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THE CONTAMINANTS OF THE BEE COLONY

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Summary

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The contamination sources of the bee colony can roughly be divided into environmental and apicul- tural ones. The environmental sources can be further divided in agricultural and non-agricultural ones. The following environmental sources were examined: heavy metals, radioactivity and pesti- cides. The contamination of bee products by these sources is relatively small and the levels found are non-toxic for humans. The bees seem to have a filtering effect, leaving honey relatively free of toxic contaminants. The contamination of pollen, wax and propolis is greater than that of honey. Pollen quality is mostly endangered by pesticides.

The apicultural sources include varroacides, antibiotics, paradichlorobenzene etc. Synthetic varro- acides are the most important sources for contamination, as they have to be used for long-term varroa control. The acaricide levels, found in the different products after treatment with the different acar- icides, decrease in the following order: brood combs > honey combs » sugar feed ~ honey. The contamination level of the brood combs, found in our study, is: bromopropylate > coumaphos and fluvalinate. Non toxic acaricides as organic acids and thymol will not endanger honey quality, if ap- plied after the prescriptions. During honey control activities of the Swiss Canton Laboratories, resi- dues of antibiotics, used for the control of American foul brood were found. The measurements in 1999 to 200 1 showed that one third of the imported honey contained antibiotics, while 6 to 9 % of the Swiss honeys were contaminated, mostly by sulfathiazole. Some beekeepers use paradichlorobenzene (PDCB) for the control of wax moth. This substance was found in about 30 % of all analysed Swiss honeys. Residues coming from wood protectants of bee hive, honey harvest and storage are also dis. cussed. The results show that the main contamination danger for bee products originates in a lesser extent from the environment, than from the apicultural practice. Antibiotics are the most likely con- taminants of honey. Acaricides are the most important contaminants of wax and propolis.

Key words: bee products, beeswax, honey quality, contamination, pollution, propolis, resi- dues

INTRODUCTION

Contamination of honey and other bee products has placed the beekeepers in the situation of Hamlet and they lead a "to bee or not bee" fight against the sea of troubles arising from the contamination sources of bee products. In order to win this struggle, the beekeepers should un- derstand the importance of the different contamination sources. In this short re- view it will be attempted to discuss the relative importance of the different con- tamination sources.

The contamination sources can roughly be divided into environmental (heavy metals, pesticides, bacteria, GM plants) and apicultural ones (Varroacides, antibiotics, paradichlorobenzene, wood protectants of bee hives etc.). The environmental sources can be further divided in non-agricultural and agricultural ones.

CONTAMINANTS COMING FROM THE ENVIRONMENT

Bees fly intensively in a radius of up to 3 km. For this reason they and their products can serve as bio-indicators for the contamination of this area (Devillers and Pham-Delègue, 2002, see there further references). The contaminations can reach the beehive:

- Through air and water they can reach the bee, which will can further transport them into the colony.
- Through air, water and soil they can reach plants, which on their part can pass them by nectar and honeydew to the bees.

Non-agricultural sources

Heavy metals in honeys. Air and water contain heavy metals from industry and traffic which can contaminate the bee colony and its products. The air can contain lead and cadmium, while cadmium can also be transported via water and soil to the plants to reach the nectar and the honeydew. The results, shown in the Table 1, illustrate the contamination of honey by two of the most important toxic heavy metals: lead and cadmium. The Pb and Cd values, measured in honey marketed in Switzerland (Bogdanov *et al.* 1986 and Wenk, 2002) are well bellow the Maximum Residues Limits (MRL), proposed for the EU. Other investigations show similar results (Porrini *et al.*, 2002). As shown in Table 1, the lead contamination has diminished, probably due to the increased world-wide use of car-engine catalysts. Heavy metal contamination is usually increased in areas of industry and heavy traffic (Höffel, 1982). The degree of contamination decreases in the following order: bees ≥ propolis > comb wax > honey (Höffel, 1982; Altmann, 1983). The lower contamination of honey is most likely due to "filtering" by the bees. Heavy metals will probably be washed off during the process of comb melting to produce pure beeswax.

Contamination by pathogenic bacteria. Honey has a very low water content, making the growth of bacteria impossible. Indeed, very few pathogens have been found in honey, as they can survive there for a very limited time (Snowdon and Cliver, 1996; Snowdown, 1999). The only source of health concern has been the presence of *Clostridum botulinum.* Spores of this bacterium can survive in honey, but

Table 1. Heavy metals in honey

Type of honey	Lead* (mg/kg)	Cadmium* (mg/kg)	Reference
Honeydew honey			
1986 (n=21)	0.200 (0.020÷0.520)	0.019 (0.004÷0.060)	Bogdanov *et al.*, 1986
2002 (n=11)	0.016 (0.000÷0.048)	0.007 (0.000÷0.017)	Wenk, 2002
Blossom honey			
1986 (n=18)	0.090 (0.020÷0.370)	0.050 (0.002÷0.020)	Bogdanov *et al.*, 1986
2002 (n=26)	0.021 (0.000÷0.274)	0.001 (0.000÷0.019)	Wenk, 2002

* average (minimum÷maximum) values; MRL proposal for the EU: 1 mg/kg for Pb and 0.1 mg/kg for Cd.

they can not build the toxin. In some rare cases, infant botulism has been explained by ingestion of honey. This has led some countries as the USA and Great Britain to prescribe a label on honey "not to be eaten by infants until one year old". On the other hand, the presence of this bacterium in natural food is ubiquitous. Recently, a scientific committee of the EU has examined the hazard of *C. botulinum* in honey (European Commision, 2002). It was concluded, that no microbiological examinations of honey are necessary, as the incidence of *C. botulinum* is relatively low and that such tests will not prevent infant botulism.

Contamination from genetically modified plants. Some genetically modified plants (GMP) as rape and maize are grown in Europe and might pose problems for bees and beekeepers (Williams, 2002). In Europe there is a wide opposition from consumer organisations against the consumption GMP derived food, while in North America this kind of food is accepted. In the EU and also in Switzerland there is a MRL of 1 % for GMP content, set for all food. Foodstuff, containing more GMP derived material than this limit should have an appellation indicating the exact GMP content. There are very sensitive methods for the determination of genetically modified plants and their parts, for instance of pollen. Indeed, the use of PCR methods allows the determination of only a few genetically modified pollen, which can be contained in honey. Pollen is endangered by GMP, while honey, which contains less than 0.1 % of pollen is not.

Other contaminants. Presently, the radioactive contamination does not present a problem for bees and their hive products (Haarmann, 2002).

Other organic chemicals, present in the environment, are the polychlorinated biphenyls (PCB's) which originate from oil and were used as coolants and lubricants before 1980. These substances are still present in the envorenment and can contaminate food, and thus also the bees and their products. The quantitites, found in honey are low and safe, while those in wax are higher (Jan and Cerne, 1993; Smith *et al.*, 2002). The contamination levels in the bee products are not high.

Agricultural sources

Pesticides. In a recent Swiss study honey and wax were examined for residues of 69 common pesticides (Bogdanov *et al.*, 2003). None of them were found in the determination range between 5 and 50 µg/kg (Table 2a). Other authors also reported that the level of non-acaricide pesticides in honey is low and safe (Fernandez-Muino *et al.*, 1995, Albero *et al.* 2001).

Pollen is significantly more contaminated than honey (Fleche, 1997) (Table 2b).

As a whole it can be stated, that the hazard when using insecticides in agriculture lies mostly in the toxicity for the bees (Devillers and Pham-Delègue, 2002). Tests for bee toxicity are carried out in the EU countries before the registration of pesticides. As stated above, the risk for contamination of honey is relatively small, but pollen is more heavily contaminated (Kubik *et al.*, 1999; 2000). Indeed, most of the modern pesticides used today are not stable and will disintegrate quickly after use. However, farmers are advised to use pesticides outside the bloom period or at least, not during the foraging time of bees.

The fire blight on fruit trees is caused by the bacterium *Erwinia amylovora.*

Table 2a. Pesticide residues in Swiss beeswax and honey (Bogdanov et al., 2003)

Object	Organochloropesticides (32 substances)	Organophosphatepesticides (37 substances)
Beeswax produced 1994–2000	nd	nd
27 honeys produced 1997–2000	nd	nd

nd = not determined.

Table 2b. Pesticide residues in French bees, pollen and honey (Fléché, 1997)

Object	Period of analysis	n	Positive results (%)	Mean values (mg/kg)
Bees	1987	148	36	0.12
Pollen	1987	146	61	0.50
Honey	1992–1996	683	3	0.03

Streptomycin is used in some countries for the control of this disease. The results shown on Table 3 demonstrate, that there is a great risk of streptomycin residues in honey. That is why streptomycin is not used in most European countries. Indeed there are other effective alternatives for the control of the fire blight.

Table 3. Residues in honey after use of streptomycin for fire blight control (*Erwinia amylovora*) in a German trial (Brasse, 2001).

Parameters	Amount
Number of samples	183
Samples with residues	38
Residues over MRL	12

The degree of contamination by pesticides from environmental sources decreases in the following order: propolis ≥ wax > pollen > honey (Fléché, 1997).

CONTAMINATION COMING FROM APICULTURAL SOURCES

Acaricides

Varroacides as a source of residues. The varroacides are the most important sources for contamination, as they have to be used for long-term varroa control. The most important varroacides are given in Table 4, but there are many more, used world-wide.

The acaricides can be divided basically into two groups. The first group is the one of the synthetic, lipophilic substances, e.g. fluvalinate. They are fatt soluble and persistent in wax, that means they do not disintegrate there for a long period. They accumulate in wax, and to a lesser extent, also in honey (Bogdanov et al., 1998b; Wallner, 1999). The second group is that of non-toxic varroacides, which are allowed for use in the frame of organic beekeeping. This group can be sub-divided into two. The first subgroup, to which belongs thymol, are volatile and fat soluble. The second one are water soluble organic acids. In the bee colony, fat soluble substances will accumulate into the beeswax, while the water soluble will accumulate more into honey. There are many different products on the market. Mites, resistant to pyrethroids and coumaphos have appeared in many countries of the world (Milani, 1999). Thus, natural substances like thymol and organic acids are increasingly used. For many acaricides there are no MRL values, for some of

Table 4. Acaricides against *Varroa*

Product	Active ingredient	Registration
Folbex VA	Bromopropylate	EU
Perizin	Coumaphos	EU
Apistan	Fluvalinate	EU
Bayvarol	Flumethrin	EU
Apitol	Cymiazol	EU
Apivar	Amitraz	EU
Thymovar, Apilife VAR	Thymol	EU *
Formic acid products	Formic acid	EU *
Lactic acid, aqueous solution	Lactic acid	EU *
Oxalic acid products	Oxalic acid	**

* allowed for organic beekeeping according to the EU Council, 1999; ** used in EU but not officially registered.

Table 5. Acaricide residues in wax and honey after normal treatments in autumn after the honey harvest (average values in mg/kg) (Bogdanov, 1998a)

Acaricide	Number of treatments	Brood combs	Honey combs	Honey	MRL
Brompropylate	1	47.8	2.4	0.01	0.1
Fluvalinate	1	2.9	0.1	nd	0.01
Coumaphos	1	3.8	0.7	0.015	0.05
Flumethrin	2	0.05	–	nd	0.005

nd = not detected.

them the MRL values differ from country to country.

Acaricide residues in wax, honey and propolis. Studies on the application of different synthetic acaricides revealed different contaminating levels in wax (brood combs and honey combs), sugar feed and honey (Table 5). The acaricides were applied in normal bee hives according to the Swiss prescription in autumn, after the honey harvest. The residues were measured during the following spring. The acaricide levels, found in the different products after treatment with the different acaricides, decreased in the following order: brood combs > honey combs >> sugar feed ≥ honey. The contamination level of the brood combs, found in this study, was: bromopropylate > coumaphos and fluvalinate. The acaricide levels in feed and honey lied well below the Swiss MRL for honey (Bogdanov et al., 1998a) (More details on synthetic acaricides and their residues could be found in Bogdanov et al., 1998a and Wallner, 1999).

Acaricide residues were also found in propolis (Bogdanov, 1998a). The fluvalinate residues were 3.4 times higher, the bromopropylate ones - two times lower than the corresponding residues found in the commercial Swiss wax of the same year. Only two samples contained flumethrine (3.7 and 1.3 mg/kg), while the other ones did not contain residues higher than the detection limit of 0.4 mg/kg.

Thus for medical purposes only propolis produced in the frame of certified organic beekeeping should be used.

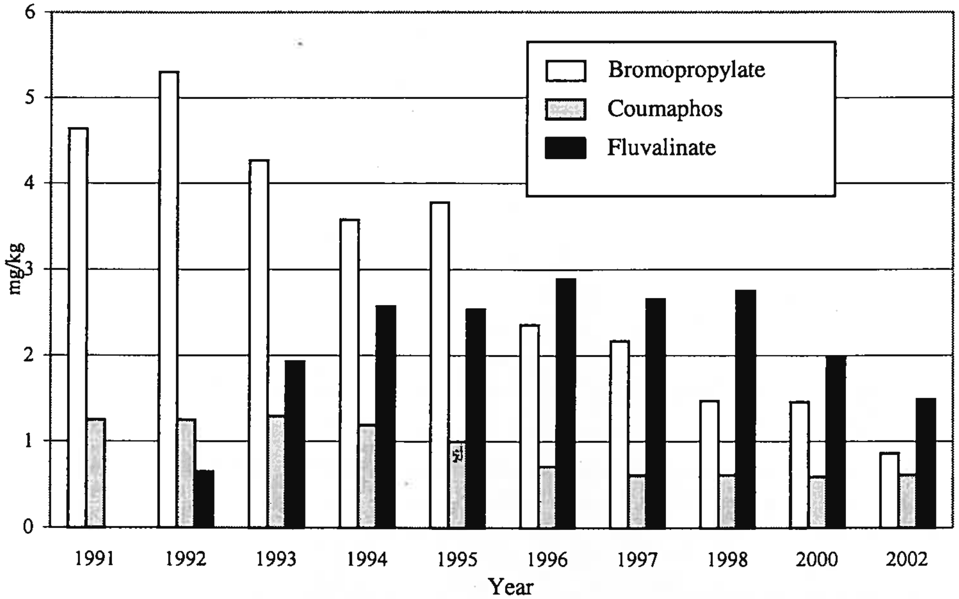

Fig. 1. Long-term monitoring of acaricides in Swiss commercial beeswax.

Long-term monitoring of acaricides in commercial beeswax. The Swiss Bee Re-seach Centre has a monitoring program for a follow up of acaricide residues in Swiss commercial wax. The results of the monitoring are shown on Fig. 1. As most of the Swiss producers of beeswax participate in this monitoring, the results can show the long-term behaviour of acaricides in commercial beeswax. The acaricides fluvalinate, coumaphos and fluvalinate are always present, while no flumethrin above the detection limit of 0.25 mg/kg could be measured. Indeed, from all acaricides, the flumethrin amounts, released into the bee colony are the smallest, with about 16 mg per treatment (Bogdanov *et al.,* 1998a). Bromopropylate will release 1.6 g of active ingredient during one treatment (Bogdanov *et al.*, 1998a). Bromopropylate was used until 1991, but 10 years after, it is still present in beeswax. Another 20 years will pass before it can fully disappear from beeswax (Bogdanov *et al.*, 1998a) . On the other hand, if a certain acaricide (e.g. fluvalinate) starts being used, its concentration in beeswax will rise immediately (Fig. 1). During the last years there is no increase in fluvalinate or coumaphos (Fig. 1). This can be explained by the increased use of alternative varroa control (for further experimental details see Bogdanov *et al.*, 1998a).

The results, shown in Fig. 1 serve as an example for the wax quality in all countries, where synthetic drugs are used for long-term *Varroa* control. Indeed, wax analysis in other countries have shown, that similar amounts of acaricides are found in wax (Wallner, 1999; Bernardini and Gardi, 2001; Menkissoglu *et al.*, 2001).

Thymol residues in honey and wax. Bee colonies were treated in August and September with Apilife VAR, which is composed mainly of thymol. During the spring of the next year the thymol residues in honey and wax were measured. The residues in honey and wax did not increase with increasing number of Apilife VAR treatments. The highest thymol value was 0.5 mg/kg, that means well below the Swiss MRL value of 0.8 mg/kg. The sensory threshold of thymol in honey is 1.1–1.5 mg/kg. The residues in beeswax were much greater, those in the brood combs being much greater than the ones in the honey combs (Table 6).

The thymol residues will evaporate from foundation upon storage under aeration or 2 weeks after placing them into the bee hive (Bogdanov *et al.*, 1998b).

If the thymol treatments are carried during the whole bee season, then the honey residues are greater and will in some cases reach levels, that will cause the change of honey taste, which is not permitted according to the international honey regulations (Bogdanov *et al.*, 1998c; 1999; Wallner, 1997).

Residues of organic acids in honey. In a three year trial bee colonies in Swiss apiaries were treated every year with two long duration formic acid treatments and with a spray containing oxalic acid. The control colonies were treated with fluvalinate (Apistan). During the spring of the next year the acid residues were measured in the honey of the treated and the control apiaries.

The honeys from the treated apiaries had on average higher formic acid content than that of the control apiaries. This increase of formic acid was not important as it lied within the natural variation of this acid (Table 7a). However, if the colonies are treated in spring, the increase of the formic acid concentration in summer honey can be high enough to change honey taste (Table 7b). Thus such treatments should be carried out only in emergency cases. After the oxalic acid treatments there was no increase of oxalic acid concentration in honey.

From the point of view of residues bee colonies can be treated in late summer with formic acid and with oxalic acid in early winter, in the absence of brood (for further details about residues of organic acids in honey see Bogdanov *et al.*, 2002).

Antibiotic residues in honey from American foul brood (AFB) treatments

Residue studies in different countries show that about 1/3 of the honey on the EU market contains antibiotics. Measurements in Switzerland from 1999 to 2001 showed that one third of imported honey contained antibiotics, while 6 to 9 % of the Swiss honeys were contaminated,

Table 6. Thymol residues in spring honey and wax after treatments with Apilife VAR in autumn or with a thymol frame for the whole season (CH-Swiss studies after Bogdanov *et al.*, 1998b, c; 1999; D-German studies after Wallner, 1997)

Treatment	Residues, mg/kg (minimum ÷ maximum)			
	Honey	Brood combs	Honey combs	Sugar feed
Apilife VAR (CH)	0.02÷0.48	500÷600	20÷30	1÷4
Thymol frame (CH)	0.08÷1.1	nt	nt	nt
Thymol frame (D)	0.09÷2.0	nt	nt	nt

nt = not tested.

Table 7a. Residues in blossom honey after treatments in a 3-year trial with formic (FA) and oxalic (OA) acids (ca. 10 apiaries, average values in mg/kg; Bogdanov *et al.*, 2002)

Residues	1996		1997		1998		Natural blossom honey content
	C	T	C	T	C	T	
FA	45	94	31	91	41	71	17–85
OA	41	33	22	18	19	19	8–51

C = control fluvalinate (Apistan) treated colonies; T = FA- or OA-treated colonies (treatment preceeding autumn).

Table 7b. Residues in honeydew honey after treatments in spring (1997, 1999) with formic acid (FA) (Bogdanov *et al.*, 2002).

FA residues	C	T	Natural honeydew honey content
Average, mg/kg	61	254	42–284
Min–max, mg/kg	20–127	68–506	38–119

C = control fluvalinate (Apistan) treated colonies; T = FA-treated colonies.

mostly by sulfathiazole (Table 8). As the control of AFB by antibiotics is forbidden, honey with antibiotic residues is not permitted to be sold on the EU market. The detection limit of modern determination methods are about 5-10 µg/kg (Bogdanov, 2003).

Table 8. Residues of antibiotics in honey in Switzerland (Swiss Cantonal Laboratories, 2002)

Honey/year	Samples	
	n	% positive
Imported honey		
1999	310	34
2001	91	31
Swiss honey		
2000	800	8
2001	88	9

Recently, residues of the antibiotic chloramphenicol were reported in the European mass media, mostly in Chinese honeys. As this substance is toxic, the MRL value is very low: 0.3 µg/kg (see for more details on AFB and its treatment in Bogdanov and Fluri, 2000).

Residues in honey and wax of paradichlorbenzene, used for moth control

Some beekeepers use paradichlorbenzene (PDCB) for the control of wax moth. The substance enters the cycle of beeswax and contaminates the whole commercial beeswax. As shown in Fig. 2, PDCB is present in Swiss beeswax for the whole monitoring period of 10 years. As PDCB is volatile it will evaporate from wax and can contaminate honey (Wallner, 1991). On the average, 30 % of the honeys samples in Switzerland contained PDCB, in 13 % of them the Swiss MRL of 0.01 mg/kg was exceeded (Swiss Cantonal Laboratories, 2002) (Table 9). PDCB is a toxic substance and is not registered for wax moth control in the EU. Thus , PDCB residues in honey are forbidden in the EU countries.

Other, even much more toxic substances as naphthalene, are also used for wax moth control. On the other hand (see below), there are other more ecological

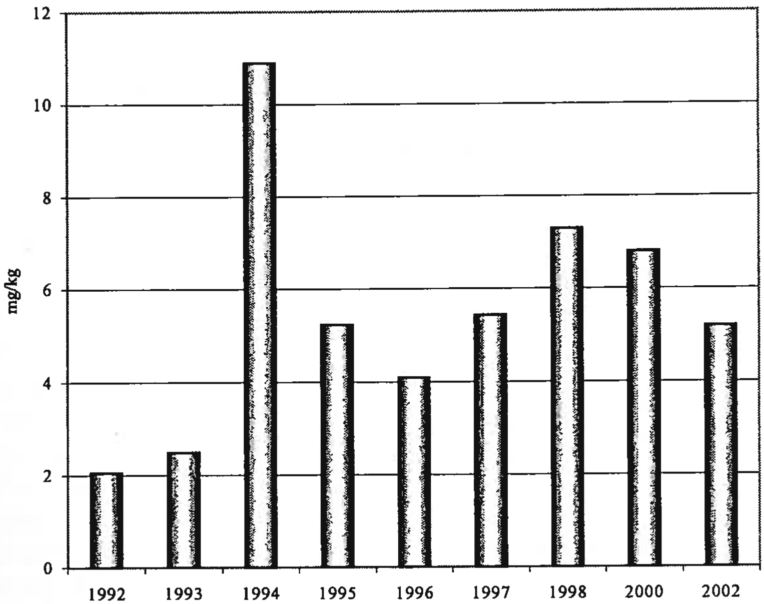

Fig. 2. Long-term monitoring of paradichlorobenzene in Swiss commercial beeswax.

Table 9. Residues of paradichlorobenzene (PDCB) used against the wax moth in Switzerland (Bogdanov *et al.*, 2002)

Year	Honey samples		
	n	Min÷max (μg/kg)	% positive
1997	28	7÷65	14
1998	13	4÷114	46
2000	23	4÷56	26
2001	16	4÷37	31
2002	93	3÷112	34

measures for wax moth control (Charrière and Imdorf, 1999):

- Regular exchange of old combs with new ones.
- Timely recycling of old combs into new beeswax.
- Storage of combs under airy, light conditions or at temperatures below 12 °C.
- Treatment with non-toxic means, which do not produce residues: sulfur, acetic acid, formic acid, *Bacillus thuringiensis*.

Other apicultural contamination sources

Residues coming from wood protectants of bee hive, honey harvest and storage. Wood protectants and paints, used to protect the bee hive against spoilage, should not contain organic chemicals, insecticides and fungicides, which might contaminate honey. Indeed, poisonous ingredients of wood protectants and paints have been detected in honey (Kalnins and Detroy, 1984).

When harvesting honey no chemical repellents should be used. Too much smoke should be avoided. The use of different chemical repellents has been reviewed (Jeanne, 1999).

Table 10. Contamination of honey from the hive, honey harvest, honey storage

Source	Contaminants
Beehive	Organic chemicals from woo protectants Insecticides and fungicides from dyes
Honey harvest	Chemical repellents Too much smoke
Honey storage in inconvenient vessels	Organic pollutants released from paraffinized recipients Zinc from galvanized vessels Collorants from coloured vessels

Storage of honey in inconvenient recipients can also lead to undesirable residues. During the storage of honey inorganic and organic components can diffuse from paraffinated, galvanized, corrosive and painted vessels and contaminate it (Table 10).

CONCLUSION

The results show that the main contamination danger for bee products originates less from the environment, but from the apicultural practice. Antibiotics are the most likely contaminants of honey. Acaricides are the most important contaminants of wax and propolis. Pollen quality is mostly endangered by pesticides.

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