



Grass samples, South Tyrol 2017

**Analysis of pesticide residues in
plant material (samples taken between 16-23.5.2017)**

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(resorption efficiency) during exposure to the lungs and skin, as well as metabolism (potential formation of metabolites). An additional problem is posed by the potential combined effects of multiple exposure to pesticides, which are not taken into consideration in current risk assessments, and are extremely difficult to appraise. This applies in particular to substances which may be endocrine disruptors.

The following study looks at two risks (reproductive toxicity and endocrine disruption) and at the pesticides which present these characteristics in more detail. It also examines the nature of fluazinam and phosmet, the most frequently found active substances, in greater detail. We will look more closely at the problem of multiple contamination at the end of the report.

Reproductive toxicity

According to the definition in Regulation (EC) 1272/2008 reproductive toxicity covers “adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring”. Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals which “is not sufficiently convincing to place the substance in Category 1”. It should be mentioned in this context that Category 1 active substances which are toxic to reproduction (“likely to be toxic to reproduction”) may not in principle be approved in accordance with Regulation (EC) 1107/2009.

The active substance penconazole is classified as a Category 2 substance toxic to reproduction. It was found in Vinschgau in the Rabland/Via Saring (0.065 mg/kg) and Goldrain/Lago di Coldrano samples (0.014 mg/kg). The classification is based mainly on findings from a study with rabbits that was submitted to the public authorities, during which malformations were found in fetuses from the high-dose group (150 mg/kg). Effects such as post-implant losses and delayed bone formation were also observed in the high-dose group of this study, as well as in other studies on rats and rabbits (RAC 2012).

As is the case with carcinogenic and mutagenic effects, attention tends to focus on effects toxic to reproduction, because in principle they are irreversible. Alongside its reproductive toxicity, penconazole is also described as being toxic to endocrine organs (see below).

Fluazinam was also classified as a Category 2 reproductive toxicity substance, because cleft palates were found in fetuses from 3 pregnant rats treated with 250 mg/kg fluazinam².

Toxicity to endocrine organs

Substances toxic to endocrine organs, also known as endocrine disruptors (EDs) are substances which either mimic hormones in the body due to their molecular structure, or effect the availability of the body’s own hormones by accelerating or retarding their synthesis and/or their degradation. Hormones themselves have 2 functions: They control numerous physical functions in developing and adult organisms. In embryos and fetuses, as well as in early childhood development, they regulate the maturation and differentiation of cells, and the functional development of organs. If EDs have an impact on these hormonal actions, the organism can be permanently damaged. This may include well-known metabolism disorders

² <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2008.137r/epdf>

(diabetes, obesity), development disorders, malformations, reproductive disorders, as well as the onset of cancer.

It is a known feature of some EDs that their effects become apparent at very low doses and/or the endocrine disruption which occurs at low doses, no longer occurs at higher doses due to a non-linear (U-shaped) dose-effect relationship curve.

No definitive regulation has yet been adopted in the EU with regard to dealing with EDs, and so this report refers to an impact assessment³ commissioned by the EU Commission, with regard to the (possible) official classification of active substances in pesticides. Annex 5 of this impact assessment lists active substances in pesticides based on the criteria of the WHO, which are considered probable endocrine disrupters (Category 1: cypermethrin, oxadiazon, tetraconazole) or possible endocrine disrupters (Category 2: fluazinam, methoxyfenozide, penthiopyrad). Further to this, academic publications also conclude that chlorpyrifos methyl, difenoconazole, imidacloprid, and penconazole may also be endocrine disrupters (Table 4).

10 of the 14 pesticides (57%) found in grass samples therefore show possible or probable endocrine disruption properties.

Fluazinam

Fluazinam is used as a fungicide and is used by fruit growers in Tyrol against scurf and mould. According to the 2017 guidelines⁴ for integrated stone fruit cultivation (issued by the working group for integrated stone fruit cultivation in South Tyrol), a maximum of 4 treatments per year with pesticides containing fluazinam are allowed.

Fluazinam was one of two active substances which were prominent in the residues found in the grass samples with regard to frequency and level. Fluazinam was found in 18 of the 71 samples. The period of the sampling conducted here (second half of May) falls within the harvest period for lettuce, spinach and strawberries. The MRL of fluazinam for these products is 0.01 mg/kg. If we accept that the residue contamination would have been comparable to that of the grass samples at a similar distance from the orchards, this results in 10 of the 18 samples exceeding the MRL by 2 to 26 times. Based on this comparison, the assumed samples which exceed this figure would be distributed as follows:

26 times: 1x
5 times: 1x
3 times: 3x
2 times: 5x

Alongside classification as a Category 2 reproductive toxic active substance, and its assessment as an endocrine disrupter – also Category 2 (see above), fluazinam has been classified by the European Food Safety Authority as harmful by inhalation, as a skin-sensitising substance (allergic reaction) and severely irritating to the eyes (see also Table 5).⁵

³ https://ec.europa.eu/health/sites/health/files/endocrine_disruptors/docs/2016_impact_assessment_en.pdf. Note: Not all active substances approved in the EU are covered by this impact assessment with regard to their endocrine disruption.

⁴ http://www.agrios.it/doc/agrios_richtlinien_2017.pdf

⁵ EFSA Scientific Report (2008) 137, 1-82, Conclusion on the peer review of Fluazinam; http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/137r.pdf.

Fluazinam is found in the products Banjo, Nando Maxi and Ohayo, where Banjo and Ohayo can cause allergic reactions.⁶

Phosmet

Phosmet is used as an insecticide and can be used in orchards in the Tyrol etc. against scale insects, codling moths and psyllids.

According to the 2017 guidelines⁷ for integrated stone fruit cultivation (issued by the working group for integrated stone fruit cultivation in South Tyrol), a maximum of 4 treatments per year with pesticides containing phosmet are allowed.

The ADI value was raised by the EU Commission Committee responsible for Phosmet on 15.07.2011 from the previous 0.003 mg/kg to 0.01 mg/kg.⁸ The basis for this was formed by the assessment conducted by the EFSA⁹ in 2011, in which phosmet was certified as being non-carcinogenic. This assessment was based on a very sparse database: The finding of liver cancer in a mouse study was dismissed as irrelevant, because according to official bodies it lay within the range of historical control data. This “historical control” consisted of just a single study with 60 animals per gender. Still more disturbing is the fact that in the other study (conducted on rats) the animals died prematurely, in other words, earlier than is permitted according to OECD guideline 453. Despite this, this study was considered definitive, and served as evidence that phosmet presented no risk of liver cancer, regardless of the fact that fatty liver disease was frequently observed in animals treated with phosmet during the rat study. A whole host of pathological changes which begin with fatty liver disease, have been documented, some leading to liver cancers via liver cirrhosis, meaning that a thorough discussion of the liver findings from the rat study would have been appropriate.

Phosmet is also well-established in the academic literature as a promoter of liver tumours and other organ tumours (Cabral et al. 1991, Hasegawa et al. 1993)

Phosmet is the active substance of the products Imidan, Spada and Suprafos.

The problem of multiple contamination

The results of the residue analyses show that pesticides do not occur in isolation, but different active substances have a concerted impact on the environment and people. The toxicological evaluation of such combined effects is complex and remains academically unresolved (cf. Hernández et al. 2012). Official efforts to address this complex matter using – relatively simple – mathematical methods, only scratch the surface of the problem, and largely ignore possible exponential effects (Solecki et al. 2014, Stein et al. 2014). An evaluation of this kind is far from becoming an integral part of the risk assessment within the approval and authorisation procedure, which would be desirable, but for the aforementioned reasons would be difficult to achieve, except in the field of ecotoxicity (cf. Altenburger et al. 2013). In light of this, the only practical solution to the problem is to avoid residues as far as possible.

⁶ <https://apps2.bvl.bund.de/psm/jsp/DatenBlatt.jsp?kennr=006899-00>

⁷ http://www.agrios.it/doc/agrios_ichtlinien_2017.pdf

⁸ <http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.ViewReview&id=448>

⁹ <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2162/epdf>

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Table 1: Calculated maximum concentration in grass samples (last column) compared with selected maximum residue levels tolerated in the EU and the ADI value

	Maximum tolerated residue value (MRL, mg/kg)				ADI value (mg/kg body weight)	Maximum value found (mg/kg), (location**)
	Min.*	Strawberries	Spinach	Lettuce		
2-phenylphenol	0.05	0.05	0.05	0.05	0.4	0.023** (Frangart/Frangarto, Strada Castelfirmiano)
Benzalkonium chloride	Not authorised as a pesticide active substance in the EU, but an ingredient of disinfectants					0.021 (Bozen/Bolzano, Casanova residential zone)
Chlorpyrifos methyl	0.01	0.5	0.05	0.05	0.01	0.250** (Girland/Cornaiano, Via dell'Agnello)
Cypermethrin	0.05	0.07	0.7	2.0	0.05	1.900** (Leifers/Laives, campo giochi Marconi)
Difenoconazole	0.05	0.4	2	3	0.01	0.015 (Neustift, Via Abazzia)
Dodine	0.01	0.01	0.01	0.01	0.1	0.091** (Leifers/Laives, campo giochi Marconi)
Fluazinam	0.01	0.01	0.01	0.01	0.01	0.260 (Rabland/Rabia, Via Saring)
Imidacloprid	0.05	0.05	0.05	2	0.06	0.023 (Natz/Naz, Vicolo Oberbrunner)
Methoxyfenozide	0.01	2	4	4	0.1	0.017** (Penon, Via In der Wies)
Oxadiazon	0.05	0.05	0.05	0.05	0.0036	0.018** (Rabland/Rabia, Via Saring)
Penconazole	0.05	0.5	0.05	0.05	0.03	0.065 (Rabland/Rabia, Via Saring)
Penthiopyrad	0.01	3	30	15	0.1	0.100 (Vahrn/Varna, "Wells")
Phosmet	0.05	0.05	0.05	0.05	0.01	0.069 (Allitz/Alliz, parte Nord del paese)
Tetraconazole	0.02	0.02	0.02	0.02	0.004	0.015** (Staben/Stava, a Ovest del paese)

*lowest listed MRL in the EU database; **only finding at the location

Table 2: Number of pesticide findings according to region and in relation to the distance from orchards and vineyards (near: up to 10-50 m; far: 55-420 m)

Region	Near		Far		Total findings	
	With	Without	With	Without	N	Percent
Eisack Valley	4	0	0	6	4 /10	40%
Etsch Valley	4	6	4	6	8/20	40%
Unterland/Überetsch	3	7	1	9	4/20	20%
Vinschgau	10	0	6	5	16/21	76%
Total	21	13	11	26	32/71	45%

Table 3: Other characteristics and number of active substances found in relation to the number of locations

Active substance	Use	Authorised until	Location with findings (total of 71 locations)
2-phenylphenol	Preservative	31.12.2019	1
Benzalkonium chloride	Disinfectant	not applicable	4
Chlorpyrifos methyl	Insecticide	31.01.2018	1
Cypermethrin	Insecticide	31.10.2017	1
Difenoconazole	Fungicide	31.12.2018	2
Dodine	Fungicide	31.05.2021	1
Fluazinam	Fungicide	28.02.2019	18
Imidacloprid	Insecticide	31.07.2022	3
Methoxyfenozide	Insecticide	31.07.2018	1
Oxadiazon	Herbicide	31.12.2018	1
Penconazole	Fungicide	31.12.2019	2
Penthiopyrad	Fungicide	30.04.2024	6
Phosmet	Insecticide	31.07.2018	18
Tetraconazole	Fungicide	31.12.2019	1

Table 4: Examples of endocrine disruption effects of active substances found, which have not yet been assigned to a category in the impact assessment document (see footnote 3).

Active substance	Described effects	Source
Chlorpyrifos methyl	Effects on thyroid glands and adrenal gland morphology, and serum level of oestradiol, testosterone and thyroid hormones after oral administration of 1, 10 and 100 mg/kg in second generation testing on rats	Jeong (2006)
	Reduced weight of prostate gland and other accessory sex glands after oral administration of 50 mg/kg over 10 days	Kang et al. (2004)
	Significant negative correlation between concentration of a metabolite (3,5,6-trichloro-2-pyridinol) of chlorpyrifos and chlorpyrifos methyl and thyroid gland hormone (T4)	Meeker et al. (2006)
Difenoconazole	Growth hormone in the blood of zebra fish increased when they lived for 7 days in water with 5 µg difenoconazole /l or more	Teng et al. (2017)
	Mosquito fish which lived in water with difenoconazole (from 1 ng/l), displayed changes in the sex hormones as well as in the number of eggs laid and in offspring	Dong et al. (2017)
	In vitro inhibition of enzyme activity (aromatase) which is responsible for the final step in the synthesis of oestrogens, at a concentration of 10 µM	Hinfray et al. (2006)
	Increased number of malformations (from 0.5 mg/l water) and lower concentration of thyroid hormone (T4, at 1 mg/l water) in zebra fishes, which were exposed in the early egg stage to difenoconazole for 120 hours.	Liang et al. (2015)
Imidacloprid	Pathomorphological changes and reduced weight of rat ovaries after 90-day oral administration of 20 mg/kg	Kapoor et al. (2011)
	Obesity and reduced glycometabolism in mice after 12-week administration of feed with a high fat content and at the same time 0.06, 0.6 or 6 mg/kg compared with a pure high fat diet.	Sun et al. (2016)
Penconazole	In vitro inhibition of an enzyme (CYP3A4) which is responsible for the biotransformation of testosterone (IC50 at 2.22 µM)	Lv et al. (2016)
	Influencing of genes which are a factor in the development of thyroid cancer (4-hour exposure of a cell culture with T-47D in four concentrations of 0.0142 to 4.26 mg/kg)	Perdichizzi et al. (2016)

Table 5: Classification of pesticide active substances found (in accordance with the EU pesticide database)

Active substance	Classification ¹⁰
2-phenylphenol	Skin irritation 2, eye irritation 2, respiratory irritation after single exposure 3, aquatic toxicity acute 1
Benzalkonium chloride	Not applicable since not approved in the EU
Chlorpyrifos methyl	Skin sensitisation 1, aquatic toxicity acute 1, aquatic toxicity chronic 1
Cypermethrin	Acute toxicity oral 4, acute toxicity inhalation 4, respiratory irritation after single exposure 3, aquatic toxicity chronic 1
Difenoconazole	No classification
Dodine	Acute toxicity oral 4, skin irritation 2, eye irritation 2, aquatic toxicity acute 1
Fluazinam	Skin sensitisation 1, eye damage 1, aquatic toxicity inhalation 4, reproductive toxicity 2, aquatic toxicity acute 1, aquatic toxicity chronic 1
Imidacloprid	Acute toxicity oral 4, aquatic toxicity acute 1, aquatic toxicity chronic 1
Methoxyfenozide	No classification
Oxadiazon	Aquatic toxicity acute 1, aquatic toxicity chronic 1
Penconazole	Acute toxicity oral 4, reproductive toxicity 2, aquatic toxicity acute 1, aquatic toxicity chronic 1
Penthiopyrad	No classification
Phosmet	Acute toxicity oral 4, acute toxicity dermal 4, aquatic toxicity acute 1, aquatic toxicity chronic 1
Tetraconazole	Acute toxicity 4, acute toxicity inhalation 4, aquatic toxicity chronic 2

¹⁰ The figures behind the respective risk type indicate the severity, with 1 indicating the most severe risk.

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