Biomonitoring of pesticides in hair

Veterinary drugs contaminate human hair

1. **Summary**.

Forty-seven pesticides were detected in hair samples taken from 21 citizens from the Netherlands. The sample results mainly concern pesticides that are banned in agriculture (such as Fipronil, Permethrin and DEET) but still authorised for the treatment of pets against fleas and ticks, which the sample results indicate as the most likely source of hair contamination. The European Medicine Agency (EMA) authorises the veterinary products containing these pesticides and doesn't take into account the indirect effects of these substances on humans through air or contact via the skin.

Hair testing is a unique method to evaluate the chronic exposure of humans to pesticides, in this case over a period of 6 months. The survey, performed by the Pesticide Action Network Europe (jointly with its Netherlands branch), shows that exposure in and around one's house is an important route of exposure to pesticides. The insect-repellent DEET was almost always detected (95% of the cases). Additionally, several fungicides could be detected in study participants living close to agricultural fields.

The detected levels of some of the pesticides are high. The risks exceed governmental standards, if daily 'cocktails'[1] are taken into account. Independent literature[2] shows that for Fipronil even brain-damaging harmful effects (behavioural effects on new-born mice) are observed at the (governmental) 'no-effect' level.

2. **Introduction**.

Hair is a stable matrix and has the advantage over traditional matrices such as blood or urine that it can also retain prolonged exposure to, for example, pesticides for a period of weeks to months, depending on the accumulated hair length. A unique tool therefore to assess chronic exposure to pesticides. On average, hair grows 1 cm per month. For example, hair of 6 cm in length, calculated from the scalp, gives an impression of the pesticide exposure of the last six months.

By controlling insects, weeds and fungi with chemical agents, we are exposed to these substances and they may be absorbed by the body. The absorption of these agents can take place through food, through respiration or skin. Agriculture is the largest consumer of pesticides, but these substances are also applied in households, consciously or unconsciously.

[1] See PAN Europe's 2019 work *Pesticide Cocktails in EU Food* (https://www.pan-europe.info/press-releases/2019/07/pesticide-cocktails-european-food)

[2] Mizuki MAEDA, Toshifumi YOKOYAMA, Sayaka KITAUCHI, Tetsushi HIRANO, Youhei MANTANI, Yoshiaki TABUCHI), and Nobuhiko HOSHI, Influence of acute exposure to a low dose of systemic insecticide fipronil on locomotor activity and emotional behavior in adult male mice, J. Vet. Med. Sci. 83(2): 344–348, 2021

The samples taken from hair have been collected by recruiting volunteers that were interested in being tested and generally cared about a healthy environment. Food consumption deviated from general consumption (the majority of the participants followed a sustainable diet, many of them mainly consuming organic or partly organic food). Many participants owned a pet. There was no attempt in this survey to monitor specific use, such as in agriculture, but rather to get a first impression of what pesticides could be expected to be present in hair in a more or less random population sample.

3. **Methodology**.

A letter with general information about the study and hair sampling was handed over to the volunteers. They were also asked to fill in a questionnaire about their residential location, their use of biocides in the house and garden, and about their possible treatment of pets with anti-tick and flea products and their type of food (conventional or organic) choices.

The volunteers were asked in the protocol to cut from their scalp a strand of hair of a total length of 6 cm and of about the thickness of a pencil (and depending on hair growth, possibly from different areas of the head). The test subjects or their hairdresser had to collect the cut hair strands parallel to each other on a piece of aluminum foil. They then had to send the foil tightly folded on all sides in an envelope to the contact person within Pesticide Action Network, after which the samples were kept in the refrigerator at 4 ° C until they were sent to the laboratory.

In total, 21 human samples were analyzed, and one dog.

Tabel 1. Summary information on the participants to the study

Sample-code	Place of living (NL)	Distance to agri-field	Pet?	Chemical treatment of the pet?	Use personal care?	Food preferences (preferneces organic/standard %)
GN20.014 01/08	Beilen (Dr)	12m	cat	Worms /ticks (june)	Hair dye	Own vegetables; organic/standard 60/40 meat 80% standard
GN20.016 02/08	Dwingeloo (Dr)	12m	dog scheep horse	Dog: Tropiclean Natural (Flea+Tick)	no	80/20 dairy standard, meat organic
GN20.017 02/08	Dwingeloo (Dr)	12m	dog scheep horse	Dog: Tropiclean Natural(Flee+Tick	no	80/20 dairy standard meat organic
MM01 04/08	Assen (Dr)	1km	no	Occasionally dog (half juli)	no	99/1 organic dairy 100% organic
JS-14/10-Haar-1	St-Jacobiparochi (Fr)	20m	no	n.a.	no	10/90, dairy standard
JDM-17/10-Haar-2	Muiderberg (NH)	3 km van weiland, 10 km van akkers	no	n.a. Occasionally dog	no	85/15; dairy 100% organic
JJK_18 /10-Haar-3	Wageningen (Gld)	1km	dog	Once per month till 3 sept. 1.0Vectra	no	100/0 (everything organic or own vegetables)
FA-21/10-Haar-4	Groningen	2km	no	n.a. Occasionally dog (halve of july)	Deet	50/50

IB-21/10-Haar-5	Bern (CH)	700m	dog	Fluralaner/Braven till 8 May	no	Vegetables, fruit20/80, Dairy standard
MS-22/10-Haar-6	Warnsveld, Zutphen (Gld)	300m	no	n.a..	no	vegetables,fruit 50/50, dairy 75/25
JdeG-24/10-Haar-7	Tholen (Zl)	Far away	dog	Adventix in March	no	vegetables, fruit 80/20; yoghurt 100% organic
AS-aug-Haar-8	Assendelft (NH)	1km	no	n.a.	no	70/30 dairy 60/40
HD-27/10-Haar-9	Barchem (Achterhoek, Gld)	100m	dog	Ectoline Duo 3aug. Dimethicom 3.oct. Milbemax 5mei.	no	40/60, dairy mixed
BW-27/10-Haar-10	Barchem (Achterhoek, Gld)	20m	dog cat 7 chicken	Dog: worms-milbemax 6sept. flees/ticks Bravecto 18mei. Cat: Fipralone-spot-on	Ecokid-Outback Jack (against mosquitoe august)	Vegetables en fruit organic; dairy 99/1
IM-27/10-Haar-11	Barchem (Achterhoek, Gld)	20m	dog cat 7chicken	Dog: worms-milbemax 6sept. flees/ticks Bravecto 18mei. Cat: Fipralone-spot-on	Ecokid-Outback Jack (against mosquitoe august)	Veg/fruit organic; dairy 99/1
LC-29/10-Haar-12	Broek in Waterland (NH)	Also lived in Adam	no	n.v.t.	n.v.t.	70/30 dairy 80/20
CdeJ-31/10-Haar-13	Assen (Dr)	500m	cat	Bravecto 1.0 250mg (flee), Milpro (1.0 worms)	care plus, DEET40%	30/70, dairy 50/50
RJ-31/10-Haar-14	Assen (Dr)	500m	cat	Bravecto 1.0 250mg (flee), Milpro (1.0 worms)	care plus, DEET40%	30/70, dairy 50/50
SA-27/10-Haar-15	Wageningen (Gld)	700m	1 dog 1 cat	Dog monthly Frontline; cat af en toe Frontline	zelden	50/50 dairy organic
NN-15/11-Haar-16	Bennekom (Gld)	600 oost/ 1000west	no	May 2019 cat imidacloprid	keratine silver-Schwarzkopf	98/2 (40% own) dairy 90!10
RL-6/11-Haar-17	Zeist (Ut)	>4km	1cat 2 cavia´s 4 chickenn fish	Dog no drugs; Cat Seresto; 5.05	no	100/0 dairy 100% organic
DOG: HA-16/11-18	Assen (Dr)	1km		Halve of July fipronil- Frontline		

The hair samples were analyzed by an accredited laboratory in Germany (Pica Lab., Berlin) by LC/MS and GC/MS for 765 different pesticides, metabolites and biocides, method LA-Pestizide-001.07 based on DIN EN 15662 L00.00-115/1. For the analysis of the hair samples, the lower limit of quantification

was lowered from 0.010 milligrams to 0.002 milligrams per kilogram of hair (i.e. from 10 µg/kg to 2 µg/kg).

4. Summary of the results.

In 20 of the 21 human hair samples examined, a total of 47 pesticides were found (11 different pesticides). On average, 2.24 different substances were detected per sample with an average content of 0.418 mg/kg hair. The insecticide/insect repellent DEET was found in 95% of the tested persons, the insecticide permethrin in almost half (48%) and the insecticide fipronil in 14%. These insecticides were also found in people who never used the agent themselves. Of the 11 substances found, two were authorized as a plant protection product, one as a biocide, one as a veterinary medicinal product, one as a plant protection product as well as a biocide, two as a biocide as well as a veterinary medicinal product, and four substances were not authorized at all. The highest content of all measured substances was found for nicotine (2 mg/kg), followed by permethrin at a concentration of 1.80 mg/kg hair as can be seen in Table 2.

Table 2. The 21 samples of humans (and 1 dog), the number of substances analysed, the total quantitative level of analysed substances and the individual levels of the substances.

NR	Sample	Number analysed substances	Total level mg/kg in hair	Detected substances (level in mg/kg)
1	GN20.014 01/08	3	0,164	Dodine 0,15 DEET 0,004 Permethrin 0,01
2	GN20.016 02/08	6	2,44	Fipronil (+sulfone) 0,31 Permethrin 0,093 DEET 0,81 Azoxystrobin 0,01 Carbendazim/benomyl 0,002 Nicotine 0,2
3	GN20.017 02/08	6	1,425	Fipronil (+sulfone) 0,11 Permethrin 0,1 DEET 0,221 Azoxystrobin 0,01 Carbendazim/benomyl 0,007 Nicotine 2,0
4	MM01 04/08	3	0,177	Fipronil (+sulfone) 0,16 Permethrin 0,008 DEET 0,009
5	JS-14/10-Haar-1	2	0,096	DEET 0,004 Permethrin 0,092
6	JDM-17/10-Haar-2	2	0,42	DEET 0,25 Permethrin 0,17
7	JJK_18 /10-Haar-3	2	1,814	Permethrin 1,80 DEET 0,014
8	FA-21/10-Haar-4	1	0,018	DEET 0,018
9	IB-21/10-Haar-5	1	0,022	DEET 0,022
10	MS-22/10-Haar-6	1	0,095	DEET 0,095
11	JdeG-24/10-Haar-7	3	0,413	Permethrin 0,36 DEET 0,047 Pentacholoanisole 0,006
12	AS-aug-Haar-8	3	0,014	DEET 0,006

				Lindane Pentacholoanisole	0,004 0,004
13	HD-27/10-Haar-9	2	0,015	Diazinone DEET	0,012 0,003
14	BW-27/10-Haar-10	2	0,24	Permethrin DEET	0,19 0,05
15	IM-27/10-Haar-11	0	0	n.a.	
16	LC-29/10-Haar-12	5	0,944	Permethrin DEET Pentacholoanisole Thiabendazole Lindane	0,92 0,007 0,004 0,004 0,009
17	CdeJ-31/10-Haar-13	1	0,029	DEET	0,029
18	RJ-31/10-Haar-14	1	0,004	DEET	0,004
19	SA-27/10-Haar-15	1	0,004	DEET	0,004
20	NN-15/11-Haar-16	1	0,01	DEET	0,01
21	RL-6/11-Haar-17	1	0,01	DEET	0,01
22	**HOND: HA-16/11-18**	2	18,542	Fipronil (+sulfone) DEET	18,5 0,042
	Average number of substances per sample (without the dog)	**2,24**			
	Average total level per sample (without the dog)		**0,418 mg/kg (418 µg/kg)**		

Most often, the insect repellent DEET (N, N-diethyl-meta-toluenamide) was found. DEET was found in 20 (95%) of the 21 hair samples. The content ranged from 0.004 to 0.81 milligrams/kg hair (mg/kg), with an average content of 0.077 mg/kg.

The insecticide permethrin was found in almost half (48%) of the samples. The quantifiable levels found ranged from 0.01 to 1.90 mg/kg. Permethrin is used for many purposes, including as a biocide to kill harmful insects in wood, home and garden and as a veterinary medicine. Permethrin also appears to be very commonly added to wool or silk garments to prevent moths from attacking textiles.

It is striking that in three of the four samples taken at the beginning of August, the very toxic insecticide fipronil was found at levels of 0.11 to 0.31 mg/kg hair. Of these, one person came into regular contact with a dog that had been treated with a fipronil-containing agent (Frontline) in mid-July. For the dog, treated with Frontline against fleas and ticks in mid-July, 18.5 mg fipronil/kg was found in its hair. The hair sample was taken 3 weeks after the treatment. The source of the fipronil found cannot be traced in the other two test subjects.

The fungicides azoxystrobin, dodine and carbendazim / benomyl were found in three test persons who live 12 meters away from a bulb growing field (lily/bulb area).

The substance pentachloroanisole (0.004 to 0.006 mg / kg) was found in 3 test persons living in Zeeland and Noord-Holland. Pentachloroanisole is a reaction product (a break-down product or metabolite) of pentachlorophenol. Pentachlorophenol was used, among other things, in wood preservation, paper and cork, in shoe polish and in many industrial processes. The insecticide lindane was also found in two of the three pentachloranisole containing hair samples (0.004 and 0.009 mg / kg).

5. Possible origin of the pesticides in hair.

Due to its highly toxic effect on humans and the environment, the insecticide fipronil is banned as a plant protection product, yet authorized as a biocide for a single covered application for professional use. The substance has been approved by the European Medicines Evaluation Board (MEB) as a veterinary medicine in 127 products to combat fleas, ticks and lice in dogs and cats. The MEB weighs the risks for the pets against the effectivity ('risk-benefit' approach)[3]. These highly toxic products are freely available in pet stores, on the Internet and through veterinarians. However, Fipronil is not approved for use in food-producing pets such as chickens and cows. For dogs or cats, a dose of 10 mg to 50 mg fipronil per kg body weight per treatment is permitted by the MEB, with the approval of the Ministry of Agriculture, Nature and Food Quality (Veterinary Medicines Information Bank). Likely, the indirect effects of its use for humans is not considered.

The insecticide permethrin is on the market in 67 products as a veterinary medicine to combat fleas, lice and ticks in dogs and cats. Both fipronil and permethrin are often marketed in combination with each other. Permethrin is also often used in conjunction with the "bee venom" imidacloprid (Imidacloprid is authorized by the MEB as a veterinary medicine in 76 products for the treatment of dogs and cats, but not detected here in this study).

The insecticide, acaricide diazinon, can be found as a veterinary medicine under 9 different product names such as flea collar for dogs and cats. This organophosphate was registered as a crop protection product until 1998.

Of the 11 substances found, five are not permitted for use as a plant protection product, biocide or veterinary medicine. The following substances are not authorized as pesticides, biocides or veterinary medicines: Pentachorophenol (PCP), the parent substance of the conversion product pentachloranisole, acts as an insecticide, fungicide, herbicide, growth regulator and is wood preservative. Since 1994, the marketing and use of PCP has been prohibited in the Netherlands.

In the Netherlands, the authorization of the fungicide carbendazim (also a conversion product of the fungicide Benomyl) was withdrawn in 2012. The marketing and use of the insecticide/acaricide Lindane have been banned in the Netherlands since 2007.

For agricultural pesticides, E. Polledri et al. (Science of the Total Environment 687 (2019) 808–816) present evidence that the quantity of applied pesticides was positively correlated with the concentration of pesticides in hair. The mechanism of incorporation of pesticides in hair is poorly understood.

6. Health risks for humans.

EMA, the European Medicine Agency, receives the dossiers that industry has to submit based on Directive 2001/82/EC. While the Directive has an objective of safeguarding public health, for veterinary products only clinical trials are required to demonstrate efficacy, side effects, contra-indications and the exposure of humans via food. The indirect effects, such as exposure of humans and the environment via pets, is not taken into account.

One way to assess the risks of (indirect) exposure through pets is the comparison with (government) health standards. Based on industry studies, European authorities have derived an Acceptable Daily

[3] See example for Fipronil (https://www.ema.europa.eu/en/medicines/veterinary/referrals/fiprex#all-documents-section)

Intake (so-called ADI's), that is, the amount of a pesticide a human can safely be exposed to. If we take the example of Fipronil and a 10 kg child, the child is allowed to be exposed to 0,002 mg daily. With one sample that contained 0,31 mg of Fipronil it can be calculated that it would take 150 days of maximum exposure to Fipronil to remain on the safe side.

The level analysed in hair, at the same time, is not equivalent to the level people are exposed to. Appenzeller et al. (Arch Toxicol (2017) 91:2813–2825) report that there is a distribution of the pesticides between urine, plasma and hair. For Fipronil f.i up tp 12,9 pg/mg in hair, up to 0,81 in urine and up to 22,8 in plasma. The comparison with the ADI should therefore calculate the total exposure of the body and will likely exceed the ADI.

Acceptable Daily Intake (ADI) in mg/kg body weight	
Azoxystrobin	*0,2*
Benomyl	*0,02*
Carbendazim	*0,02*
Diazinon	*0,002*
Dodine	*0,1*
Fipronil	*0,0002*
Lindaan	*0,008*
Nicotine	*0,0008*
Permethrin	*0,01*
Thiabendazool	*0,1*
Pentachooranisole	*0,01*

For Permethrin, a similar calculation can be done. A child weighing 10 kg would be allowed to be exposed to 0,1 mg of Permethrin on a daily basis. One sample contained 1,8 mg/kg of Permethrin and it would take 18 days of maximum exposure to remain on the safe side. If we take into account that people are exposed to dozens of pesticides at the same time and many other chemicals, or chemical 'cocktails', the conclusion is that the government system on ADIs is not safe, especially since the total exposure of the body will be higher than in hair alone.

Additionally, consumers are exposed to pesticide residues in food, pesticides and chemicals via drugs and cosmetics, as well as in the air. The toxicity of chemicals with the same toxicity as Permethrin (which is especially toxic to the nervous system and the brain) should be added and ADIs lowered. If we include a default factor (10), revising the ADI to 0,1x ADI to account for the cocktail effects, in both examples of Fipronil and Permethrin, the exposure is unsafe.

The dog shows very high levels of Fipronil in hair, 18,5 mg/kg. This is 740x higher than the 'no effect' level of 0,025 mg/kg, based on animal studies. It can be doubted whether this high dose is really safe for the pet on the long run. Via this route of animal drugs, the EMA allows very high levels of pesticides in the environment, while risks are not evaluated.

On top of this, the reliance of governments on industry studies includes a big conflict of interest. Industry has an interest in finding a 'desired' outcome. If available, independent studies from academia should be consulted. Taking a look at independent literature another picture emerges. Again concerning the pesticide Fipronil, Maeda et al. (J. Vet. Med. Sci. 83(2): 344–348, 2021) show that effects (behavioural effects on new-born mice) can be seen at the (governmental) 'no-effect' level, which is the basis for the ADI.

Kim et al. (International Journal of Hygiene and Environmental Health 222 (2019) 524–532) demonstrate that Fipronil-sulfone, the metabolite of Fipronil, exposes the foetus in a study conducted on urban parents, and that this (background) Fipronil exposure adversely affects infantile health outcome, including measurements of thyroid function and 5-min Apgar scores (reflexes, heart tone, etc.).

7. Conclusion and recommendations.

- The hair of the majority (95%) of the people tested, from both rural and urban areas, was contaminated with pesticides
- Insecticides were found in the hair of 95% of tested people, even in people who never use these agents such as DEET, permethrin or fipronil themselves
- Fungicides were also found in the hair of people living in arable areas
- The occurrence of certain banned and persistent pesticides in hair such as lindane or pentachloranisole appears to be a regional matter (North Holland, Zeeland)
- The source of the found agents such as lindane, carbendazim or pentachloroanisole is usually not traceable; veterinary products are the likely source of fipronil and permethrin contamination
- All dog and cat owners surveyed in this study treat their animal against fleas and ticks; 83% of the agents used are based on one or more highly effective insecticides, which are partly prohibited for use in agriculture or as biocides due to excessive risks for humans and nature
- Veterinary products constitute a massive gate of emission of pesticides
- EMA authorises the veterinary products without evaluating indirect effects on humans and the environment
- The risks for humans very likely exceed acceptable government standards, especially if daily cocktails are taken into account
- Independent scientific research shows that substances like Fipronil can be harmful, even at governmental “no effect” levels

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