

## Contaminants of bee products<sup>1</sup>

Stefan BOGDANOV\*

Agroscope Liebefeld Posieux, Swiss Bee Research Centre, Liebefeld, 3003 Bern, Switzerland

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**Abstract** – Bee products can be contaminated from different sources. The contamination can arise from beekeeping practices or from the environment. Environmental contaminants are covered in the first part of the review. They are: the heavy metals lead, cadmium and mercury, radioactive isotopes, organic pollutants, pesticides (insecticides, fungicides, herbicides and bactericides), pathogenic bacteria and genetically modified organisms. The second part of the review discusses contaminants from beekeeping. The main ones are acaricides: lipophylic synthetic compounds and non-toxic substances such as organic acids and components of essential oils; and antibiotics used for the control of bee brood diseases, mainly tetracyclines, streptomycine, sulfonamides and chloramphenicol. Other substances used in beekeeping play a minor role: para-dichlorobenzene, used for the control of wax moth and chemical repellents. The degree of contamination of honey, pollen, beeswax, propolis and royal jelly by the different contaminants is reviewed.

**Honey / beeswax / propolis / royal jelly / contamination / residues**

### 1. INTRODUCTION

Honey and bee products have the image of being natural, healthy and clean. However, today bee products are produced in an environment, polluted by different sources of contamination. In the recent past, news about “contaminated honey” has been distributed by the mass media. The most recent example is the news about antibiotic-contaminated honey. Such messages will damage the good image of honey. Thus, it is of utmost importance for beekeepers to localise and exclude the different contamination sources.

In this work an attempt will be made to cover all major contaminants of bee products. A complete coverage of all published works is not possible, as such an undertaking can only be made with the help of monograph on the subject. Here, the main works and issues will be

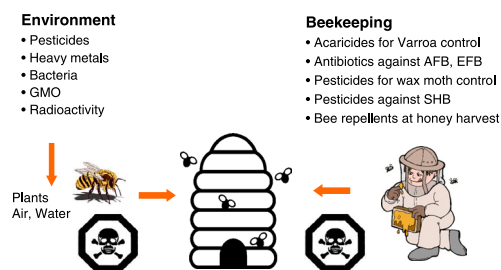
discussed, by choosing the most relevant references. Further details and references can be found in the cited publications. The present review addresses first of all apicultural and food scientists. This knowledge should be then transferred to the beekeepers, so that they can produce clean bee products. A popular article for beekeepers, summarising the most important contamination issues, without going into details, has been published elsewhere (Bogdanov et al., 2002b).

There are very few special residue limits for honey (Piro and Mutinelli, 2003), making it difficult to discuss the toxicological importance of residues. However, an attempt will be made to evaluate the residue hazards of the different residues.

The contamination sources can be roughly divided into environmental and apicultural ones (Fig. 1).

\* Corresponding author: stefan.bogdanov@alp.admin.ch

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**Figure 1.** The contamination sources for the bee colony. GMO: genetically modified organisms; AFB: american foul brood; EFB: european foulbrood, SHB: small hive beetle.

## 2. CONTAMINANTS FROM ENVIRONMENTAL SOURCES

Contaminants can reach the raw materials of bee products (nectar, honeydew, pollen, plant exudates) by air, water, plants and soil and then be transported into the bee hive by the bees.

In this part, the contamination levels and causes will be discussed, without going into details of the contamination paths, which are often discussed in the cited publications.

## 2.1. Heavy metals

Air and soil contain heavy metals, mainly from industry and traffic which can also contaminate the bee colony and its products. Lead (Pb) and cadmium (Cd) are considered the principle toxic heavy metals and are thus most frequently studied. Lead, contained in the air and originating mainly from motor traffic can contaminate air and then directly nectar and honeydew. Generally, Pb is not transported by plants. On the other hand, Cd originating from metal industry and incinerators, is transported from the soil to plants and can then contaminate nectar and honeydew. Only a small portion of Cd might reach honey by air, mainly in the vicinity of incinerators. The great majority of the Pb and Cd values in honey, reviewed in Table I are well below the MRL (Maximum Residue Limit) values of 0.1 mg/kg for Cd and 1 mg/kg, proposed for the EU (EC, 2000a). However, today there are no established MRL values for honey. Pb contamination is expected to diminish, due to the increased world-wide use of car-engine catalysts. Indeed, a decrease of the Pb honey concentration was documented in Switzerland. In honey harvested in 1984 the average Pb concentration in honey was 0.2 mg/kg (Bogdanov et al., 1986). In honey harvested

**Table I.** Contamination of honey with cadmium and lead.

	Range in mg/kg	honey	Reference
<b>Pb</b>	0.001–0.37	blossom	(Höffel, 1982; Bogdanov et al., 1986; Uren et al., 1998; Petkov et al., 1998; Fakhim-Zadeh, 1998; Devillers et al., 2002b; Kantonales Laboratorium Basel-Stadt, 2002)
	0.02–0.8	blossom	(Altmann, 1983; Yazgan et al., 2002)
	0.02–0.52	honeydew	(Bogdanov et al., 1986; Uren et al., 1998)
	1.0–1.4	honeydew	(Altmann, 1983)
	0.02–0.56	unspecified	(Conti et al., 1998)
	0.003–0.045	unspecified	(Conti and Botre, 2001; Kantonales Laboratorium Basel-Stadt, 2002)
	1.7–1.8	unspecified	(Leita et al., 1996)
<b>Cd</b>	0.001–0.07	blossom	(Höffel, 1982; Bogdanov et al., 1986; Uren et al., 1998; Fakhim-Zadeh, 1998; Devillers et al., 2002b)
	0.011–0.113	blossom	(Altmann, 1983)
	0.001–0.10	honeydew	(Altmann, 1983; Bogdanov et al., 1986; Uren et al., 1998)
	0.001–0.10	unspecified	(Conti et al., 1998; Conti and Botre, 2001; Kantonales Laboratorium Basel-Stadt, 2002; Porrini et al., 2002)

between 2000 to 2002 it was 0.04 mg/kg (Kantonales Laboratorium Basel-Stadt, 2002). The results, published in different studies show, that lead contamination of honey in polluted and non polluted areas is not significantly different, due to the considerable natural variation of the data (Höffel, 1982; Altmann, 1983; Bogdanov et al., 1986; Jones, 1987; Conti et al., 1998; Yazgan et al., 2002). However, the highest Pb values were often found in honey from polluted areas. The relatively low contamination of honey is probably due to “filtering” by the bees since honey has a considerably lower heavy metal content than bees (Leita et al., 1996; Fakhim-Zadeh and Lodenius, 2000; Porrini et al., 2002).

There are very few published studies on heavy metal contamination of pollen, beeswax and propolis (Altmann, 1983; Cesco et al., 1994; MAFF, 1995; Leita et al., 1996; Conti and Botre, 2001; Madras-Majewska and Jasinski, 2003). The values in mg/kg vary across a rather broad range:

Pb, mg/kg: honey: 0.01–1.8; pollen: 0.02–3.9; beeswax: 0.06–6.2; propolis: 0.003–461.0; Cd, mg/kg: beeswax: 0.01–0.1; honey: 0.03–2.1; pollen: 0.05–2.3; propolis: 0.006–3.8.

Bees fly intensively in a radius of up to 3 km. For this reason they, together with their products can serve as bio-indicators for the heavy metal contamination, mostly by Pb, of this area (Höffel, 1982; Altmann, 1983; Bromenshenk et al., 1985; Bogdanov et al., 1986; Jones, 1987; Balestra et al., 1992; Cesco et al., 1994; Conti and Botre, 2001; Yazgan et al., 2002; Porrini et al., 2002; Devillers et al., 2002b; Lebedev and Murashova, 2004).

Other heavy metals like mercury (Hg) and nickel (Ni) have been much less frequently studied. World-wide there are no specific MRL levels for these heavy metals in honey. The MRL levels in Switzerland for Hg vary in different food between 0.01 mg/kg (fruit juices and jellies) and 0.5 mg/kg (fish). The Swiss MRL for Ni varies between 0.1 mg/kg (beer) to 0.2 mg/kg (edible fat). Following levels have been found in honey: Ni: 0.004–3.23 mg/kg (Porrini et al., 2002); < 0.001 mg/kg, (Devillers et al., 2002b); 0.09–0.34 mg/kg (Devillers et al., 2002c). In honey samples from contaminated and uncontaminated areas of Slovakia Hg levels ranged from 0.050 to 0.212 mg/kg and from

0.001 to 0.003 mg/kg, respectively (Toporèak et al., 1992). In two recent studies no Hg could be found in 150 French acacia honeys above the detection limit of 0.0005 mg/kg (Devillers et al., 2002b) or in 86 honey samples of different botanical and geographical origin sold in France (Devillers et al., 2002c). In a Polish study, Hg levels were measured in different bee products were studied and the following values in mg/kg were reported: propolis: 0.001–0.07, beeswax: 0.0001–0.06, honey: 0.00001–0.006 (Madras-Majewska et al., 2002).

On average, the Pb concentration was low and unproblematic in honey but the lead residues found in propolis are often too high and care should be taken to harvest it in areas that are at least 3 km away from motor car traffic and incinerators. Bee products are less suitable to serve as indicators for the measurement of Pb and Cd pollution due to considerable natural variation. Bees themselves seem to be better candidate for this purpose (Porrini et al., 2002).

## 2.2. Radioactivity

Presently the main radioactive isotopes found in honey are  $^{40}\text{K}$  and  $^{137}\text{Cs}$ , the first being of natural origin, the latter originating from the Chernobyl atomic power station accident in 1986. Radioactivity is expressed in Becquerel (Bq) per kg. The  $^{40}\text{K}$  radioactivity varied in different types of honey from Poland varied from 39 to 123 Bq/kg (Borawska et al., 2000).  $^{137}\text{Cs}$ , with a half-life of thirty years, is the most studied isotope, and the results reported hereafter refer to this isotope. A maximum limit of 370 Bq/kg for milk and 600 Bq/kg for all other products was set in the EU (EC, 1990). Most works, cited below, studied the effect of the Chernobyl atomic power station accident in 1986 on bee products. In an Ukrainian study Alexenitser and Bodnarchuk (1999) found an average concentration of 4430 Bq/kg radioactive caesium in honey harvested in the Ukraine between 1986 and 1989. The radioactive contamination, measured in other countries was much smaller, due to the distance from Chernobyl. In Germany measurements in about 6000 honeys were carried out in the period between 1986 and 1991 (Horn and Vorwohl, 1986, 1987, 1988). Honey initially had relatively high contamination, averaging 51 Bq/kg. This level dropped by about 50% one

year later and remained at an average level of about 10 Bq/kg in subsequent years. German heather honey was highly contaminated, with an average of 532 Bq/kg in 1987 (Dustmann and von der Ohe, 1988). These high values could be due to different affinity of  $^{137}\text{Cs}$  to the plants or to differences of the isotope concentrations in the soil (Kubik et al., 1991). There are extensive data on honey harvested between 1987 and 1995 in Croatia, Slovenia, Austria and Germany (Barišić et al., 2002). The values in Slovenian honeys varied between 8 and 51 Bq/kg honey and in Croatian honeys: between 1 and 21 Bq/kg. The values of 46 honeys harvested between 1986 and 1989 were between 1 and 425 Bq/kg, with a median around 30 Bq/kg, the highest value being for a single honeydew honey in 1986 (Devillers et al., 2002a), while in honeys harvested in 2000 and 2001 the average radioactivity was about 2 Bq/kg. In Italian honeys harvested between 1994 and 1996 the values varied between 1 and 5 Bq/kg (Porrini et al., 2002).

The radioactivity in other bee products has been less investigated. Measurements of  $^{137}\text{Cs}$  radioactivity in the Ukraine showed very high averages for products harvested between 1986 and 1989: pollen: 11070 Bq/kg, propolis: 34310 Bq/kg. In France considerably lower values were measured at the same time: honey, 29 Bq/kg; pollen, 283 Bq/kg (Canteneur, 1987). In Italy, measurements of honey and pollen harvested in 1986 showed  $^{137}\text{Cs}$  values in honey between 30 and 360 Bq/kg, while those in pollen varied between 1000 and 2500 Bq/kg (Porrini et al., 2002). Pollen and propolis are considered to be better indicators for radioactive contamination than honey (Alexenitser and Bodnarchuk, 1999).

In measurements in Croatia in 1990 the  $^{137}\text{Cs}$  values in honey varied from 1 to 15.9 Bq/kg, those in pollen were from 20.1 to 32.3 Bq/kg (Barišić et al., 1992).

In a more recent study the radioactivity due to different radioisotopes was measured (Handa et al., 1997).  $^{226}\text{Ra}$ ,  $^{214}\text{Pb}$ ,  $^{214}\text{Bi}$  and  $^{40}\text{K}$  were present in all 13 studied honeys originating from Japan, China, Hungary, Italy, and Australia,  $^{137}\text{Cs}$  was present in 4 samples (acacia honey from Hungary, chestnut honey from Italy, lime honey from Japan) while  $^{134}\text{Cs}$  was detected only in one Italian chestnut honey (10.97 mBq/g). The concentration of  $^{40}\text{K}$  was:

minimum: 15 Bq/kg, (Acacia) maximum: 211 Bq/kg (chestnut), while that of  $^{226}\text{Ra}$  was minimum 24 Bq/kg, maximum: 45 Bq/kg.

Radioactivity is not currently a problem for honey and for other bee products. However, after thermo-nuclear incidents, bee products should be controlled before consumption.

### 2.3. Organic contaminants

An example of organic chemicals present in the environment are PCB's, (polychlorinated biphenyls) which originate from motor oil, coolants and lubricants, produced before 1980. These substances are still present in the environment and can contaminate plants and thus, the bees and their products. The quantities, found in honey are low and safe, while those found in wax are higher (Estep et al., 1977; Gayger and Dustmann, 1985; Anderson and Wojtas, 1986; Morse et al., 1987; Jan and Cerne, 1993).

Small residues of polyaromatic compounds originating from oil were found in honeys produced near an oil factory in Germany (Horn and Martius, 1997).

### 2.4. Pesticides used in agriculture

#### 2.4.1. Insecticides

The most common insecticides that have been examined in European honeys include:

- Organochlorines (OC) such as lindane and its isomers, hexachlorocyclohexane (HCH), aldrin, dieldrin, endrin, DDT isomers, heptachlor, heptachlor epoxide, methoxychlor, endosulfan. Many OC are no longer used in agriculture, but are still present in the environment.
- Organophosphorus pesticides (OP) like dialiphos, trichlorophos, and dichlorvos.
- Carbamates: pesticides containing an amino group:  $\text{R}^1\text{-NH-CO-OR}^2$ .

Six hundred and fifteen French honeys were analysed between 1986 and 1996 and pesticide residues were found in 17.5% of the samples (Fléché et al., 1997).

In 101 Spanish honeys 10 OC pesticides were found in 1 to 57% of the samples (Fernandez-Muino et al., 1995). In another Spanish study

6 OP pesticides were found in 7 to 24% of the samples, the values varied between 0.001 and 0.116 mg/kg (Garcia et al., 1995).

In a recent study using 50 honey samples from Spain and Portugal, residues of 42 different pesticides (OC, OP and carbamates) were examined (Blasco et al., 2003). Most of the pesticides found in honey were organochlorines. Among them, gamma-HCH was found in 50% of the samples and was the most frequently detected substance, followed by HCB in 32% of the samples and the other isomers of HCH (alpha-HCH and beta-HCH) in 28 and 26% of the samples, respectively. The values found varied between 0.03 and 4.31 mg/kg, but most of them were below 0.5 mg/kg. The study concludes that honey makes a minimal contribution to the acceptable daily intake (ADI) of pesticides.

In an Indian study 27 market samples were examined for residues of organochlorine (OC), synthetic pyrethroids (SP), organophosphates (OP) and carbamate insecticides (Anju et al., 1997). All honeys were contaminated with at least one pesticide. Residues of 10 different OC pesticides varied between 0.01 and 6 mg/kg, with most values being below 0.5 mg/kg. The residues of 8 different OP and 3 carbamates varied between 0.1 and 9 mg/kg, with the values being generally higher than those of the OC.

In an Italian study 11 OC pesticides were analysed (Roggi et al., 1990). In 24 out of 26 honeys residues ranging from traces to 0.15 mg/kg were found, with most of the active ingredient residues present below 0.05 mg/kg.

In a Jordanian study, 50 OP and OC pesticides were examined in 27 honey samples (Al-Rifai and Akeel, 1997). Low levels of OC residues (lindane,  $\alpha$  HCH,  $\beta$  HCH and heptachlor) with values between 0.004 and 0.21 mg/kg were detected in some of the samples.

In a Finnish multi-residue study no measurable residues were found (Moilanen et al., 1986). In a recent Swiss study honey and wax were examined for residues of 69 common OP and OC pesticides (Bogdanov et al., 2003). Non of them were detected above the detection limit, which varied between 0.005 and 0.050 mg/kg.

Imidacloprid is a systemic insecticide known under the name of Gaucho. Very small residues of 0.001 to 0.005 mg/kg (Schmuck et al., 2001;

Bonmatin et al., 2003; Maus et al., 2003) or no residues above 0.002 mg/kg (Rogers and Kemp, 2003; Schöning and Schmuck, 2003; Stadler et al., 2003; Faucon et al., 2004) were found in various studies on honey, nectar or pollen. There are no specific MRL values for bee products. The Swiss tolerance values for imidacloprid are 0.01 mg/kg in maize and 0.05 mg/kg for fruits. Thus, the residues, found in honey and pollen are not considered to be a problem for human health. On the other hand, the effect of imidacloprid on bee health is highly controversial (Maus et al., 2003; Faucon et al., 2004).

Residues from microencapsulated insecticides as methylparathion, which is toxic to bees, were found in US studies on honey and pollen (Atkins and Kellum, 1984; Russell et al., 1998).

#### 2.4.2. Other pesticides: herbicides, bactericides, fungicides

Honey seems to be especially susceptible to residues from fungicides used against pests in fruit trees and rape. Levels ranging between traces and 0.11 mg/kg of some systemic fungicides have been found in honey (Rexilius, 1986; Kubik et al., 1999). In studies in Poland where 5 different fungicides (vinclozolin, iprodione and methyl tiophanate, captan and difenoconazole) were applied in cherry trees, the residue level of vinclozolin in honey was the highest, with an average of 0.11 mg/kg, was the highest, while the other substances produced residues from 0.001 to 0.06 mg/kg (Kubik et al., 1999). Residues in pollen were higher than in honey (Fléché et al., 1997; Kubik et al., 1999). Pesticides, especially herbicides, seem to contaminate mostly bees and pollen, and only rarely, also honey (Tab. II). The fungicides dithianon, pyrifenox, penconazol and cyproconazol applied in fruit trees in spring in Switzerland caused honey residues between 0.02 and 0.09 mg/kg (Rüegg, 1995). In a German study with 5 different fungicides used in rape, only carbendazim caused substantial residues, with a maximum of 0.12 mg/kg (Büchler and Volkmann, 2003).

Another class of pesticides are antibiotics used against bacterial plant pests. The fire blight on fruit trees is caused by the bacteria *Erwinia amylovora*. This bacterium can be controlled by streptomycin. Residue studies in

**Table II.** Pesticide residues in French bees, pollen and honey, (Fléché et al., 1997). Means values are computed from the positive values only.

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

Germany have shown that honey was often contaminated. Out of 183 samples, 21% contained streptomycin residues (Brasse, 2001). Because of this contamination risk, streptomycin is not registered for such use in most countries of the EU.

In two recent Swiss studies, residues arising from the treatment with the herbicide asulam were examined (Kaufmann and Kaenzig, 2004; Bogdanov and Edder, 2004). Unexpectedly, considerable amounts of the asulam breakdown product sulphanilamide were found (minimum 0.005, maximum 0.70 mg/kg), together with asulam residues themselves (minimum 0.005, maximum 0.23 mg/kg).

#### 2.4.3. Pesticides: conclusions

Bees, more than bee products, have been used as biological monitors for pesticide contamination of geographic regions (Celli et al., 1996). The relatively low concentration of pesticides in honey seems to be due to a filtering effect of bees. Indeed, bees decrease initially high pesticide nectar concentrations, so that the final concentration in honey was much lower, mostly by a factor of about 1000 (Schur and Wallner, 1998; Schur and Wallner, 2000). Also, many of the modern pesticides used today are unstable and disintegrate quickly after use. Generally, there are no MRL's for pesticides in honey. In the EU an action level of 0.01 mg/kg is often considered for pesticides with no fixed MRL. In Switzerland, for most foodstuffs, pesticide levels was much lower than 1% of the acceptable daily uptake (Kuchen et al., 1998). Considering the fact that pesticide residues found in honey are comparable to the ones found in other foodstuffs (Kuchen et al., 1998) and taking into account the relatively low consumption of honey, it can be concluded, that pesticide residues in honey are safe from toxicological point of view.

To avoid residues pesticides should be used outside the bloom period or at least, not during the foraging time of bees. Beekeepers can also avoid residues by placing their hives more than 3 km from agricultural plants treated with pesticides.

#### 2.5. Pathogenic bacteria

Honey has a very low water activity, preventing the multiplication and, in most cases, even the survival of bacteria. Also, very few pathogens have been found in honey (Snowdon and Cliver, 1996; Snowdon, 1999). However, there is a health concern concerning the presence of *Clostridium botulinum* in honey. Spores of this bacterium can survive in honey, but they can not build the toxin. In some rare cases, infant botulism has been explained by ingestion of honey. This has led some honey packers (e.g. the British Honey Importers and Packers Association) to place a warning on the honey label, that "honey should not be given to infants under 12 months of age". 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 *Clostridium botulinum* in honey (EC, 2002). It has concluded, that no microbiological examinations of honey are necessary, as the incidence of *Clostridium botulinum* is relatively low and that tests will not prevent infant botulism.

From the other bee products only pollen might pose problems for bacterial contamination and control of the bacteriological safety is necessary.

#### 2.6. Genetically modified plants

Genetically modified organisms (GMO), such as rape and maize, are grown in some countries and might pose problems for bees and

beekeepers (Williams, 2002a, b). In some countries such as USA and Canada genetically modified plants are commonly grown and accepted by the public, while in the European Union there is a wide opposition against the consumption of GMO – containing food. In the European Union the appellation of GMO content in food is compulsory above 1% (EC, 2000b). There are very sensitive methods for the determination of genetically modified plants and also of pollen. Indeed, the use of polymerase chain reaction (PCR) methods allows the determination of only a few grains of genetically modified pollen (Ramsay et al., 1999). Bee pollen can be thus significantly contaminated, while honey, which contains less than 0.1% of pollen, will require no specific appellations.

### 3. CONTAMINANTS FROM BEEKEEPING

The most important contaminants are the substances used for the control of bee pests. At present, two of the most important pests worldwide are varroa and American Foul Brood.

#### 3.1. Acaricides

Varroacides are an important contamination source, as they have to be used for long-term *Varroa destructor* control. In 1987 there were more than 90 products used for control worldwide (Wienands, 1987).

The acaricides can be divided into two groups (see Tab. III):

- Synthetic, persistent substances
- Natural non-toxic substances.

A more complete list of more acaricides such as tetradion, chlorodimiform, cymiazole, can be found elsewhere (Wallner, 1999). The great majority of beekeepers world-wide use the acaricides mentioned here (Tab. III).

##### 3.1.1. Synthetic acaricides

Synthetic acaricides are mostly fat-soluble and persistent in wax. After acaricide treatments, they accumulate in wax and contaminate honey to a much lesser extent. In a review article on acaricides (Wallner, 1999) and in a detailed work with the main synthetic acari-

**Table III.** Acaricides used for varroa control and legal limits in the EU and in Switzerland or only for Switzerland (\*).

n.n. = not necessary according to the EU legislation. MRL = Maximum Residue Limit.

Active ingredient (commercial name)	MRL mg/kg
<i>Synthetic drugs</i>	
Cymiazol (Apitol)	1
Fluvalinate, (Apistan)	n.n.; 0.05*
Amitraz (Apivar)	0.2
Flumethrin (Bayvarol)	n.n.; 0.005*
Coumaphos (Perizin)	0.1; 0.05*
<i>Non-toxic drugs</i>	
Thymol (Apiguard, Apilife-VAR, Thymovar)	n.n., 0.8*
Lactic acid (aqueous solution)	n.n.
Oxalic acid (different applications)	n.n.
Formic acid aqueous solution	n.n.

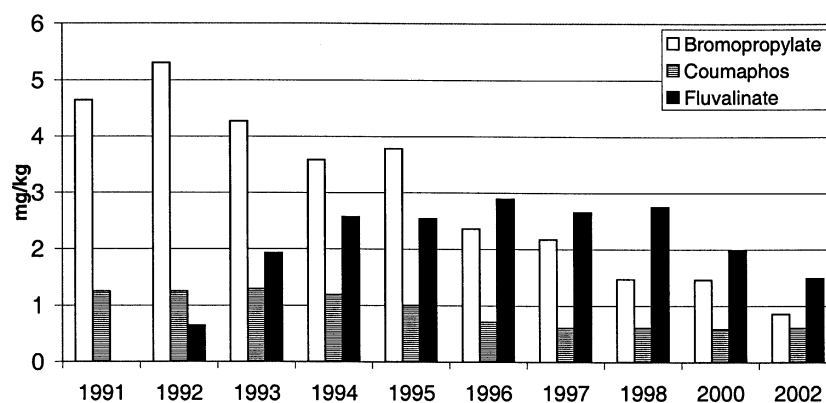
cides (Bogdanov et al., 1998b) the main issues and publications concerning the acaricide residues in bee products were discussed and the reader should refer to these works for additional references. Here the main issues will be summarised, and important recent works will be dealt with. The main acaricides are shown in Table III but many different active ingredients are used, many of which are not registered as varroacides. The application of different synthetic acaricides results in different contamination levels in wax (brood combs and honey combs), sugar feed and honey. Table IV shows the contamination of wax and honey after a normal application according to the Swiss prescription in autumn, following the honey harvest. The residues were measured during the following spring. The acaricide levels, found in the different products after treatment with the acaricides, decreased in the following order: brood combs > honey combs >> sugar feed ≥ honey (Tab. IV). Similar findings are expected for most synthetic lipophylic acaricides.

The acaricide levels found in honey are generally lower than the accepted MRL levels (see references cited in Muino et al., 1997; Bogdanov et al., 1998a; Wallner, 1999). On the other hand, residues accumulate in wax (Bogdanov et al., 1998a; Wallner, 1999). The

**Table IV.** Acaricide residues in wax and honey in spring honey of the following year, after normal treatments following the honey harvest in the previous autumn, average values in mg/kg (after Bogdanov et al., 1998a).

\* = MRL for honey in Switzerland; \*\* – since 2000 there is no longer a MRL.

Acaricide	Number of treatments	Wax of brood combs	Wax of honey combs	Honey	MRL*
Bromopropylate	1	47.8	2.4	0.01	0.1**
Fluvalinate	1	2.9	0.1	≤ 0.001	0.05
Coumaphos	1	3.8	0.7	0.015	0.05
Flumethrin	2	0.05	≤ 0.001	≤ 0.001	0.005



**Figure 2.** Monitoring of acaricide concentration in Swiss commercial beeswax (after Bogdanov et al., 1998a) and unpublished data.

residue amount depends on the number of treatments, with more treatments leading to higher the wax residues (Bogdanov et al., 1998b). The widely used acaricide amitraz is metabolised not only in honey (Bogdanov, 1988), but also in wax (Korta et al., 2001, 2002). Residue studies in wax should be carried out on the main amitraz metabolite (Korta et al., 2002).

A theoretical model on the contamination of the bee colony by coumaphos was recently published (Tremolada et al., 2004). This model could successfully predict the contamination level by coumaphos of different colony matrices as bees, wax, pollen and honey. The model could be also used for predicting the contamination by other acaricides.

The Swiss bee research centre has a monitoring program for a follow up of acaricide residues in Swiss commercial wax (Bogdanov et al., 1998a). As all Swiss producers of beeswax participate in this monitoring, the results can serve as a model for the long-term behaviour of acaricides in commercial beeswax. During the monitoring period, the acaricides bromopropylate, coumaphos and fluvalinate were always present, while no flumethrin below the detection limit of 0.25 mg/kg was measured (Fig. 2). Residues of bromopropylate, an acaricide used until 1991, have been found to be steadily decreasing. However, another 20 years will pass before it is expected to fully disappear from beeswax. If a certain acaricide (e.g. fluvalinate) starts being used, its



**Table V.** Residues of thymol in honey and beeswax after treatments with thymol containing acaricides. Min, max: minimum and maximum values found.

Treatment	min-max mg/kg	References
<i>Normal Apilife VAR, Thymovar treatments</i>		
Honey	≤ 0.02–0.48	(Lodesani et al., 1992; Bogdanov et al., 1998c; Bollhalder, 1998; Bernardini and Gardi, 2001)
Wax of honey combs	4–20	(Bogdanov et al., 1998c)
Wax of brood combs	169–1989	(Bogdanov et al., 1998c)
Wax foundations	≤ 0.7–50	(Bogdanov and Kilchenmann, 2003)
<i>Thymol frames used during the whole bee season or non-specified thymol treatments</i>		
Honey	≤ 0.02–2.65	(Wallner, 1997a; Bogdanov et al., 1998b; Nozal et al., 2002)

concentration will rise immediately in the commercial wax. Since the appearance of fluvalinate-resistant mites in Switzerland the use of alternative acaricides such as thymol and organic acids has increased. This practice is probably the reason of the decreased fluvalinate concentrations, encountered during the last monitoring periods. Wax analyses in other countries have shown, that similar amounts of acaricides are found (Wallner, 1995, 1997b; Menkissoglu-Spiroudi et al., 2001; Bernardini and Gardi, 2001).

Acaricide residues have been found also in propolis (Bogdanov et al., 1998a; Wallner, 1999). The fluvalinate residues found were higher than those in wax. For medical purposes only propolis produced in the frame of certified organic beekeeping should be used. A MRL level, similar to that established for honey should apply for propolis.

### 3.1.2. Non-toxic natural acaricides

Due to the persistence of synthetic acaricides, mites, resistant to pyrethroids and coumaphos have appeared in many countries of the world (Milani, 1999). This has led to the increased use of alternative control measures with non-toxic substances such as thymol and organic acids. Thymol is fat soluble and volatile, while organic acids are water soluble and non-volatile. These substances are natural honey and plant constituents. In the concentration range found in honey they are non-toxic

and safe. Therefore, no MRL values have been fixed in the European Union (Mutinelli, 2003). Natural substances like thymol and organic acids are increasingly used for *Varroa destructor* control in many European countries, and also world-wide.

#### 3.1.2.1. Thymol

In Table V, the data from different residue studies involving different thymol preparations are summarised. If bee colonies were treated with thymol-based acaricides under moderate climate conditions after the honey harvest in August and September, the residues in honey will be low and safe (Tab. V). The residues in beeswax were much greater than those found in honey. Both did not increase with an increasing number of Apilife VAR treatments (Bogdanov et al., 1998c). Thymol residues will evaporate upon storage of combs and wax foundations (Bogdanov et al., 1998a). However, if thymol treatments are carried during the whole bee season, then, in some cases the residues in honey reach levels, that will cause the change of honey taste, which is not permitted according to the international honey regulations (Wallner, 1997a; Bogdanov et al., 1998b, 1999). The sensory threshold of thymol in honey is 1.1–1.5 mg/kg (Bogdanov et al., 1999). As international honey regulations do not allow that the natural taste of honey be changed, a MRL of 0.8 mg/kg has been fixed in Switzerland.

### 3.1.2.2. Organic acids

Oxalic and formic acids are natural honey constituents, widely used in the frame of alternative *V. destructor* control. They have a GRAS status (Generally Recognised As Safe) and the establishment of a MRL is not necessary (Mutinelli, 2003). Residue studies, carried out under controlled experimental conditions after single treatments with formic acid showed that the residue levels was within the natural variation of these acids (Stoya et al., 1986; Hansen and Guldborg, 1988; Krämer, 1994; Capolongo et al., 1996; Radtke and Hedtke, 1998; Prandin et al., 1999; Bogdanov et al., 2002a). Long-term use of formic acid, applied according to the prescriptions, does not lead to an acid increase, but if used during the honey flow, residues might be high enough to change the honey taste (Bogdanov et al., 2002a).

Single or repeated treatments with oxalic acid do not lead to accumulation of residues in honey (Mutinelli et al., 1997; del Nozal et al., 2000; Bernardini and Gardi, 2001; Radetzki and Bärmann, 2001; Prandin et al., 2002; Bogdanov et al., 2002a; del Nozal et al., 2003; Moosbeckhofer et al., 2003).

The results summarised in this section show that *Varroa* control with natural acaricides like organic acids and thymol is a good alternative to treatments with synthetic substances. If used properly, the residues in honey are low and safe.

## 3.2. Antibiotics used against Foul Brood

Antibiotic residues can originate from treatments against the brood diseases American Foul Brood (AFB) or European Foul Brood (EFB). Treatments with antibiotics are not allowed in the EU, while in many other countries they are widely used. Thus, in most EU countries there are no MRL levels for antibiotics, which means that honey containing antibiotic residues are not permitted to be sold. As no residues are permitted, no MRL are established. However some countries, e.g. Switzerland, UK and Belgium, have established Action Limits, which generally lie between 0.01 to 0.05 mg/kg for each antibiotic group.

For the correct detection of antibiotics in honey, the sensitivity and the precision of the detection method play a very important role.

On the other side, the sensitivity of the methods, used to detect antibiotics can vary, as different, mostly non harmonised methods are used for honey analysis. These difficulties should be resolved by establishing a Minimum Required Performance Limit (MRPL), valid for testing laboratories. At present, for most antibiotics the MPRL lies around 10 µg/kg, for chloramphenicol it is about 0.3 µg/kg, and for nitrofurans about 1 µg/kg. Routine analysis of antibiotics is carried out according to the "classical approach" in two steps. In the first one screening is carried out by the Charm test (Salter, 2003) or ELISA (Heering et al., 1998). In a second step the residues of the positively tested samples are quantified mostly by HPLC or LC-MS (Kaufmann et al., 2001). The possibilities and the limits of the different analysis methods were reviewed recently (Bogdanov, 2003). In spite of the present analytical difficulties, there is no doubt, that a substantial part of the presently marketed honey contains residues of antibiotics. Indeed, different reports on antibiotic residues in honey were made at the recent Apimondia symposiums in Celle in 2003 and 2004. Twenty to 50% of the honey, imported in France (Morlot and Beaune, 2003), Belgium (Reybroeck, 2003) and Switzerland (Bogdanov and Fluri, 2000) contained antibiotics, mostly streptomycin and sulfonamides, but also tetracyclines and chloramphenicol. On the other hand, honey produced in Switzerland (Bogdanov and Fluri, 2000), Belgium (Reybroeck, 2003) and Germany (Wallner, 2003) had a lower residue level, varying from 1 to 7%. For Italian honey contradictory results have been reported. In one publications about 5% of the samples were reported to contain tetracycline (Sabatini et al., 2003), while according to another 50% of the Italian samples contained the same antibiotic (Tantillo et al., 2000). A recent publication reported that 2 to 7% of the Italian honey samples tested contained sulfonamides, tetracycline or tylosin (Baggio et al., 2004). During the 2004 Celle conference no substantial change was reported. Results from different laboratories showed that a great part of Chinese honey, but also of honey from various countries, contains chloramphenicol in quantities greater than the EU MRL of 0.3 µg/kg (e.g. Reybroeck, 2003; Verzegnassi et al., 2003). Recently, nitrofurans were also found in honey (Stiftung Warentest, 2004). The different

**Table VI.** Antibiotic residues in honey.

Antibiotics	References
<b>Sulfonamides</b> sulfathiazole, sulfamerazine, sulfamethazine, sulfamethaxazole, sulfadiazine, sulfamethoxypyridazine, sulfadoxine, sulfadimidine, sulfanilamide	(Martel and Zeggane, 2003; Reybroeck, 2003; Wallner, 2003; Kaufmann and Känzig, 2004)
<b>Aminoglycosides</b> streptomycine, dihydrostreptomycine	(Morlot and Beaune, 2003; Reybroeck, 2003; van Bruijnsvoort et al., 2004)
<b>Tetracyclines</b> tetracycline, oxytetracycline, chlortetracycline, doxycycline	(Argauer and Moats, 1991; Tantillo et al., 2000; Morlot and Beaune, 2003; Reybroeck, 2003; Sabatini et al., 2003)
<b>Amphenicols</b> chloramphenicol	(Dharmananda, 2003; Reybroeck, 2003; Verzegnassi et al., 2003; Ortelli et al., 2004)
<b>Macrolides</b> tylosine myrosamine	(Baggio et al., 2004; Feldlaufer et al., 2004) (Nakajima et al., 1998)
<b>Beta-lactams</b> penicillins	(Nakajima et al., 1997; Reybroeck, 2003)
<b>Nitrofurans metabolites</b> AOZ, SC	(Stiftung Warentest, 2004; Jenkins and Young, 2005)

AOZ: 3-amino-2-oxazolidinone; SC: semicarbazide.

antibiotics, which were studied or found in honey are summarised in Table VI.

Although most studies concern residues in honey, antibiotic use in the colony can contaminate also royal jelly (Matsuka and Nakamura, 1990). Chloramphenicol residues has been also found in royal jelly produced in China (Dharmananda, 2003; Reybroeck, 2003).

The residues of antibiotics, encountered in honey, and also in royal jelly are not very problematic from toxicological point of view, as higher MRL for many of them have been established in many foods of animal origin. On the other side, residues of chloramphenicol and nitrofurans are very toxic and practically a zero tolerance has been established for them. For this reason, Chinese honey and royal jelly, which contained often chloramphenicol, were banned from the European and North American markets. In 2004, the ban from the EU market was lifted.

At present, the problem with antibiotics in honey is the most serious for honey trade. How-

ever, the use of antibiotics for the control of AFB is not necessary and does not lead to a control of this pest. Antibiotic residues can be avoided, as AFB can be successfully controlled without the use of antibiotics (Waite et al., 2003; von der Ohe, 2003). Indeed, the experience in different EU countries and New Zealand shows that long-term efficient AFB control can be carried out without the use of antibiotics.

### 3.3. Other substances, used in beekeeping

Some beekeepers use para-dichlorobenzene (PDCB) for the control of wax moth. The substance enters the cycle of beeswax and contaminates commercial beeswax and also honey (Wallner, 1992; Seiler et al., 2003; Bogdanov et al., 2004). Indeed, on average 34% of the honeys samples produced in Switzerland contained PDCB, 13% of these samples exceeded the Swiss MRL of 0.01 mg/kg (Seiler et al., 2003; Bogdanov et al., 2004).

Other, more toxic substances as naphthalene, are also used for wax moth control and residues in honey have been found (EC, 2001).

Wood protectants and paints, used to protect the bee hive against spoilage, should not contain insecticides and fungicides, that might contaminate honey. It has been shown, that when hives were treated with preparations, containing metal-organic substances and the preservative pentachlorophenol, residues of these compounds were found in honey and beeswax (Kalnins and Detroy, 1984).

The use of chemical repellants is another source of contamination, the different chemical repellents been reviewed (Jéanne, 1999). Residues of phenol, one widely used repellent, have been detected (Daharu and Sporns, 1985; Kwan and Sporns, 1988) but the interpretation of the residue data must be made carefully, as phenol is a natural honey constituent (Beckh and Lüllmann, 1998).

Storage of honey in inappropriate containers can also lead to undesirable residues of heavy metals. During the storage of honey, inorganic and organic components can diffuse from the inner surface of paraffinated, corrosive and painted vessels and contaminate it. Increased iron concentrations due to storage of honey in metal containers is a common problem, although iron is a beneficial mineral. Darkening of tea upon addition of honey is due to the high iron content of honey (Morse and Lisk, 1980; Merin et al., 1998).

Recently small amounts of semicarbazide (0.003 to 0.005 mg/kg) originating from the twist-off cover of honey glass jars, were found in honey (Stiftung Warentest, 2004).

Another very serious contamination source is the chemical control of the small hive beetle (SHB). The residue levels in honey and beeswax after the use of check-mite (coumaphos) for the SHB control in the USA are much higher than those found after the use of acaricides containing coumaphos (Nasr and Wallner, 2003).

#### 4. CONCLUSIONS

The results presented in this review show, that the main contamination danger for bee

products originates more from apicultural practices than from the environment.

The main contamination risks for the different bee products are:

- Honey: antibiotics
- Wax: persistent lipophylic acaricides
- Propolis: persistent lipophylic acaricides, lead
- Pollen: pesticides
- Royal jelly: antibiotics.

Beekeepers can take successful measures to prevent the contamination of bee products from beekeeping source, as in all cases there are ecological alternatives. A HACCP (Hazard Analysis and Critical Control Point) system for the control of contamination sources should be developed and applied to beekeeping. The present review provides the foundations for the establishment of such a system, as it covers all major bee product contaminants. Alternative bee pest control strategies and minimal use of synthetic chemicals in beekeeping can keep bee products clean and safe. The introduction of organic beekeeping is an ecological means to avoid all major contamination sources for the production of high quality bee products, free of toxic contaminants.

**Résumé – Les contaminants des produits du rucher.** Les sources de contamination des produits du rucher peuvent provenir soit de l'environnement, soit des pratiques apicoles (Fig. 1). La première partie de cette revue de synthèse décrit les principaux contaminants d'origine environnementale : les métaux lourds, tels le cadmium, le plomb et le mercure, les isotopes radioactifs, les polluants organiques tels les biphényles polycycliques, les pesticides (insecticides, fongicides, herbicides et bactéricides), les bactéries pathogènes et le matériel génétiquement modifié. La contamination par le plomb et le cadmium, trouvée par divers auteurs, est résumée dans le tableau I. Le tableau II présente la contamination des abeilles, du pollen et du miel par les pesticides, d'après l'article de Fléché et al. (1997). La pertinence des risques possibles de contamination est discutée pour chacun des contaminants, de même que le rôle du miel et des autres produits du rucher comme marqueurs de l'environnement. De tous les contaminants issus de l'environnement, les pesticides sont les plus importants pour la contamination du miel et du pollen, tandis que le plomb compromet la qualité de la propolis.

Dans la seconde partie on discute des divers contaminants issus des pratiques apicoles. Des acaricides de synthèse lipophyles et persistants sont utilisés

dans de nombreux pays contre l'acarien *Varroa destructor*. Les acaricides les plus utilisés sont présentés dans le tableau III avec leur limite maximale de résidu. Ces substances polluent principalement la cire d'abeille, alors que le miel est relativement peu touché (Tab. IV). Des acaricides non toxiques tels que les acides organiques et les composés d'huiles essentielles (thymol par ex.) ne compromettent pas la qualité du miel. Les taux trouvés dans le miel sont faibles et sans danger, mais ne compromettent pas la qualité du miel alors que ceux trouvés dans la cire d'abeille sont bien plus élevés (Tab. V). Les antibiotiques utilisés contre la loque américaine et la loque européenne sont aujourd'hui une source importante de contamination du miel. Divers antibiotiques, appartenant à 7 classes différentes, sont actuellement détectés dans le miel (Tab. VI) : sulfonamides, aminoglycosides, tétracyclines, amphénicols, macrolides, beta-lactams et les métabolites du nitrofurane. Actuellement on trouve principalement à l'échelle mondiale des tétracyclines, de la streptomycine, des sulfonamides et du chloramphénicol. L'apiculture fournit aussi d'autres contaminants : le para dichlorobenzène, utilisé contre la fausse-teigne, des insecticides (par ex. le coumaphos) contre le Petit Coléoptère des ruches, des répulsifs utilisés lors de la récolte du miel, des pesticides, pour protéger le bois, et des substances qui diffusent dans le miel à partir des fûts. Les contaminants les plus importants pour les produits du rucher et qui proviennent des pratiques apicoles sont les antibiotiques pour le miel et les acaricides pour la cire et le pollen. Les niveaux de résidus trouvés dans les produits du rucher ne présentent en général pas de risques pour les consommateurs, mais altèrent l'image des produits du rucher comme produits naturels et sains. La conclusion de la synthèse est que les contaminants issus des pratiques apicoles sont actuellement plus importants pour la qualité des produits du rucher que ceux issus de l'environnement.

**miel / cire d'abeille / propolis / gelée royale / pollen / contamination / résidu**

**Zusammenfassung – Kontaminantien der Bienenprodukte.** Die Bienenprodukte können durch verschiedene Quellen kontaminiert werden: von der Umwelt oder von der Imkereipraxis (Abb. 1). Im ersten Teil des Reviews werden alle Umweltkontaminantien besprochen: Schwermetalle wie Kadmium, Blei und Quecksilber; radioaktive Isotope; organische Kontaminantien wie polycyclische Biphenyle; Pestizide (Insektizide, Fungizide, Herbizide und Bakterizide); pathogene Bakterien und genetisch modifizierte Materialien. Die Honigkontamination durch Pb und Cd, die von verschiedenen Autoren gefunden wurde, ist in Tabelle I zusammengefasst. In Tabelle II ist die Kontamination von Bienen, Pollen und Honig nach Fléché et al. (1997), wiedergegeben. Die Relevanz der verschiedenen Kontaminationsrisiken, sowie die Rolle des

Honigs und der anderen Bienenprodukte als Umweltmonitoren wird diskutiert. Von allen Umweltkontaminantien am wichtigsten ist die Kontamination von Honig und Pollen durch Pestizide und diejenige der Propolis durch Pb.

Im zweiten Teil der Übersicht werden die Kontaminantien aus der Bienenzucht besprochen. Persistente lipophile Akarizide, werden in vielen Ländern für die langfristige Kontrolle der Varroatose angewendet. Die wichtigsten Akarizide und ihre MRL-Werte sind in Tabelle III wiedergegeben. Diese Substanzen kontaminieren in erster Linie das Bienenwachs während der Honig wenig belastet wird (Tab. IV). Nicht-toxische Akarizide wie organische Säuren und Komponenten von essentiellen Ölen wie Thymol gefährden nicht die Honigqualität. Die Thymolbelastung von Honig und Wachs ist in Tabelle V wiedergegeben. Die Werte von Thymol in Honig sind tief und unproblematisch, diejenigen in Wachs sind viel höher, stellen aber keine Kontaminationsgefahr für den Honig dar. Antibiotika, die für die Bekämpfung der Amerikanischen und der Europäischen Faulbrut verwendet werden, sind gegenwärtig die wichtigste Kontaminationsquelle für den Honig. Antibiotika verschiedener Klassen wurden in Honig gefunden: Sulfonamide, Tetracycline, Aminoglykoside, Ampheningole, Makrolide, Beta-Laktame und Nitrofurane (Tab. VI). Verschiedene Antibiotika, in erster Linie Tetracycline, Streptomycin, Chloramphenicol und Sulfonamide wurden in Honigen aus verschiedenen Ländern der ganzen Welt nachgewiesen. Andere Kontaminantien aus der Bienenzucht sind: Paradichlorbenzol für die Bekämpfung der Wachsmotte; Insektizide (z.B. Coumaphos) für die Kontrolle des Kleinen Beutenkäfers; Pestizide aus Holzschutzmitteln und Substanzen, die von Honigbehältern in den Honig hinausdiffundieren. Die wichtigsten Kontaminantien aus der Bienenzucht sind: Antibiotika für Honig und Gelée Royale und Akarizide für Wachs und Propolis.

Die Kontamination der Bienenprodukte stellt im allgemeinen kein Gesundheitsrisiko für Konsumenten dar aber gefährdet ihr Image als natürlich und rein. Die Schlussfolgerung des Reviews ist, dass das Verschmutzungsrisiko aus der Bienenzucht gegenwärtig grösser ist als dasjenige aus der Umwelt.

**Honig / Wachs / Propolis / Gelée Royale / Pollen / Kontamination / Rückstände**

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