ossification at maternally toxic doses; delayed sexual development" (lowest relevant NOAEL 5 mg/kg). It did not assess academic studies on reproductive toxicity. JMPR concluded that atrazine is not teratogenic and that developmental effects in rats and rabbits were observed only at maternally toxic doses.

In contrast, several authorities have classified atrazine as a reproductive toxin: the Japanese Government updated its GHS classification and classified atrazine as a category 2 reproductive toxin[15]; effective 15 July 2016, the Californian Office of Environmental Health Hazard Assessment (OEHHA) listed atrazine as a reproductive toxicant[16] and the U.S. EPA concluded that exposure to atrazine (and other triazines) "results in reproductive and developmental effects in laboratory animals that are considered relevant to humans" (EPA, 2018, p.7). EPA (2018) identified the disruption of the estrus cycle as the most sensitive endpoint with NOAELs in rats of 1.8 mg/kg (Morseth et al. 1996, cited by EPA, 2008) and 1.56 mg/kg (Cooper et al, 2010, cited by EPA, 2008). Moreover, it seems to be fair to conclude that the animal tests conducted seem to underestimate the risk of reproductive effects, because a number of epidemiological studies demonstrated statistically significant associations between atrazine exposure and detrimental reproductive effects.

Although the JMPR report contained a chapter on "Observations in humans", it dealt exclusively with carcinogenicity. JMPR completely ignored epidemiological studies relating to birth defects and adverse pregnancy outcomes. However, at the time of JMPR's September 2007 meeting, 10 epidemiological studies on the association between atrazine exposure and birth defects or altered pregnancy outcomes had already been published. Three of them described a statistically significant association between atrazine exposure and birth defects (Gary et al. 1996, Mattix et al. 2007, Munger 1997 et al.), and 2 of them described statistically significant adverse pregnancy outcomes (Savitz et al. 1997, Munger et al. 1997).

After the 2007 JMPR meeting further epidemiological studies were published on the reproductive effects of atrazine. All studies published before May 2013 were summarized in a Syngenta-sponsored review. The authors concluded that "claims about a causal link between ATR[17] and adverse pregnancy outcomes[18] are not warranted" (Goodman et al. 2014, p. 231). Contrary to the facts (see Table 4, Table 5), they claimed that the epidemiologic evidence was largely negative. While formally in line with the criteria of a systematic review, the review was biased. For instance, it questioned the relevance of a high-quality study (Chevrier et al. 2011)[19] "because most of the samples for that study were collected after ATR was banned in France" (Goodman et al. 2014, p. 230) - an irrelevant statement, because the authors referred to urine concentrations in pregnant women, not to atrazine use data. Another bias consisted of using studies that dealt with triazines in general instead

of atrazine: some of these studies explicitly investigated other triazines, but not atrazine (Rull et al. 2006) or mixed the results for cyanazine (not part of the chlorotriazine class) and atrazine (Weselak et al. 2008). Remarkably, 3 out of 4 of these studies showed no effect (Dabrowski et al. 2003; Rull et al. 2006; Weselak et al. 2008), thereby helping to create the impression that the epidemiological results for an association between atrazine and birth defects/adverse pregnancy outcomes were inconclusive.

BIRTH DEFECTS

Table 4 summarizes the available epidemiological publications on potential associations between atrazine and birth defects. It should be noted that the transplacental transfer of atrazine from the mother to the fetus has been demonstrated in animal studies. Model simulations suggest that the fetus is exposed to atrazine and its main metabolite didealkylatrazine to the same extent as the mother (Lin et al. 2013).

Statistically significant positive associations were reported for abdominal wall defects, cardiac defects, choanal atresia/stenosis, early menarche, limb defects and urogenital defects. Epidemiological studies on pesticides have advantages and disadvantages one should be aware of. The major advantage compared with studies in laboratory animals is that the extrapolation of results to a different species (typically allowed for with an uncertainty factor of 10) is unnecessary. A significant disadvantage is that while such studies deal with "real life exposures", exposure estimates are often difficult and imprecise. In the case of atrazine, this was one of the more frequently encountered problems, potentially introducing exposure misclassification resulting in allocating the study participants to the wrong group (Weselak et al. 2007, Goodman et al. 2014). However, it should be acknowledged that such misclassifications can go in both directions, i.e. overestimation and underestimation. Further, if independent studies have different types of methodological weaknesses but yield the same result, it is not very likely that the result was a false positive.

These considerations need to be taken into account with regard to the significant association between abdominal wall defects and atrazine that were identified in three independent studies (Mattix et al. 2007, Waller et al. 2010, Agopian et al. 2013a). Likewise, two studies identified a statistically significant association between urogenital defects and atrazine (Agopian 2013c and Munger et al. 1992), while in two others the association was not significant (Meyer et al. 2006, Chevrier et al. 2011). But even one of the two negative studies, while not being statistically significant, demonstrated an increased risk (see Table 4). An increased risk was also identified in two studies for limb defects (Munger et al. 1992, Ochoa-Acuña and Carbajo 2009).

In conclusion, from the available epidemiological literature, the strongest evidence exists for an association between prenatal atrazine exposure and abdominal wall defects, although other birth defects should also be taken into consideration.

ADVERSE PREGNANCY OUTCOMES

Reports about adverse pregnancy outcomes (e.g. low body weight, preterm delivery, small head circumference) are summarized in Table 5. Eight of the 9 studies on possible associations between adverse pregnancy outcomes and atrazine showed one or several statistically significant associations with atrazine. Most frequently, one or the other form of fetal growth retardation was identified (Munger et al. 1997, Villanueva et al. 2005, Ochoa-Acuña et al. 2009, Chevrier et al. 2011, Almberg et al. 2018), while two other studies did not identify a significant association with atrazine exposure for this end point (Savitz et al. 1997, Stayner et al. 2017). A similar number of studies reported a positive association between atrazine exposure and preterm birth (Savitz et al. 1997, Rinsky et al. 2012, Stayner et al. 2017), while no association was identified by Villanueva et al. (2005), Ochoa-Acuña et al. (2009), and Albouy-Llaty et al. (2016). Other endpoints (small head circumference, abortion) were less frequently investigated.

CONCLUSION

With regard to the WHO guideline value of 100 µg/liter atrazine in drinking water, it should be noted that a number of epidemiological studies have shown positive associations between atrazine concentrations in water and reproductive effects, where the "high" exposure groups were one or several orders of magnitude lower than this WHO guideline value. More specifically, high exposure was defined as ≥0.08 µg/liter drinking water in the Rinsky et al. (2012) study, as 2.1 µg/liter drinking water (median value) in the Munger et al. (1997) study, and as > 3

µg/liter surface water in the Waller et al. (2010) study. Mattix et al. (2007) correlated incidences of abdominal wall defects with surface water concentrations, where peak values were 11 µg/liter. Villanueva et al. (2005) compared adverse pregnancy outcomes of "high" vs. "low" season. The high season was characterized by raw water concentrations (geometric mean) of between 0.06 and 0.1 µg/liter.

In other words, these epidemiological studies showed statistically significant effects at tremendously lower concentrations than the WHO guideline value.

The only conclusion possible from these studies is that the WHO guideline value (100 µg/liter) cannot be considered as "safe". The association of abdominal wall defects, urogenital defects and limb defects seen in human fetuses/newborns with atrazine exposure warrant the use of an additional safety factor of at least 10.

5
Other non-cancer observations in humans

Further epidemiological studies have been published in recent years investigating possible immunological and neurological effects.

PARKINSON'S DISEASE

In a study analyzing the association between Parkinson's disease and groundwater levels of pesticides, the authors calculated that for every 1 µg/liter of pesticide the risk of Parkinson's disease increased by 3% (James and Hall 2015). Four pesticides were taken into consideration in this study: atrazine, simazine, alachlor and metolachlor. The mean atrazine level was 0.14 µg/liter (ranging from 0.0005 to 10 µg/liter) as compared to an average concentration of 0.17 µg/liter for all 4 pesticides together. In other words atrazine exposure was the absolutely dominating pesticide in this investigation, contributing to more than 80% of the average residue burden.

The underlying mechanism of Parkinson's disease is a disturbance and gradual destruction of the brain's nigrostriatal dopaminergic system. Remarkably, atrazine-induced effects in the dopaminergic system were demonstrated in experiments after oral administration to rats (Bardullas et al. 2011, Bardullas et al. 2013, Li et al. 2014a, 2014b, 2015, Song et al. 2015, Walters et al. 2015) and mice (Coban and Filipov 2007, Lin et al. 2013, 2014).

The fact that the association between atrazine and Parkinson's disease (identified at groundwater concentrations in the 1 ppb-range) was corroborated by the findings of reduced concentrations of dopamine in the nigrostriatal system in rats and mice can be considered as an alert concerning the WHO guidance value for atrazine in drinking water. At the same time, this epidemiological finding underscores the relevance of the effects seen in animal studies.

RHEUMATOID ARTHRITIS

As part of the Agricultural Health Study, a recent analysis investigated possible associations between pesticide exposure and the risk for rheumathoid arthritis in licensed male pesticide applicators (Meyer et al. 2017). These authors found a "robust" dose-response association with atrazine (OR = 1.62 for the third tertile, 95% CI: 1.09, 2.40) and discussed this finding in the context of immunotoxic effects of atrazine seen in animal studies (see Section 2.3.3., above), including the observation of a dose-dependent increase of apoptotic leukocytes in mice occurring concomitantly with an atrazine-induced apoptosis of splenocytes (Zhang et al. 2011).

6
Low-dose effects

The WHO current guideline value of 100 ppb is based on a NOAEL of 1.8 mg/kg derived from a 6-month rat study. In this study a disruption of the estrous cycle subsequent to a suppression of the surge of the luteinizing hormone was observed at 3.6 mg/kg. JMPR failed to take into consideration the studies in female pigs by Gojmerac et al. (1996, 1999). There, a disruption of the estrous cycle was demonstrated at 2 mg/kg body weight when pigs were fed with atrazine for 19 days (Gojmerac et al. (1996), an observation that was replicated in a similarly designed study at 1 mg/kg body weight (Gojmerac et al. 1999). The effect at 1 mg/kg was again replicated in a later study (Gojmerac et al. 2004), which also elucidated the mode of action by showing the relationship between disruption of the pulsatile release of the gonadotropin-releasing hormone and the attenuation of the surge of the luteinizing hormone. Though these pig studies were not regulatory studies, the findings are highly relevant, and their replication adds weight to the evidence that a NOAEL is to be expected at an unknown dose clearly below 1 mg/kg body weight. Assuming a dose spreading at least by factor 3, which would be the typical best case in regulatory studies, a putative NOAEL of 0.3 mg/kg could be assumed for the Gojmerac et al. (1999, 2004) studies.

In a well-designed, comprehensive study Enoch et al. (2007) investigated the effect of atrazine and four of its metabolites (designated AMM) on reproductive endpoints in offspring after oral (gavage) treatment of pregnant Long Evans rats from gestational day 15-19. It should be noted that this study built on previous work (Rayner et al. 2005). The most prominent effect was a dose-dependent, statistically significant delay in mammary gland development investigated on postnatal days 4, 25, 33, 40 and 60. On most of the postnatal days the effects were dose-dependent. The dose of 0.09 mg/kg body weight was the lowest dose at which a statistically significant delay was observed. This mixture contained 25% atrazine (corresponding to 22.5 µg/kg body weight), and was designed in proportions and at levels reported in a survey of ground and surface water (Enoch et al. 2007). Other doses tested were 0.87 mg/kg AMM, 8.73 mg/kg AMM and 100 mg/kg atrazine. No NOAEL was reached in this study. The delay in mammary gland development (using microscopic preparations) was independently scored by two separate persons. A dose-dependent effect was seen (in different offspring) on postnatal days 33, 40 and 60.

JMPR dismissed this important study, using evidently false or unscientific arguments and ignoring *Codex Alimentarius* guidance, which is supposed to govern its work. First of all, it is wrong to state that "it was not reported whether the scoring was done blind" (JMPR 2009, p. 99). In the paper it is clearly stated that "two individuals *without knowledge of the treatment group scored*" the preparations (Enoch et al. 2007, p. 543, emphasis added).

Secondly, while JMPR acknowledged the existence of a dose-response relationship, it cautioned that this "relationship appears flat or variable". Terminal end bud (TEB) scores for mammary gland development is an endpoint of endocrine disruption. As with many other endpoints, biological variability is not uncommon and should not be used to dismiss

an effect that was replicated in different animals on three different postnatal days. Besides, for the TEB scores on postnatal day 40 and 60 the effects are not variable at all (Table 3).

JMPR (2009) stated that it is uncertain what adverse consequences or functionally relevant toxic effects could be associated with the morphological changes seen in the study by Enoch et al. (2007). However, according to a more recent review paper on screening for chemical contributions to breast cancer risk, TEB scores represent a relevant endpoint for hazard identification of breast carcinogens (Schwarzman et al. 2015). In addition, Rudel et al. (2011) pointed out that in the U.S. alone an estimated 3-6 million mothers per year are unable to produce milk or have difficulty with breast-feeding. Altered mammary gland development is considered one of the reasons.

In direct violation of the precautionary principle laid down in Appendix IV of the procedural manual of the Codex Alimetarius Commission (2003), JMPR concluded: "Further work is needed to clarify and repeat these observations before concluding that exposure to a mixture of atrazine and its metabolites can cause alterations in development of the mammary gland at doses as low as 0.09 mg/kg bw per day, which would lead to an adverse public health consequence" (JMPR 2009, p. 99). This "further work" was actually mentioned in the paper by Enoch (2007), because they referred to such "studies being under way", but obviously they have never been published.[20] Taking the findings at 0.09 mg/kg seriously, the precautionary principle should be applied until these important follow-up investigations have been completed. The lowest-observed-adverse effect level (LOAEL) of 0.09 mg/kg should only be abandoned if the findings by Enoch et al (2007) are proved to be of no concern. A recent review criticized the EPA for failing to take these findings into consideration and expressed concern "that the EPA assessments are not protective for mammary gland effects" (Rodgers et al. 2018).

Four years after Enoch et al. (2007), a Syngenta-sponsored study was published (Hovey et al. 2011). They assessed the same endpoint (mammary gland development), also using Long Evans rats, but employed quantitative morphometric analyses, while Enoch et al. (2007) "relied on a holistic assessment of the mammary tissue" (EPA 2011, p. 31). In the Hovey et al. (2011) study no treatment-related effects were seen. It should be noted that they used atrazine whereas Enoch et al. (2007) used the mixture of atrazine and metabolites as mentioned above. Finally, Davis et al. (2011) conducted a study with a similar design and applied both quantitative morphometric analyses and the semi-quantitative scoring system employed by Enoch et al. (2007). No treatment-related effects on mammary gland development were seen in this study either, regardless of the method of assessment. However, in this study Sprague Dawley rats were used, while Enoch et al. (2007) and Hovey et al. (2011) used Long Evans rats. Further, atrazine was used as a single compound, not as a mix of atrazine and metabolites. Therefore both subsequent studies (Hovey et al. 2011 and Davis et al. 2011) were not suited to replicating (or dismiss) the findings by Enoch (2007).

JMPR (2009) failed to review a paper showing neurodegenerative changes in offspring (striatal and cortical brain regions) from pregnant/lactating CD-1 mice that were treated orally with atrazine from gestational day 14 to postnatal day 21, when the pups were weaned (Giusi et al. 2006). At a dose of 0.1 mg/kg body weight neuronal damage was demonstrated histopathologically in female but not in male offspring in the hippocampal region. The observed sexual dimorphism concurred with sex-dependent differences in the mRNA expression in this brain region. It should be noted that EPA (2010) identified methodological flaws in this study and criticized besides other things the bad quality of the micrographs used in the publication to demonstrate neuronal damage.

In a similarly designed study (CD-1 mice receiving 0.001 or 0.1 mg/kg body weight from gestational day 14 to postnatal day 21), statistically significant behavioral changes (a "feminization" of behavior), effects on sperm quality and on testosterone metabolism were shown in offspring (Belloni et al. 2011). In yet another study, pregnant mice were exposed to atrazine via drinking water (3 mg/L) from gestational day 6 to offspring's postnatal day 23. The average dose estimated from body weight development and water consumption was 1.4 mg/kg. Statistically significant behavioral changes were seen in both pregnant dams (on gestational day 21) and offspring (different tests, performed on postnatal day 35 or 70). These effects were accompanied by neurochemical

changes in the striatum and other brain regions in dams and offspring (Lin et al 2014).

In summary, atrazine effects at doses below the NOAEL used to determine the WHO guideline value have been shown in three different mammalian species.

7
General conclusions

The JMPR (2009) evaluation of atrazine is insufficient, incomplete and, with regard to important aspects, not up-to-date. New and/or neglected evidence includes insights into the mode of action of atrazine, epidemiological studies on cancer, birth defects, adverse pregnancy outcomes, and atrazine effects at doses lower than the lowest NOAEL used by JMPR to derive the ADI. Non-cancer observations in humans were not taken into account.

New insights from epidemiological and mechanistic studies support possible associations between atrazine exposure and thyroid cancer, ovarian cancer, NHL and hairy-cell leukemia. Of particular concern with regard to thyroid and ovarian cancer is recently published evidence that atrazine can activate estrogenic pathways through the non-genomic receptor GPR30. This mechanism provides plausibility for the observed 4.84-fold increase in thyroid cancer risk seen in the AHS study.

In addition, it represents the alternative mode of action which EPA claimed in 2011 to be non-existent, thereby strengthening the "weakly suggestive" association between atrazine and ovarian cancer acknowledged by EPA. Newer publications on genotoxicity, oxidative stress and immunotoxicity offer potential mechanisms to explain the development of lymphohematopoietic cancers (NHL, hairy-cell leukemia) that were reported in several epidemiological studies.

As described in Section 6 of this report, atrazine affects endpoints not covered by regulatory studies at doses below the NOAEL of 1.8 mg/kg body weight used by JMPR to calculate the ADI. This relates to studies in pigs showing disruption of the estrous cycle at 1 mg/kg body weight, a delay in mammary gland development of Long Evans rats at 22.5 µg/kg atrazine body weight (administered together with a representative mix of atrazine metabolites at a total dose of 90 µg/kg), and possibly neurodegenerative changes in CD-1 mice after repeated oral administration of 1 or 100 µg/kg atrazine to dams during pregnancy and early postnatal development.

Non-cancer epidemiological studies demonstrated associations between atrazine and Parkinson's disease, adverse pregnancy outcomes, menstrual cycle irregularities and rheumatoid arthritis.

Taken together, important new insights have accumulated since JMPR's evaluation of atrazine in 2007 (JMPR 2009), concerning cancer epidemiology and related mechanistic evidence, and also low dose effects on several endpoints in independent studies, and non-cancer epidemiological studies. Atrazine is a known endocrine disrupting compound. Therefore, the results of epidemiological studies demonstrating adverse pregnancy outcomes and effects on reproductive endpoints are particularly alarming.

It should be noted that:

Applying the precautionary principle laid down in the Codex Alimentarius, the NOAEL used for the calculation of the WHO guideline value for the maximum tolerable atrazine concentration in drinking water should be revised. Appendix 1 contains several specific considerations for the necessary revision of the current guideline value for drinking water of 100 ppb (WHO 2011a).

The elimination of atrazine would be beneficial not only for human and environmental health, but also in economic terms. Based on calculations conducted for the United States context, its ban would prompt the development of more sustainable agricultural practices and increase farmers' revenues, while the impact on consumer prices would be in the range of pennies (Ackerman et al. 2014).

In summary, this report is an urgent call for a thorough re-review of the data on hazard assessment of atrazine, taking into consideration new evidence as well as studies ignored or misinterpreted during JMPR's review (JMPR 2009).

8 References

Abarikwu, S.O. et al. (2014): Toxicological Mechanisms and Methods 25: 70–80; DOI: 10.3109/15376516.2014.989349

Abass, M.A. et al. (2016): Environmental Science and Pollution Research; DOI: 10.1007/s11356-016-6637-x

Ackerman, F. et al. (2014): International Journal of Occupational and Environmental Health 20: 61-70: DOI: 10.1179/2049396713Y.0000000054

Adesiyan, A.C. et al. (2011): Experimental and Toxicologic Pathology 63: 201–207; DOI: 10.1016/j.etp.2009.11.008

Adeyemi, J.A. et al. (2015): Comparative Biochemistry and Physiology, Part C; DOI: 10.1016/j.cbpc.2015.04.001

Agopian, A.J. et al. (2013a): Maternal and Child Health Journal; DOI 10.1007/s10995-012-1196-3

Agopian, A.J. et al. (2013b): Journal of Pediatrics 162: 581–586

Agopian, A.J. et al. (2013c): American Journal of Medical Genetics Part A 161A: 977–982

Alavanja, M.C.R. et al. (2005): Scandinavian Journal of Work Environment and Health 31, Supplement 1:39–45

Albanito, L. et al. (2015): Environmental Health Perspectives 123: 493-499; DOI: 10.1289/ehp.1408586

Albouy-Llaty, M. et al. (2016): International Journal of Environmental Research and Public Health 13: 796; DOI:10.3390/ijerph13080796

Almberg, K.S. et al. (2018): International Journal of Environmental Research and Public Health 15: 1889; DOI:10.3390/ijerph15091889

Ashby, J. et al. (2002): Regulatory Toxicology and Pharmacology 35**:** 468–473, DOI:10.1006/rtph.2002.1571

Bardullas, U. et al. (2011): Neurotoxicology and Teratology 33: 263–272; DOI: 10.1016/j.ntt.2010.09.001

Bardullas, U. et al. (2013): Neurotoxicology and Teratology 40: 59–66; DOI: 10.1016/j.ntt.2013.10.003

BAuA (2016): Federal Institute for Occupational Safety and Health. Proposal for harmonized classification and labelling. Substance name: N-phosphonomethyl)glycine; Glyphosate (ISO), https://www.echa.europa. eu/ documents/10162/23665416/clh_glyphosate_rcom_6621_en.pdf/df1f047b-478d-daca-02b1-66e559da42ab

Beane Freeman et al. (2011): Environmental Health Perspectives 119:1253–1259; DOI: 10.1289/ehp.1103561

Belloni, V. et al. (2011): Toxicology 279: 19–26; DOI: 10.1016/j.tox.2010.07.002

Bergman, Å. et al., eds. (2013): State of the science of endocrine disrupting chemicals 2012, ISBN: 978-92-807-3274-0 (UNEP); 978-92-4-150503-1 (WHO)

Bhatti, J.S. et al. (2011): Molecular and Cellular Biochemistry 353:139–149; DOI: 10.1007/s11010-011-0780-y

Blahova, J. et al. (2013): Food and Chemical Toxicology 61: 82–85; DOI: 10.1016/j.fct.2013.02.041

Blomberry, P.A. et al. (2012): Haematologica 97:780-783; DOI: 10.3324/haematol.2011.054874

Bode, G. et al. (2010): Journal of Pharmacological and Toxicological Methods 62: 196–220. DOI: 10.1016/j.vascn.2010.05.009

Bofetta, P. et al. (2013): European Journal of Cancer Prevention 22:169–180; DOI: 10.1097/CEJ.0b013e32835849ca

Campos-Pereira, F.D. et al. (2012): Ecotoxicology and Environmental Safety 78: 170–177; DOI: 10.1016/j.ecoenv.2011.11.

Cavas, T. (2011): Food and Chemical Toxicology 49: 1431–1435; DOI: 10.1016/j.fct.2011.03.038

Chen, J.Y. et al. (2013): Journal of Environmental Science and Health, Part B 48: 637–645; DOI: 10.1080/03601234.2013.777308

Chen, J. et al. (2015): Food and Chemical Toxicology 76: 61–69; DOI: 10.1016/j.fct.2014.11.026

Chevalier, N. et al. (2016): Environmental Health Perspectives 124: A64

Chevrier, C. et al. (2011): Environmental Health Perspectives 119: 1034–1041

Clausing, P. et al. (2018): J Epidemiol Community Health; DOI:10.1136/jech-2017-209776

Clavel, J. et al. (1996): Scandinavian Journal of Work Environment and Health 22:285-93; DOI:10.5271/sjweh.143

Coban, A. and Filipov, M.N. (2007): Journal of Neurochemistry 100, 1177–1187; DOI: 10.1111/j.1471-4159.2006.04294.x

Codex Alimentarius Commission (2003): Procedural Manual, Thirteenth edition, Appendix IV. Working Principles for Risk Analysis for Application in the Framework of the Codex Alimentarius, ISSN 1020-4091

Connor, K. et al. (1996): Fundamental and Applied Toxicology 30, 93-101

Cragin, L.A. et al. (2011): Environmental Research 111: 1293–1301; DOI: 10.1016/j.envres.2011.09.009

Dąbrowski, S. et al. (2003): International Journal of Occupational Medicine and Environmental Health 16: 31-39

Donna, A. et al. (1984): Carcinogenesis 5: 941-942

Donna, A. et al. (1989): Scandinavian Journal of Work Environment and Health 15: 47-53; DOI: 10.5271/sjweh.1882

Enoch, R.R. et al. (2007): Environmental Health Perspectives 115: 541–547; DOI:10.1289/ehp

EPA (2007): Atrazine. Chemical summary. https://archive.epa.gov/region5/teach/web/pdf/atrazine_summary.pdf

EPA (2010): Re-Evaluation of Human Health Effects of Atrazine: Review of

Experimental Animal and *In vitro* Studies and Drinking Water Monitoring Frequency. April 26-30, 2010

EPA (2011): Re-Evaluation of Human Health Effects of Atrazine: Review of Cancer Epidemiology, Non-cancer Experimental Animal and In vitro Studies and Drinking Water Monitoring Frequency. EPA Issue Paper, Presented On: July 26-29, 2011

EPA (2018): Atrazine. Draft Human Health Risk Assessment for Registration Review, https://www.regulations.gov/document?D=EPA-HQ-OPP-2013-0266-1159

EU (2009): Regulation (Ec) No 1107/2009 of the European Parliament and of the Council of 21 October 2009

Concerning the Placing of Plant Protection Products on the Market and Repealing Council Directives 79/117/EEC and 91/414/EEC

EU (2016): Impact Assessment. Defining criteria for identifying endocrine disruptors in the context of the implementation of the plant protection products regulation and biocidal products regulation. Main Report. SWD(2016) 211 final

European Commission (2000): Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption. European Commission DG ENV, http://ec.europa.eu/environment/archives/docum/pdf/bkh_main.pdf

European Commission (2003): Review report for the active substance atrazine. SANCO/10496/2003-final

Ewalt, M. et al. (2011): Hematological Oncology DOI: 10.1002/hon.1023

Feldman, R.D. and Limbird, L.E. (2016): Annual Review of Pharmacology and Toxicology 57: 24.1–

24.18; DOI: 10.1146/annurev-pharmtox-010716-104651

Florian, C.P. et al. (2016): DOI: Biochemistry Research International; 10.1155/2016/2984081

Gao, S. et al. (2016): Oxidative Medicine and Cellular Longevity; DOI: 10.1155/2016/7978219

Garry, V.F. et al. (1996): Environmental Health Perspectives 104: 394-399.

Giusi, G. et al. (2006): Toxicological Sciences 89: 257–264; DOI: 10.1093/toxsci/kfj012

Gojmerac, T. et al. (1996): Toxicology Letters 85: 9-15

Gojmerac, T. et al. (1999): Acta Veterinaria Hungarica 47: 129-135

Gojmerac, T. et al. (2004): Veterinary and Human Toxicology 46: 245-247

Goncalves, M.W. et al. (2017): Chemosphere 182: 730-737; DOI: 10.1016/j.chemosphere.2017.05.078

Goodman, M. et al. (2014): Birth Defects Research (Part B) 101:215–236; DOI: 10.1002/bdrb.21101

Hayes, T.B. et al. (2011): Journal of Steroid Biochemistry and Molecular Biology 127: 64–73; DOI: 10.1016/j.jsbmb.2011.03.015

Hoar, S.K. et al. (1986): JAMA 256:1141-1147

Hoar Zahm, S. et al. (1993): Scandinavian Journal of Work Environment and Health 19:108-14

Hovey, R.C. et al. (2011): Toxicological Sciences 119: 380–390

IACHR (2017): Inter-American Court of Human Rights. Environment and Human rights. Advisory Opinion OC-23/17 of November 15, 2017

IARC (1991): Atrazine. In: Occupational Exposures in Insecticide Application and Some Pesticides. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 53. IARC Press, Lyon.

IARC (1999): Atrazine. In: Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances. Vol. 73, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. IARC Press, Lyon.

IARC (2015): IARC Monographs Questions and Answers. https://www.iarc.fr/en/media-centre/iarcnews/pdf/Monographs-Q&A.pdf

James, K.A. and Hall, D.A. (2015): International Journal of Toxicology 34: 266-273; DOI: 10.1177/1091581815583561

Jin, Y. et al. (2014): Environmental Toxicology and Pharmacology 37: 782–790; DOI: 10.1016/j.etap.2014.02.014

JMPR (2009): Pesticide Residues in Food – 2007. Joint FAO/WHO Meeting on Pesticide Residues. Atrazine, p. 37-138; ISBN 978 92 4 166523 0

Kortenkamp, A. et al. (2011): State of the Art Assessment of Endocrine Disrupters. Final Report; Project Contract Number 070307/2009/550687/SER/D3

Lappano, R. et al. (2016): The AAPS Journal 18: 305-310; DOI: 10.1208/s12248-016-9881-6

Laws, S.C. et al. (2003): Toxicological Sciences 76**:** 190–200

Lee, D.-J. et al. (2016): Environmental Toxicology; DOI: 10.1002/tox.22109

Li, Y. et al. (2014a): Environmental Toxicology and Pharmacology; DOI: 10.1016/j.etap.2014.04.023

Li, Y. et al. (2014b): Mutation Research/Genetic Toxicology and Environmental Mutagenesis 763: 23–29; DOI: 10.1016/j.mrgentox.2013.12.009

Li, Y.-S. et al. (2015): Birth Defects Research (Part B) 104:184–189; DOI: 10.1002/bdrb.21151

Lin, Z. et al. (2013): Life Sciences 92: 81–90; DOI: 10.1016/j.lfs.2012.10.027

Lin, Z. et al. (2014): Toxicological Sciences 141: 90–102; DOI: 10.1093/toxsci/kfu107

Luzhna, L. (2015): Mutation Research/Genetic Toxicology and Environmental Mutagenesis 779:39-56. DOI: 10.1016/j.mrgentox.2014.07.006

MacLennan, P. et al. (2003): Journal of Toxicology and Environmental Health, Part A: Current Issues 66:501–517; DOI: 10.1080/15287390306356

Mattix, K.D. et al. (2007): Journal of Pediatric Surgery 42: 947–949

Meyer, A. et al. (2017): Environmental Health Perspectives; DOI: 10.1289/EHP1013

Mills, P.K. (1998): Archives of Environmental Health 53:6, 410-413; DOI: 10.1080/00039899809605729

Monnereau, A. et al. (2014): Journal of the National Cancer Institute Monographs 48:115–124; DOI: 10.1093/jncimonographs/lgu004

Munger, R.G. et al. (1997): Environmental Health Perspectives 105: 308-314

Namulanda, G. et al. (2017): Environmental Research 156: 420–425

NCI (1980): Bioassay of reserpine for possible carcinogenicity, NCI Publication No.NCI-CG-TR-193, NTP Publication No. NTP-80-16. 1980, National Cancer Institute Carcinogenesis Testing Program.

Neuberger, J.S. (1996): Journal of Agromedicine 3: 9-30, DOI: 10.1300/J096v03n02_03

Nowsheen, S. et al. (2012): Current Molecular Medicine 12: 672-680

Nwani, C.D. et al. (2011): Environmental Toxicology and Pharmacology 31: 314–322; DOI:10.1016/j.etap.2010.12.001

Nwani, C.D. et al. (2014): Drug and Chemical Toxicology 37: 370–377; DOI: 10.3109/01480545.2013.866138

Ochoa-Acuña, H. and Carbajo, C. (2009): Science of the Total Environment 407: 4447–4451

Ochoa-Acuña, H. et al. (2009): Environmental Health Perspectives 117: 1619-1624

OECD (2012): Guidance document 116 on the conduct and design of chronic toxicity and Carcinogenicity studies, supporting test guidelines 451, 452 and 453. 2nd edition. France, Paris: OECD Publishing, 2012.

Orsi, L. et al. (2009): Occupational and Environmental Medicine 66:291–298; DOI:10.1136/oem.2008.040972

Phillips McDougall (2018): AgrAspire. Agribusiness Intelligence. https://agribusinessintelligence.informa.com/products-and-services/data-and-analysis/phillips-mcdougall/agraspire

Pogrmic-Majkic, K. et al. (2012): Environmental Toxicology and Pharmacology 34: 495-501; DOI: 10.1016/j.etap.2012.06.004

Pogrmic-Majkic, K. et al. (2016): Toxicology; DOI:10.1016/j.tox.2016.08.016

Rayner, J.L. et al. (2004): Toxicology and Applied Pharmacology 195 (2004) 23– 34

Rayner, J.L. et al. (2005): Toxicological Sciences 87: 255–266; DOI: 10.1093/toxsci/kfi213

Rinsky, J.L. et al. (2012): Public Health Reports 127: 72–80

Rodgers, K.M. et al. (2018): Environmental Research 160: 152–182; DOI: 10.1016/j.envres.2017.08.045

Rohr, J.R. and McCoy, K.A. (2010): Environmental Health Perspectives 118: 20–32

Rooney, A.A. et al. (2003): Toxicological Sciences 76: 366–375; DOI: 10.1093/toxsci/kfg250

Rowe, A.M. et al. (2006): Toxicology Applied Pharmacology 214: 69–77

Rudel, R.A. et al. (2011): Environmental Health Perspectives 119: 1053-1061; DOI: 10.1289/ehp.1002864

Ruiz-Guzman, J.A. et al. (2017): Mutation Research; DOI: 10.1016/j.mrgentox.2017.10.002

Rull, R.P. et al. (2006): American Journal of Epidemiology 163:743–753

Rusiecki, J.A. et al. (2004): Journal of the National Cancer Institute 96: 1375-1382; DOI: 10.1093/jnci/djh264

Sanderson, J.T. et al. (2001): Environmental Health Perspectives 109: 1027–1089

SAP (2011): SAP Minutes No. 2011-05; Re-evaluation of the Human Health Effects of Atrazine: Review of Non-Cancer Effects, Drinking Water Monitoring Frequency and Cancer Epidemiology; FIFRA Scientific Advisory Panel Meeting; July 26 – 28, 2011

Sass, J.B. and Colangelo, A. (2006): International Journal of Occupational and Environmental Health 12: 260-267

Sathiakumar, N. and Delzell, E. (1997): Critical Reviews in Toxicology 27:599-613

Savitz, D.A. et al. (1997): American Journal of Epidemiology 146: 1025–1032

Schwarzman, M.R. et al. (2015): Environmental Health Perspectives 123:1255–1264; DOI: 10.1289/ehp.1408337

Simpkins, J.W. et al. (2011): Toxicological Sciences 123: 441-459.

Singh, M. et al. (2008): Journal of Biochemistry and Molecular Toxicology 22: 363-369; DOI 10:1002/jbt

Singh, M. et al. (2011): Experimental and Toxicologic Pathology 63: 269–276; DOI: 10.1016/j.etp.2010.01.005

Smith, M.T. et al. (2016): Environmental Health Perspectives 124:713–721; DOI: 10.1289/ ehp.1509912

Son, H.Y. et al. (2003): Food and Chemical Toxicology 41: 1811–1816; DOI: :10.1016/S0278-6915(03)00234-5

Song, Y. et al. (2014): Biomedical and Environmental Sciences 27: 281-288; DOI: 10.3967/bes2014.050

Song, X.-Y. et al. (2015): International Journal of Molecular Sciences 16: 13490-13506; DOI: 10.3390/ijms160613490

Stayner, L.T. et al. (2017): Environmental Research 152: 294–303; DOI: 10.1016/j.envres.2016.10.022

Swan, S.H. et al. (2003): Environmental Health Perspectives 111: 1478-1484

Tennant, M.K. et al. (1994): Journal of Toxicology and Environmental Health 43: 197-211; DOI: 10.1080/15287399409531915

Tennant, A.H. et al. (2001): Mutation Research 493: 1–10

Thomas, P. and Dong, J. (2006): Journal of Steroid Biochemistry and Molecular Biology 102: 175–179; DOI: 10.1016/j.jsbmb.2006.09.017

Thueson, L.E. et al. (2015): Toxicological Sciences 143: 418–429; DOI: 10.1093/toxsci/kfu242

Tiacci, E. et al. (2011): New England Journal of Medicine 364: 2305-2315; DOI: 10.1056/ NEJMoa1014209

Torres, C. et al. (1992): Mutation Research 280: 291-295

Villanueva, C.M. et al. (2005): Occupational and Environmental Medicine 62: 400–405

Vivacqua, A. et al. (2006): Molecular Pharmacology 70:1414–1423; DOI: 10.1124/mol.106.026344

Waller, S.A. et al. (2010): American Journal of Obstetrics and Gynecology DOI: 10.1016/j.ajog.2010.01.023.

Walters, J.L. et al. (2015): Toxicology and Applied Pharmacology; DOI: 10.1016/j.taap.2015.09.026

Wang, X. et al. (2013): Food and Chemical Toxicology 62: 148–158; DOI: 10.1016/ j.fct.2013.08.044

Weselak, M. et al. (2007): Journal of Toxicology and Environmental Health, Part B: Critical Reviews, 10: 41-80

Weselak, M. et al. (2008): Reproductive Toxicology 25: 472–480

WHO (2003): Atrazine in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality, WHO/SDE/WSH/03.04/32, 10 pp.

WHO (2011a): Atrazine and Its Metabolites in Drinking-water, Background document for development of WHO Guidelines for Drinking-water Quality, WHO/HSE/WSH/10.01/11/Rev/1, 15 pp.

WHO (2011b): Guidelines for Drinking-water Quality, Fourth Edition; 541 pp. ISBN 978 92 4 154815 1

WHO (2017): Guidelines for Drinking-water Quality, Fourth Edition, Incorporating the First Addendum; 631 pp. ISBN 978-92-4-154995-0

Wirbisky, S.E. and Freeman, J.L. (2015): Toxics 3: 414-450

Wirbisky, S.E. et al. (2016): Food and Chemical Toxicology; DOI: 10.1016/j.fct.2016.03.027

Xing, H. et al. (2012): Chemosphere 88: 377–383; 10.1016/j.chemosphere.2012.02.049

Young, H.A. et al. (2005): Journal of Occupational and Environmental Medicine 47:1148–1156

Zhang, X. et al. (2011): BioMed Central Medicine 9:117

Zhao, S. et al. (2013): BioScience Trends 7:193-201; DOI: 10.5582/bst.2013.v7.4.193

Zhao, F. et al. (2014): International Journal of Clinical and Experimental Pathology 7: 2780-2789

Zhu, L. et al. (2010): Environmental Toxicology 26: 480–488; DOI 10.1002/tox.20575

Ziech, D. et al. (2010): Chemico-Biological Interactions 188: 334–339; DOI: 10.1016/j.cbi.2010.07.010

9
Tables

Table 1: Case-control studies with significant or borderline-significant associations between triazines (T) or atrazine (A), reviewed by JMPR (2009), including citations from review papers

Cancer type	Exposure*	No. of Exposed Cases/Controls	OR (95% CI)	Reference
NHL	T	59/1,245	1.6 (1.0-2.6)	Cantor et al. (1992) presented in review by Neuberger (1996)
NHL	A	27/64	1.2 (0.9-1.8)	Cantor et al. (1992) presented in review by Sathiakumar and Delzell (1997)
NHL	T	14/43	2.5 (1.2-5.4)	Hoar et al. (1986) presented in review by Sathiakumar and Delzell (1997)
NHL	A	130/249	1.4 (1.1-1.8)	Hoar Zahm et al. (1993)
NHL	T	Fate 2,213 persons of production plant analyzed by standardized mortality ratio	4 deaths observed vs. 1.1 expected	MacLennan et al. (2003) **
Ovarian	T	21/126	2.7 (1.0-6.9)*	Donna et al. (1989), presented in review by Neuberger (1996)

OR = Odds ratio expressing the increased risk (1.6 = 1.6-fold); CI = Confidence Interval (a lower limit at or above 1.0 is considered statistically significant. * The authors used a 10% CI. **JMPR cited from the publication that "one of the four decedents whose death certificate included a diagnosis of non-Hodgkin lymphoma had medical records including a biopsy report that indicated a diagnosis of poorly differentiated nasopharyngeal cancer. This case was not removed from our analysis." JMPR failed to cite the next sentence in this publication where the authors explain the scientific reasons why they did so.

Table 2: Summary of Non-Hodgkin lymphoma (NHL) data in epidemiological studies reviewed by EPA (2011). Studies assessing triazines (T) or atrazine (A) are mentioned, and the odds ratio (OR) is listed. The odds ratio is an estimate of the risk. For instance, an OR of 1.6 describes a 60% higher risk for exposed persons as compared to the control group. Note: A statistically significant association is identified when the lower limit of the confidence interval (CI), i.e. the first value in the parentheses, is 1.0 or greater.

Exposure*	Number of Exposed Cases/ Controls	OR (95% CI)	Remarks	Reference
T	14/43	2.5 (1.2-5.4)	Case-control study	Hoar et al. (1986)
T	59/1,245	1.6 (1.0-2.6)	Case-control study	Cantor et al. (1992)
A	130/249	1.4 (1.1-1.8	Case-control studies, meta-analysis	Hoar Zahm et al. (1993)
A	90/185	1.5 (1.0-2.2)	Case-control studies, meta-analysis	De Roos et al. (2003)
T	8/20	2.1 (0.8-5.0)	Case-control-study	Orsi et al. (2009)
T	20/17	1.75 (0.73-4.20)	Cohort-study	Rusiecki et al. (2004)
A	34/38	0.93 (0.58-1.50)	Cohort-study	Beane Freeman et al. (2011)

* A = atrazine, T = triazines

Table 3. Terminal end bud scores of mammary glands in Long-Evans rat offspring after oral (gavage) treatment of pregnant rats with a representative mixture of atrazine and atrazine metabolites from gestational day 15 to 19. Percent scores of vehicle treated controls (= 100%) are depicted. Note: different ages represent different animals.

Age (postnatal day)	0.09 mg/kg body weight/day	0.87 mg/kg body weight/day	8.73 mg/kg body weight/day
4	77	61	75
25	75	65	67
40	68	63	54
60	74	63	56

Table 4: Epidemiological studies concerning birth defects. OR = Odds ratio, RR = Rate ratio, CI = 95% Confidence Interval, USGS = U.S. Geological survey.

RESULTS STATISTICALLY SIGNIFICANT				
Endpoint	**Location, study period**	**Exposure assessment**	**Result**	**Reference**
All birth anomalies combined	Minnesota, 1989-1992	County clusters of pesticide use (high vs. low)	OR 1.13 (CI 1.04-1.24)	Garry et al. (1996)
Abdominal wall defects (AWD)	Indiana, 1990-2002	USGS data for atrazine levels in surface water (monthly averages)	Significant positive correlation (r=0.69. p=0.0125) between AWD rate and atrazine levels	Mattix et al. (2007)
	Washington State, 1987-2006	USGS data for atrazine levels in surface water, proximity to high atrazine areas (>3µg/L)	Gastroschisis: OR 1.60 (CI 1.10-2.34) for <25 km distance to high atrazine; OR 1.41 (CI 1.19-1.66) for 25-50 km to high atrazine	Waller et al. (2010)
	Texas, 1999-2008	Atrazine use estimated from USGS data (based on crop type and acreage) high vs. low use counties	Gastroschisis: OR 1.97 (CI 1.19-3.26) for newborn from mothers ≥ 25 years old	Agopian et al. (2013a)
Cardiac defects	U.S. 1983-1989	Residence in 18 communities with atrazine contamination: yes versus no	RR = 3.1 (2.1–4.6)	Munger et al. (1992), cited by Goodman et al. (2014)
Choanal atresia/stenosis	Texas, 1999-2008	Atrazine use (pounds per square mile) according to USGS data	Adjusted OR 1.79 (CI 1.17-2.74)	Agopian et al. (2013b)
Early menarche	Bristol area, UK, April 1991 to December 1992	Atrazine and metabolites in urine samples of mothers	OR 1.86 (CI 1.03-3.38) for diamino-chlorotriazine (DACT) for the subset with complete data	Namulanda et al. (2017)
Limb defects	U.S. 1983-1989	Residence in 18 communities with atrazine contamination: yes versus no	RR 6.9 (CI 4.2–11.0)	Munger et al. (1992), cited by Goodman et al. (2014)
	Indiana, birth records 01 May to 31 August for the years 2000 through 2004	Yearly determination of proximity to corn crops (living within 500 to at least 3.4 ha corn)	Adjusted OR 1.76 (CI 1.12-2.78)	Ochoa-Acuña & Carbajo (2009)

Table 4, continued

RESULTS NOT STATISTICALLY SIGNIFICANT				
Endpoint	**Population**	**Exposure assessment**	**Result**	**Reference**
Urogenital defects	Texas, 1999-2008	County level estimated atrazine exposure according to USGS data	Adjusted OR 1.20 (CI 1.11-1.29)	Agopian et al. (2013c)
	U.S. 1983-1989	Residence in 18 communities with atrazine contamination: yes versus no	RR = 3.5 (2.2–5.3)	Munger et al. (1992), cited by Goodman et al. (2014)
Congenital heart defects (CDH)	Texas, 1999-2005	Drinking water levels of atrazine (high vs. low contamination water districts)	Estimated atrazine exposure was not positively associated with CDH in general or any of the eight CDH subtypes assessed	Kim et al. (2017)
Male genital anomalies	Brittany region, France, 2002-2006	Atrazine and metabolites in urine samples of mothers	OR 1.4 (CI 0.6-3.2) for atrazine or one of its metabolites OR 2.3 (CI 0.6-8.4) for atrazine alone	Chevrier et al (2011)
Urogenital defects (hypospadia)	Arkansas, 1998-2002	Georeferenced pesticide use (no use vs. more than 1,63 kg use within 500 m of maternal residences during or persisting into the critical developmental window)	OR 1.02 (CI 0.58-1.79)	Meyer et al. (2006)

Table 5: Epidemiological studies concerning adverse pregnancy outcome. OR = Odds ratio, RR = relative risk, CI = 95% Confidence Interval.

RESULTS STATISTICALLY SIGNIFICANT				
Endpoint	**Location, study period**	**Exposure assessment**	**Result**	**Reference**
Preterm birth	Ontario, 1986	Questionnaires about individual **male** farm activities, "exposed" vs. "not exposed" groups	Adjusted OR 4.9 (CI 1.6-15) for yard use	Savitz et al. (1997)
	Kentucky, 2004-2006	Atrazine levels in drinking water, high (0.081 µg/L) vs. low (0 µg/L, i.e. below limit of detection)[21]	OR 1.22 (CI 1.16-1.29)	Rinsky et al. (2012)
	Indiana, Iowa, Missouri, Ohio, 2004-2008	Atrazine levels in drinking water, RR increase per 1 ppb atrazine increase	RR 1.10 (CI 1.01-1.20) for exposure during entire gestation	Stayner et al. (2016)
Small for gestational age, fetal growth restriction or low birth weight	Iowa, 1984-1990	Atrazine levels in drinking water, median value, high (2.1 µg/L) vs. low (0.7 µg/L)	RR 1.8 (CI 1.3-2.7) for high vs. low contamination of drinking water	Munger et al. (1997)
	Indiana, 1993-2007	Atrazine levels in drinking water during pregnancy, geocoded residence of mothers	Mean atrazine levels during entire pregnancy >0.644 µg/L versus < 0.1µg/L associated with higher adjusted prevalence rate 1.14 (CI 1.03-1.24)	Ochoa-Acuña et al. (2009)
	Brittany region, France, 2002-2006, live-born infants in hospital	Atrazine and metabolites in urine samples of mothers	OR 1.5 (CI 1.0-2.2)	Chevrier et al. (2011)
	Brittany, France, October 1997-September 1998	Atrazine levels in drinking water, matching pregnancy trimesters with high (May – September) and low (October – April) exposure periods	OR 1.37 (CI 1.04-1.81) for third trimester exposure	Villanueva et al. (2005)
Small head circumference	Brittany region, France, 2002-2006, live-born infants in hospital	Atrazine and metabolites in urine samples of mothers	OR 1.7 (CI 1.0-2.7)	Chevrier et al. (2011)

Table 5, continued

RESULTS NOT STATISTICALLY SIGNIFICANT				
Endpoint	**Location, study period**	**Exposure assessment**	**Result**	**Reference**
Abortion/ miscarriage	Ontario, 1986	Questionnaires about individual **male** farm activities, "exposed" vs. "not exposed" groups	Adjusted OR 1.2 (0.6-2.3) for yard use	Savitz et al. (1997)
Preterm birth	Brittany, France, October 1997-September 1998	Atrazine levels in drinking water, matching pregnancy trimesters with high (May – September) and low (October – April) exposure periods	All ORs (calculated separately for first, second and third trimester)	Villanueva et al. (2005)
	Indiana, 1993-2007	Atrazine levels in drinking water during pregnancy, geocoded residence of mothers	No significant association with atrazine exposure during first or last month of pregnancy. Mean atrazine levels varying between < 0.057 µg/L (low) and > 0.507 µg/L (high)	Ochoa-Acuña et al. (2009)
	Deux-Sèvres, France, 2005-2010	Drinking-water levels of atrazine metabolites, low (<0.013 µg/L), medium (0.013-0.020 µg/L) and high (> 0.020 µg/L)[22]	Adjusted OR 1.625 (CI 0.975-2.710) for high vs. low; all other comparisons not significant either	Albouy-Llaty et al. (2016)
Small for gestational age, fetal growth restriction or low birth weight	Ontario, 1986	Questionnaires about individual **male** farm activities, "exposed" vs. "not exposed" groups	Adjusted OR 0.5 (0.2-1.5) for yard use	Savitz et al. (1997)
	Indiana, Iowa, Missouri, Ohio, 2004-2008	Atrazine levels in drinking water, RR increase per 1 ppb atrazine increase	No significant association with atrazine exposure	Stayner et al. (2016)
	Ohio, 2006-2008	Atrazine levels in finished drinking water: 0.16-017 µg/L (median); 4.23-15.66 µg/L (annual maximum)	OR 1.27 (95% CI 1.10-1.45) for low term birth weight	Almberg et al. (2018)

10 Acronyms

ADI	Acceptable daily intake
AHS	Agricultural Health Study
AMM	Atrazine metabolite mixture
CI	Confidence interval
CRH	Corticotropin-releasing hormone
DACT	Diaminochlorotriazine
EPA	Environmental Protection Agency of the United States
FAO	Food and agricultural Organization of the United Nations
GnRH	Gonadotropin-releasing hormone
GRP30	G-protein coupled receptor 30
HCD	Historical control data
HPA axis	Hypothalamic-pituitary-adrenal axis
IARC	International Agency for Research on Cancer
JMPR	Joint Meeting on Pesticide Residues of the FAO/WHO
LH	Luteinizing hormone
LOAEL	Lowest-observed adverse effect level
NHL	NonHodgkin's lymphoma
NOAEL	No-observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
OR	Odds ratio
SAP	Scientific Advisory Panel
TDI	Tolerable daily intake
TEB	Terminal end buds
UNEP	United Nation's Environment Programme
WHO	World Health Organization of the United Nations

APPENDIX

CONSIDERATIONS FOR THE REVISION OF THE WHO GUIDELINE VALUE FOR ATRIZINE IN DRINKING WATER (CURRENTLY 100 PPB)

EVIDENCE THAT REQUIRES AN ADDITIONAL SAFETY FACTOR

NEW EVIDENCE CONCERNING CARCINOGENICITY

A significantly increased risk was identified as part of the AHS study for the association of thyroid tumors and atrazine exposure. Furthermore, EPA acknowledged a "weakly suggestive" association between atrazine and ovarian cancer. In contrast, based on the available evidence, EPA's Scientific Advisory Committee did not consider the association between atrazine and these tumors as "weakly suggestive", but as "suggestive" without caveat (SAP 2011). In addition, SAP's evaluation is supported by new insight that the mode of atrazine's action could be mediated via the G protein-coupled receptor 30 (GPR30).

In two hospital-based case-control studies an association was shown between triazine exposure and hairy cell leukemia. Likewise, a positive association between atrazine and NHL has been described. A growing number of studies, mostly published after JMPR's (2009) report and reviewed in this report show that atrazine is genotoxic *in vivo*, elicits oxidative stress and alters the immune system, providing mechanistic evidence for the development of these two cancer types.

EVIDENCE CONCERNING REPRODUCTIVE TOXICITY

According to WHO (2017, p. 162) "studies in which the end-point is malformation of a fetus" might warrant an additional uncertainty factor. As reviewed in this report, three epidemiological studies associated atrazine exposure of pregnant women with abdominal wall defects in their fetuses, two with urogenital effects and two with limb defects.

In summary, two considerations exist which independently warrant the application of an additional uncertainty factor of at least 10.

THE NOAEL IS BELOW 1.8 MG/KG

First of all, it needs to be stated, that JMPR neglected its own data base by ***not*** using the lowest NOAEL in its own report: On page 53, referring to the carcinogenicity studies by Hazelette and Green of 1987 in mice, JMPR that the NOAEL was 1.2 mg/kg body weight in male mice (JMPR 2009). Nevertheless, JMPR used the NOAEL of a 1.8 mg/kg from a six-week rat study to derive its ADI.

However, three lines of evidence exist that the lowest NOAEL is even lower than the 1.2 mg/kg body weight which JMPR (2009) preferred not to use:

- Two studies have demonstrated a disruption of the estrous cycle in pigs at doses as low as 1 mg/kg body weight. This finding has particular weight, because the pig is considered a species particularly representative for human beings (Bode et al. 2010) and because it was corroborated by findings in humans as described above. The LOAEL of the two studies was 1 mg/kg body weight. While a NOAEL was not determined, it is reasonable to use a 3-fold dose-spacing. Therefore

a NOAEL of 0.3 mg/kg body weight or lower can be assumed.

- Two studies have shown behavioral, morphological or hormonal effects in CD-1 mice at doses of 1 or 100 µg/kg body weight. A NOAEL was not established in these studies, because effects were seen at both doses. A conservative approach would be to use the neurodegenerative changes demonstrated by silver staining of neurons seen at 100 µg/kg body weight as the point of reference. Again, the finding of neurodegenerative effects in animal experiments was corroborated by the results of an epidemiological study demonstrating an increased risk of Parkinson's disease associated with exposure to atrazine. Using the same approach as above (a 3-fold dose-spacing to extrapolate a potential NOAEL), such a level can be assumed for 0.03 mg/kg body weight or lower. The conservative character of this assumption is further supported by the observation of slight, but statistically significant effects at 1 µg/kg body weight.
- In a well-designed, comprehensive study, a delay of mammary gland development was shown for Long Evans rats at 22.5 µg/kg. Following a conservative approach, this value is not used directly for NOAEL considerations, but only as additional evidence showing effects in the range of the NOAEL extrapolated above. This is done because the study used a mix of atrazine and atrazine metabolites, with atrazine representing only 25% of the mixture, and because the effect seen in this study was not directly corroborated by epidemiological findings.

GUIDELINE VALUE CONSIDERATIONS

To recap, the previous WHO guideline value for atrazine in water was 2 ppb (WHO 2003). Seven years ago it was raised to 100 ppb (WHO 2011a).

In summarizing the evidence reviewed in this report, two independent lines of evidence are followed, both resulting in an ADI (or TDI) value of 0.0003 mg/kg.

In the first line of evidence, it is proposed to start from a NOAEL of 0.03 mg/kg body weight. As explained above, such a NOAEL can be considered a conservative estimate because it has been extrapolated from LOAEL values of 100 µg/kg body weight and slight, but significant effects have been seen at doses below 0.03 mg/kg. In this approach the use of a safety factor 100 yields an ADI of 0.0003 mg/kg.

In the second line of evidence, the extrapolated NOAEL of 0.3 mg/kg body weight (or lower) from the pig studies is used as the point of departure, but the new evidence concerning possible carcinogenic effects is taken into account. Therefore, a safety factor of 1,000 is applied, again yielding an ADI of 0.0003 mg/kg.

It could be argued that the safety factor of 1,000 should also be applied to the estimated NOAEL of 0.03 mg/kg body weight, but to be conservative, this approach is not followed here.

The guideline value can be derived as follows using the algorithm explained in the introduction:

ADI:	0.0003 mg/kg
Body weight:	60 kg
P (drinking water contribution)	0.1
C (amount of water consumed)	2 liters

Guideline value = 0.0003 x 60 x 0.2, divided by 2 = **1.8 ppb**.

Different P-values have been used by different authorities and on different occasions. Here, a P-value of 0.2 has been applied, which was used in the most recent WHO calculation (WHO 2011a).

When applying the same algorithm, but taking into account children as a vulnerable part of the population, a 10 kg body weight and consumption of 1 liter water applies, according to WHO rules. The particular vulnerability of children can be assumed because of the known endocrine disrupting properties of atrazine and its interference with endocrine endpoints after administration during the peripubertal phase of life in animal studies. Therefore the guideline value derived as follows should be applied:

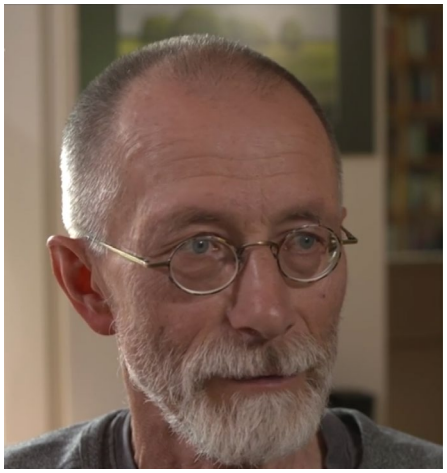
0.0003 x 10 x 0.2, divided by 1 = **0.6 ppb**

The particular vulnerability of children was also taken into account by U.S. EPA, although using a different approach: "The 10x FQPA[23] safety factor was applied to account for the uncertainties associated with atrazine's toxic effects on the developing child and the extent and magnitude of exposure to atrazine in drinking water" (EPA 2006, p. 89).

Endnotes

1 µg/liter and ppb (parts per billion) are used synonymously in this text.
2 Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:01998L0083-20151027&from=EN
3 Unpublished, see JMPR (2009) for reference
4 A database of 4 appropriate studies was considered "relatively small" by the authorities during the assessment of tumor incidences associated with glyphosate in Europe (BAuA 2016, p.71)
5 Unpublished, see JMPR (2009) for reference
6 Unpublished, see JMPR (2009) for reference
7 Support by A. Safer is gratefully acknowledged who performed these calculations using SAS software
8 In cohort studies the participants of the study (e.g. pesticide applicators) are first selected and their pesticide use and health records are then followed for many years. In case-control studies the medical history of the participants and their pesticide use are established retrospectively using questionnaires. A so-called recall bias occurs in case of errors when participants "recall" what pesticides they have used in the past.
9 More recently this receptor was termed "G protein estrogen receptor" (GPER) and recognized as being expressed at the cell membrane as well as (and predominantly) intracellularly, triggering a plethora of effects which "are of profound physiological significance" (Feldman and Limbird 2016).
10 In general, a 95% confidence interval (CI) is considered as statistically significant. Therefore, the results with a 10% CI are considered with reservation.
11 It should be noted that this contradiction has not been resolved yet because the EPA evaluation, which started with the publication of its issue paper (EPA 2011), is still ongoing.
12 SAP (2011) referred to Rusiecki et al. (2004) with regard to the AHS database, to Hoar et al. (1986) and Zahm-Hoar (1993) in the Midwest U.S. and Orsi et al. (2009) in France, though in France the association between atrazine and NHL was positive, but statistically non-significant.
13 i.e. in living animals
14 EPA (2011)
15 http://www.safe.nite.go.jp/english/ghs/10-mhlw-2007e.html
16 https://oehha.ca.gov/proposition-65/crnr/atrazine-propazine-simazine-and-their-chlorometabolites-dact-dea-and-dia-0
17 ATR = atrazine
18 In the review by Goodman et al. (2014) this included birth defects.
19 Chevrier et al. (2011) matched individual maternal urinary atrazine concentrations with adverse pregnancy outcomes (statistically significant for fetal growth retardation and small head circumference). Studies matching individual exposure data with individual effect data are considered of particularly high quality.
20 In the Enoch et al. (2007) paper it was stated that "Studies are under way to investigate the particular metabolite — or combination of metabolites — responsible for the effects observed in the present study; also, studies are being conducted to address whether these are direct effects on mammary cells or an indirect effect."
21 Three different methods were used to define exposure. High exposure was statistically significant for all three methods.
22 The very low levels of contamination and the narrow spacing of the contamination groups should be noted
23 FQPA = Food Quality Protection Act

About the Author



DR. PETER CLAUSING

Dr. Peter Clausing graduated as an agronomist at the University of Leipzig and earned his doctoral degree in 1974. After post-graduate studies in toxicology he became a board-certified toxicologist in 1988 and held positions at two research institutes in the former East Germany. As a postdoctoral scientist he worked at the US FDA's National Center for Toxicological Research from 1994–1996. Thereafter, until retirement in 2010 he was employed as a senior toxicologist in the pharmaceutical industry. Since 2014 he is a member of the Pesticide Action Network (PAN) Germany.