RESIDUES OF VETERINARY DRUGS IN HONEY AND POSSIBLE APPROACHES TO DERIVE MRLS FOR THIS COMMODITY

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The 66th meeting of the Committee discussed the report of the 2005 meeting of the Joint FAO/RIVM/WHO workshop "Updating the Principles and Methods of Risk Assessment: Maximum Residue Levels (MRLs) for Pesticides and Veterinary Drugs" (FAO/WHO, 2005). With regard to the workshop recommendation on honey, that JECFA should investigate a specific approach for MRLs in honey and prepare a paper to consider if a separate approach for honey is warranted, the 66th meeting of the Committee agreed that a paper should be prepared for consideration at the next meeting of the Committee devoted to residues of veterinary drugs in food. The paper should consider the relevant scientific issues specific to honey, and accordingly, develop draft recommendations for consideration by the Committee and by CCRVDF. It should be noted that the 52nd meeting of the Committee (FAO/WHO, 2000) first considered the subject and requested that the 12th Session of the CCRVDF comment on the matter. The report of the 12th Session of the CCRVDF does not include any comments on the matter.

There is substantial production and trade in honey worldwide; however, there are very limited numbers of standards for residues (MRL) in honey. Table 1 shows five year honey production figures for the different parts of the world. Asia is the greatest honey producer. Other major honey producing regions or countries are the European Union, the United States and Argentina. Honey production does naturally vary from year to year depending on all manner of environmental factors such as cropping or the weather and the impact of pests and pathogens.

Table 1: World honey production by region 2001-2005 (1000t)

	2001	2002	2003	2004	2005
Africa	145	153	152	152	154
North and Central America	193	188	191	187	188
South America	127	132	137	133	133
Asia	458	497	525	543	545
Europe	311	294	320	328	332
Oceania	29	23	29	29	29
Total	1,264	1,287	1,354	1,372	1,381

Source FAO

A list of products used world-wide, including the active ingredient where it is available is included in Table 2. ADIs established either by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) or the Joint FAO/WHO Expert Committee on Food Additives (JECFA) are indicated in the table.

Table 2: List of products used in apiculture

Substance	Proprietary product	ADI [mg/kg bw/day] JECFA	JMPR
Acrinathrine	Yes		
Amitraz	Yes		0-0.01
Bromopropylate	Yes		0-0.03
Chlorobenzilate	No		0-0.02
Chlortetracycline	No	0-0.003	
Coumaphos	Yes		[2]
Cymiazole hydrochloride	Yes		
Enilconazol (imazalil)	No		0-0.03
Erythromycin	No	0-0.0007	
Fenproximate	Yes		
Fipronil	No		0-0.0002
Flumethrin	Yes		0-0.004
Formic acid[1]	Yes	0-3	
Fumagillin	Yes		
Lactic acid[1]	No	Not limited	
Lincomycin hydrochloride	?	0-0.03	
Malathion	No		0-0.3
Menthol[1]	Yes	0-4	
Methyl bromide	No		
Monensin	No	0-0.01	
Oxalic acid[1]	Yes		
Oxytetracycline	Yes	0-0.003	
Paradichlorobenzene	No		
Permethrin	yes		0-0.05
Propargite	?		0-0.01
Rifampicin	No		
Spinosad	No		0-0.02
Streptomycin/ dihydrostreptomycin	No	0-0.05	
Sulfathiazole	No	No ADI allocated	
Tau-Fluvalinate	Yes		
Thymol[1]	Yes	Acceptable	
Tylosin tartrate	Yes	0-0.03	

[1]. Substances considered by many national authorities as generally recognized as safe
[2]. Temporary ADI withdrawn in 1980; no ADI allocated in 1990

Points to consider

Several factors have to be considered in developing a process to address the need for recommending MRLs pertaining to the use of veterinary drugs and pesticides for bee health and honey production submitted to the Committee for evaluation. They include but may not be limited to the following points:

- The recommendations must be within the Committee's terms of reference with adequate flexibility to meet differing conditions and availability of information.

- There is a need to accommodate a robust yet conservative approach to facilitate MRL recommendations and encourage sponsorship for studies that the Committee considers necessary for recommending MRLs.

- The drug is available as a commercial product, and the commercial product containing the active ingredient is currently registered by a national or regional authority.

- Honey production and honey bees in most countries are considered as a minor use and/or minor species food product, and the availability of active sponsors to provide studies suitable for recommendations on MRLs is likely to be limited.

- Honey is widely used as a sweetener and glazing agent in confectionary products, breakfast cereals and baked goods, in addition to direct consumption of liquid and set honey, and these uses must be accounted for in intake estimates.

- Several substances use to manage bee health are unlikely to raise public health concern, because intake of residues resulting from effective use are far below internationally established ADIs.

- Some proprietary products are not registered for use in bee colonies, and therefore approved dosages and conditions of use do not exist.

- For a number of substances registered for use by national authorities, no contemporary toxicological evaluation may have been performed, or the review did not result in the establishment of a health-based guidance value, such as an ADI.

- The main groups of substances that typically leave residues in edible bee products are antibiotics (residues in honey and royal jelly) and persistent lipophilic acaricides (residues in wax and propolis).

- Royal jelly should be the subject of a separate and later consideration.

- Residues in both honey and wax need to be considered in exposure estimates. The ratio honey to wax is typically 9:1.

Substances with an ADI and/or MRL in a food producing animal or food commodity

The main groups of substances which typically leave residues in edible bee products are antibiotics (residues in honey and royal jelly) and persistent lipophilic acaricides (residues in wax and propolis). Of the products known to be used for treatment of bee diseases listed in Table 2, most, but not all have a national registration, a JECFA or JMPR evaluation with an ADI and/or MRL, or the equivalent, in national legislation, for either a food producing animal or other food commodity, and usually the active ingredients are substances with a long history of use.

Where an established ADI or MRL exists for use in other species as either a veterinary medicine or pesticide, application to a minor use/minor species (e.g., bee and honey products) would generally require a smaller set of additional data as an ADI (or MRL) exists in a generally recognized major food producing animal or bird and their products (e.g., milk and eggs). A critical issue, however, are the studies necessary to provide the relevant data. Because of the complexity of honey production by bees, this may be difficult, as discussed below.

Veterinary drugs (or pesticides) for apiculture use submitted to the JECFA for evaluation should meet general criteria for evaluation, including that the use of the drug will result in drug residues in honey and other edible products obtained from bees, that may constitute a potential public health concern and/or cause impediments in trade. The submitted dossier should include confirmation of

authorization and a precise description of approved dosages and conditions of use. The dossier ought to include data suitable for the establishment of a health based guidance value (for substances yet without an international established ADI) and for the evaluation of residues including the recommendation of MRLs.

<u>Substances generally regarded as safe</u>

Several substances are unlikely to raise public health concerns because any use in food producing animals or especially the use in bees is generally regarded as safe. Examples of such substances include formic acid, lactic acid, oxalic acid, thymol and menthol. In the case of a substance that has clear documentation to support the designation as "generally regarded as safe" by national regulatory authorities and not requiring a MRL, a similar designation can be made. It would require a proviso that equivalence can be demonstrated in honey and that the ADI is sufficient so that no MRL should be required and the ADI is not exceeded. In the case of a new substance not previously considered for registration by national authorities, substances would have to be evaluated as new animal drugs or pesticides and subject to a full food safety risk assessment.

<u>Use of non-approved veterinary drugs or pesticides</u>

In the situation where a substance is not approved for use in food producing animals (e.g., chloramphenicol or nitrofurans), no exception for honey would be applied.

<u>Suggested tools for data generation</u>

Account must be taken of the unique nature of honey and how the residue behaves in honey as well as the numerous factors noted above. It should be noted that all the drug residues and metabolites collect in the honey and the only mechanisms for reduction are dilution as more honey is produced or removed from the hive, photochemical or thermal degradation of the residues in the honey or through such factors as pH and environmental conditions.

The biological variability of residue concentrations found under seemingly the same conditions in a trial or under similar conditions in different trials may be very high, as bees commonly move honey around the hive as required, and this can lead to significant variation in residue concentration even across the same frame in all three hive dimensions. This suggests the need for trials to be conducted over more than one honey producing season to take account of seasonal variability. Likewise, a number of active substances are not stable in honey.

Residue Study Design

For substances leaving residues in honey and related products, well designed residue trials under the established practical conditions of use should be conducted. The trials should preferably be performed under GLP compliant conditions and use of data from non-compliant studies would need to be justified. Design criteria for residue data studies should include the following considerations:

- the number of apiaries involved representing a variety of honey types;

- number of hives per apiary sampled;

- number of frames per hive sampled;

- number of samples of wax and honey to be taken from a frame;

- number and spacing of time points to describe the kinetics of formation and depletion of honey in the edible products;

- estimates of amounts of surplus honey present at the beginning, during and after the treatment until the end of the trial;

- scheme for the analysis of individual and bulk samples;

- climatic information for the duration of the trial including season of the year (e.g. rainfall);

- crop on which bees forage;

- temperature profile within the hive;

- data on honey flow periodicity;

- data on any supplemental feed given to bees;

- data on bee health and bee/parasite mortality during the study

- a protocol for the analysis of individual and bulk samples

- studies on storage stability of residues in honey.

- analytical methods should be suitable for the purpose and validated in the different matrices (honey, wax, etc.)

Should the Committee make a recommendation on sampling parameters, it should include something comparable to a minimum of 10 samples each of 20 g taken from random positions within one hive.

The quality of the data should allow a statistical evaluation to determine the confidence intervals necessary to recommend the setting of MRLs. The data should show with 95% statistical confidence that 95% of all honey samples from treated bees would be below the MRL and that the estimated intake of residues (considering all other sources of intake) remains below the ADI. As the design of the study depends on many factors, it has to be developed on a product by product basis, depending on the use pattern.

Marker residue

The marker residue concept may not be normally or easily applied in honey scenarios. However, it is important to sufficiently identify and, where feasible, quantify metabolites and degradation products in honey. If a marker residue is proposed and it can be demonstrated that it is appropriate it may be a practical consideration. Analysis of data in shown in Annex 2 on products used in honey bees indicates it may not work in all cases – it could work with amitraz as all residues are hydrolyzed to a single substance for residue analysis. For tylosin it may not, as one of the tylosin metabolites also has antimicrobial activity and it would require the analysis of both microbiologically active substances for exposure considerations (Annex 2 provides more detailed explanations). The ratio of marker to total residue concentrations needs to be established for the whole time period from drug application to the end of the withdrawal time for each matrix.

Dietary intake considerations

The internationally accepted daily dietary intake of honey is generally taken to be 20 g per person per day, as per the JECFA model diet. A review of the WHO GEMS Food 13 cluster diets indicates that median daily intakes are equal to or less than 2 grams per day. These data have been generated based on the FAO food balance sheets and may underestimate consumption by honey eaters. In discussing the criteria for the establishment of an estimate of chronic intake, the Committee concluded that such a figure should be derived from consumption data for "chronic" honey eaters. A number of countries have data specific to consumption in their areas that may be very useful for national standards.

Adequate information regarding how dietary surveys have been conducted is necessary to properly assess dietary consumption figures. Comprehensive intake data are available from the UK on honey consumption across the population and information on key groups is given in the table below. The mean consumption for these groups is within the 20 g per person per day in the standard JECFA diet. However, there are extreme consumers with a chronic exposure in excess of this figure. Table 3 does not show ingestion on a bodyweight basis and this figure will be higher for children and infants.

Table 3: UK consumption of honey in 2000 (UK Food Standards Agency data)

Consumer group	Exposure type	Mean consumption (g/day)	97.5 Percentile consumption (g/day)	Maximum consumption (g/day)
Adult	Acute	7.8	41.2	152
Adult	Chronic	2.4	15.0	77.1
Free living elderly adult	Acute	7.8	42.0	115
Free living adult	Chronic	4.7	32.2	97.8
School children	Acute	4.9	26.4	90
Infant	Acute	2.6	16	64
Infant	Chronic	1.0	7.0	45.1

Explanation: Chronic = the amount consumed by individuals over 7 days computed as the average for 1 day. Acute = the highest days consumption for each individual in the survey. Mean Consumer = the total consumption in grams of the food divided by the number of consumers of that food in the survey

The Committee used a study conducted in 1986-1989 in Germany as the basis for the study of methodological aspects of deducing a figure for daily intake of honey, because consumption data were available for more than 9000 consumers of honey. Germany has one of the largest populations of honey consumers in Europe. The data set includes records on more than 23,000 individuals (approximately 47% ≥ 14 years old). Of the more than 23,000 individuals, about 9,000 consumed honey at least once a day. In this data set, the median portion consumed was 18 g per person with a range of 0.1 to 222 g/day. The total consumption of the people over the whole observation period was from 0.54 to 576 g. The results are given in the tables below.

Table 4: Statistics of the individual portions and of the daily intakes

Number of portions:	25730	Number of people:	9019
	Size of portion [g]		Daily intake[1]
Min	0.1	Min	0.08
Max	222	Max	82.29
Median	18	Median	5.57
P90	40	P90	17.14
P95	48	P95	23.44
P97.5	57	P97.5	29.08
P99	75	P99	37.99
P99.9	120	P99.9	67.07
P99.95	144	P99.95	75.02
P99.99	207	P99.99	81.51

[1.] Averaged over 7 days observation period

The data in Table 4 indicate that for a high percentile portion, suitable for use as an estimation of acute dietary intake, consumption is between 100-150g. Table 4 indicates the current JECFA intake

value is between the 90th and 95th percentile of daily intakes. The median daily intake of all eaters (Table 4) is below the current dietary consumption of 20 g/day. The data provided by the UK and Germany suggest that an acute dietary consumption factor needs to be determined by the Committee.

Table 5: Statistics of the average portions consumed by a person (only days where honey is consumed)

Portions of honey consumed in 7 days:	1	2	3	4	5	6	7	8	9 to 12
Number of people	2983	1886	1374	914	693	571	521	47	30
	Average size of a portion [g]								
Min	0.5	0.5	1.2	2.0	2.3	0.8	4.5	7.5	3.6
Max	144.0	85.0	96.0	98.0	80.8	96.0	81.4	58.8	30.0
Median	18.0	16.5	17.2	16.0	16.2	17.3	18.0	14.8	14.9
P95	48.0	45.0	44.0	40.5	39.6	40.0	47.6	Sample size too small for meaningful calculations	
P97.5	54.0	50.0	54.0	46.6	47.9	48.1	55.3		
P99	72.0	60.0	65.5	53.8	52.2	60.0	67.3		
P99.9	100.0	80.5	90.3	86.1	68.2	89.2	81.0		

Table 6: Statistics of the averaged daily intakes of a person (total amounts consumed averaged over 7 days)

Portions of honey consumed in 7 days:	1	2	3	4	5	6	7	8	9 to 12
Number of people	2983	1886	1374	914	693	571	521	47	30
	Daily consumption [g]/person								
Min	0.08	0.15	0.53	1.17	1.66	0.73	4.50	8.57	5.15
Max	20.57	24.29	41.14	56.00	57.71	82.29	81.43	67.14	38.57
Median	2.57	4.71	7.36	9.14	11.57	14.86	18.00	16.86	20.27
P95	6.86	12.86	18.86	23.14	28.29	34.29	47.57	Sample size too small for meaningful calculations	
P97.5	7.71	14.29	23.14	26.65	34.22	41.25	55.29		
P99	10.29	17.14	28.05	30.75	37.26	51.43	67.29		
P99.9	14.29	22.99	38.70	49.22	48.72	76.42	80.98		

Additional data to collaborate the tables and discussions above is provided in Annex 1.

RECOMMENDATIONS

In considering the matters of interest noted in this report and the complex and unique nature of honey and honey bees, the Committee may not be able to take any specific approaches without further guidance from CCRVDF. The Committee therefore made the following recommendations to CCRVDF:

1. That CCRVDF with the aid of member countries compile a comprehensive list of all veterinary drugs registered for honey production and bee health and develop a priority list of veterinary drugs for use in honey bees to be considered for risk assessment by JECFA.

2. That CCRVDF and member countries be encouraged to provide data on honey consumption, considering both direct and indirect honey intake, for purposes of improved intake assessments as part of the risk assessment for recommending MRLs.

3. That CCRVDF consider extension of good veterinary practice guidelines to include honey production.

4. That the CCRVDF ad hoc Working Group on Methods of Analysis and Sampling consider analytical methods for residues in honey.

5. That the CCRVDF provide guidance on the appropriate percentile for an estimation of acute intake.

The Committee further makes the following recommendation to the JECFA Joint Secretariat:

1. That the JMPR Joint Secretariat be advised of the Committee's report regarding residues in honey and considerations of residues from use of pesticides in honey production and bee health.

REFERENCES

FAO/WHO (2000). Evaluation of Certain Veterinary Drug Residues in Foods (Fifty-second Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 893.

FAO/WHO (2005). Final report from Workshop on Update of Principles and Methods of Risk Assessment – MRLs for pesticides and veterinary drugs. FAO/RIVM/WHO, Bilthoven, the Netherlands, 7-11 November, 2005. Available at the FAO JECFA website at: ftp://ftp.fao.org/ag/agn/jecfa/bilthoven_2005.pdf .

ANNEX 1

Supporting Documentation - Honey Consumption

The Committee reviewed the adequacy for the estimation of acute and chronic intakes of its currently used consumption figure of 20 g honey per person per day. When the original food basket was established at early meetings of the Committee and at the first sessions of the CCRVDF care was taken that the consumption figures protected the preferential eater of foods of animal origin. With the data available in the late 1980's, the approximately 97.5[th] percentile of daily consumption by the consumers of a commodity from a country with a known high consumption of honey was chosen. It was furthermore considered necessary to derive figures which would also cover the intake resulting from consumption of processed products containing the raw commodity. There was concern that the consumption figure for honey did not meet the above criteria.

For the review at the seventieth meeting of the Committee, very limited non-aggregated recent consumption data was available in the public domain. However, the Committee considered as a basis for studying the methodological aspects to find an appropriate honey intake a study conducted in 1986-1989 in Germany (NVS 1989), because from this study non-aggregated consumption data of more than 9000 consumers of honey were available in an electronic public use file. Additional information from the United Kingdom, the Netherlands and Germany was made available by experts participating in the meeting.

Germany is one country with high honey consumption. Of the 23209 individuals (10985 $\geq$ 14 years old) delivering valid data in the above mentioned study, 9019 had consumed honey at least once on one day of the 7 day observation period. For a few persons data for more than 7 days were available. A total of 25730 portions were consumed with a median of 18 g per portion and a range of 0.1 to 222 g per portion. The total consumption of the participants over the whole observation period was from 0.54 to 576 g and was slightly higher for males compared to females (for example, in seven days the following total amounts were consumed: median for 3845 males 42 g, median for 4753 females 36 g; 97.5[th] percentile for males 225g, 97.5[th] percentile for females 189 g). When consumption was compared in relation to body weight of the participants consumption was slightly higher for females (for example, in seven days the following total amounts were consumed on a body weight basis: median for 3845 males 0.253 g/kg bw, median for 4753 females 0.268 g/kg bw; 97.5[th] percentile for males 0.952 g/kg bw, 97.5[th] percentile for females 0.968 g/kg bw). Some persons consumed up to 12 portions during the observation time, which means 2 portions on several days; however, the median consumption frequency was 2 portions in seven days. A small subpopulation of individuals ate honey every day. The results of an initial statistical evaluation are given in the tables below.

The statistics of the individual portions (left part of table 1) could serve as a basis for the determination of the *"acute intake"*. The acute intake represents a *high single intake* or a *high amount consumed over a short period of time*, such as one day. Since only a few people consumed honey more than once a day, a statistics of the 25580 individual daily intakes would yield almost exactly the same values for the median and higher percentiles as the above statistics of portion sizes. Thus, the left part of table 1 can be directly used to determine an appropriate figure for an estimated acute intake. The right part of table 1, however, is not suitable to estimate the "chronic intake". Most persons consumed honey only twice in seven days, and this cannot be considered chronic intake.

Table 1: Statistics of the individual consumed honey portions and daily intakes

Number of portions:	25730	Number of persons:	9019
	Portion size [g]		Daily intake*[g]
Min	0.1	Min	0.08
Max	222	Max	82.29
Median	18	Median	5.57
P90	40	P90	17.14
P95	48	P95	23.44
P97.5	57	P97.5	29.08
P99	75	P99	37.99
P99.9	120	P99.9	67.07
P99.95	144	P99.95	75.02
P99.99	207	P99.99	81.51

*averaged over the whole observation period

Therefore, it was necessary to look at the subpopulation of individuals who consume honey more regularly in order to obtain a suitable estimate of chronic intake. Such an approach is discussed on the basis of the following two tables 2 and 3. In table 2, it is investigated whether or not the portion size of those who consumed honey occasionally and of those who consumed it more regularly is similar. The 9019 consumers were grouped into nine groups according to the number of portions they ate during the observation period. The results obtained for the first seven groups are shown in the tables. Intake was calculated as total amount consumed divided by the number of portions eaten. It can be seen, that the statistics of the median and of the higher percentiles of the average portions sizes are more or less similar for all groups. This means that - if some people consumed more honey than others – this was primarily due to higher frequencies of consumption.

Table 2: Statistics of the averaged daily amounts consumed by a person
(Average calculated for the days where the persons consumed honey)

Portions of honey consumed in 7 days:	1	2	3	4	5	6	7
Number of persons	2983	1886	1374	914	693	571	521
	Daily consumption [g]/person						
Min	0.5	0.5	1.2	2.0	2.3	0.8	4.5
Max	144.0	85.0	96.0	98.0	80.8	96.0	81.4
Median	18.0	16.5	17.2	16.0	16.2	17.3	18.0
P95	48.0	45.0	44.0	40.5	39.6	40.0	47.6
P97.5	54.0	50.0	54.0	46.6	47.9	48.1	55.3
P99	72.0	60.0	65.5	53.8	52.2	60.0	67.3
P99.9	100.0	80.5	90.3	86.1	68.2	89.2	81.0

The median portion size is more or less independent on the frequency of honey consumption.

However, if one now calculates (see table 3) the daily intakes for the same groups and consumption averaged over the 7 days observation period, the median intake and the higher percentiles increase from the group with the lower consumption frequencies to those with higher consumption frequencies. Compared with the right part of table 1 giving an overall median of 5.57 g/person/day one now obtains a median of 18.0 g/person/day for the 521 individuals who consumed honey seven times during seven days. This finding is trivial, but typically ignored in the evaluation of many studies. Column 7 is identical in both tables 2 and 3. The values of column 7 of table 3 represent the best estimate of chronic intakes which can be obtained from the data of this study. Nearly the same numerical values are obtained if the total consumption of the individual persons is divided by the number of the corresponding days on which honey was consumed. If this is done for all 9019 consumers the median, 95th and 97.5[th] percentiles are 17.8, 48, and 55.3 g per person and day, respectively.

Table 3: Statistics of the averaged daily amounts consumed by a person
(Average calculated from total consumption of a person divided by 7)

Portions of honey consumed in 7 days:	1	2	3	4	5	6	7
Number of persons	2983	1886	1374	914	693	571	521
	Daily consumption [g]/person						
Min	0.08	0.15	0.53	1.17	1.66	0.73	4.50
Max	20.57	24.29	41.14	56.00	57.71	82.29	81.43
Median	2.57	4.71	7.36	9.14	11.57	14.86	18.00
P95	6.86	12.86	18.86	23.14	28.29	34.29	47.57
P97.5	7.71	14.29	23.14	26.65	34.22	41.25	55.29
P99	10.29	17.14	28.05	30.75	37.26	51.43	67.29
P99.9	14.29	22.99	38.70	49.22	48.72	76.42	80.98

Some studies (including the more recent UK study discussed below) found that infants and young children have a significantly higher daily intake in relation to their body weights than adults. In order to investigate this finding, on the basis of the data from the study in Germany, the consumers were grouped according to sex and age (from 4 years to 80 years in steps of one year), and a separate group for the individuals >80 years). For each person the total consumption was divided by the number of days on which honey was consumed and by the body weight. Body weights were available only for 3845 males and 4753 females. Sample sizes for individual age groups were ranging from 9-92 for males, 12-104 for females and 21-196 for both sexes combined. If the size of an age subgroup was $\geq$ 30, a median of the consumption, expressed as g honey/kg of bw/day was calculated; in cases where the group size was $\geq$ 50, a 95th percentile was also estimated.

The results are given in the figure 1, separately for each sex and for both sexes combined. For those age groups with less than the required sample sizes for the calculations a symbol is placed on the zero consumption line representing a missing value. There were sufficient age groups eligible to perform the calculations and to establish a trend. The figure clearly indicates that there is a sharp decline in honey consumption per kg of body weight during the first years of life.

Figure 1: Consumption of honey, expressed in g/kg bw as function of age
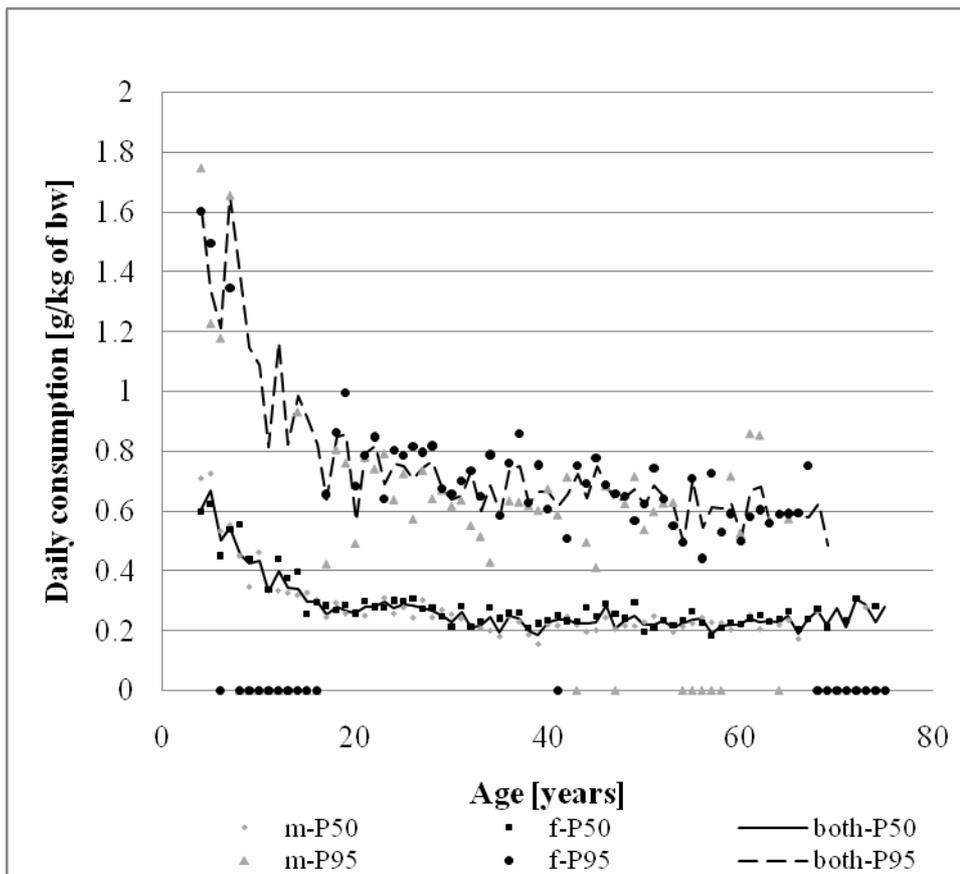

m: males, f: females, P50: 50th percentile, P95: 95th percentile

It was important to find out whether this decline is due to decreasing consumption, increasing body weight or to both influencing factors. Therefore the figure 2 shows the absolute amounts of daily intakes as function of age for consumer between the ages of four years and twenty years. Three of 8698 available data points have been omitted from figure 2 (consumption < 1 gram/day) in order to improve the format of presentation.

For the purpose of figure 2 the total consumption reported for the 7 day reporting period was divided by the number of days on which honey was consumed. The figure shows that both the median and the 95th percentile of daily intakes increase moderately over the first years of life. Therefore, an intake figure which is properly selected from the previously shown statistics for the calculation of JECFA EDIs would be applicable to all age groups. The decrease in consumption expressed on a body weight basis is primarily attributable to increasing body weights.

Figure 2: Variation of median and 95th percentile intakes of honey as function of age.
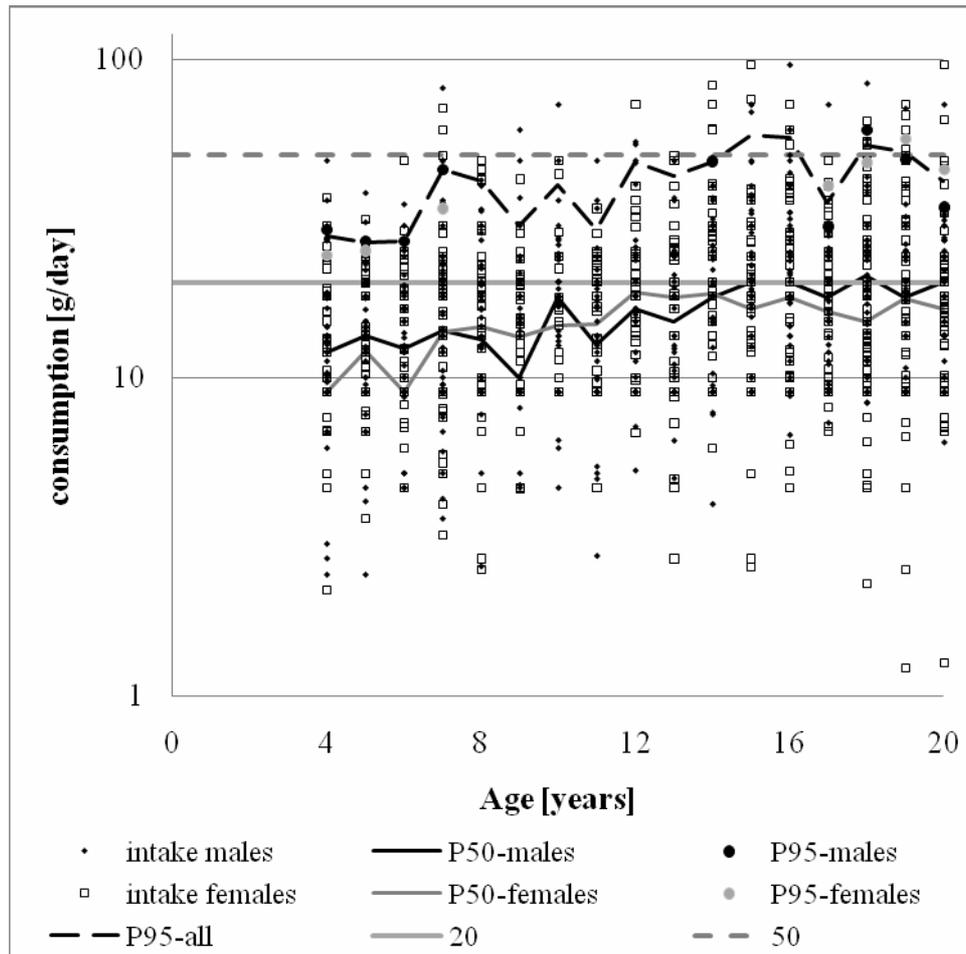
More recently the Food Standards Agency of the United Kingdom (FSA-UK) carried out a survey yielding also data on honey consumption (National Dietary and Nutrition Survey). Aggregated data were available for the groups defined in table 4.

Table 4: Group design of the FSA study

Group	Type	Explanation
Adults	Chronic	The amount consumed by individuals over 7 days then divided to get the average for 1 day.
Free living elderly		
Infants		
Adults	Acute	The amount consumed on the highest day's consumption for each individual in the survey.
Free living elderly		
Infants		

Except for the chronic intakes of infants all data were expressed in gram per day as well as in gram per kg of body weight per day. The results are summarised in tables 5 a-d.

Table 5a: Acute intake of honey in the FSA study

	Consumer Group		
	Adults	Free living elderly	Infants
Participants	1724	1275	488
Consumers	747	464	216
	Intake [g/day]		
min	0	0	0.001
median	2.973	2.123	0.609
P97.5	41.197	41.985	16
P99	62.313	71.852	26.72
max	152	115	64

The ratios consumers/participants are very similar to those observed in the German study (around 0.4). A direct comparison with the German data is not possible. However, the median "acute" intake of adults seems to be very much lower and the higher percentiles are only moderately lower in the FSA study compared to the German study.

Table 5b: Chronic intake of honey in the FSA study

	Consumer Group		
	Adults	Free living elderly	Infants
Participants	1724	1275	488
Consumers	747	464	216
	Intake [g/day]		
min	0	0	0
median	0.711	0.838	0.113
P97.5	15.006	32.204	6.971
P99	24.849	52.695	14.48
max	77.143	97.75	45.145

The estimated median chronic intakes are extremely low. It is not known whether the individuals participating in this survey consumed honey regularly. The higher percentiles are similar in both studies. A possible explanation could be that the lower percentiles are heavily influenced by the data of the occasional eaters. For the higher percentiles, the influence of the data for the people who eat honey every day is significant.

Table 5c: Acute intake of honey, in relation to body weight in the FSA study

	Consumer Group		
	Adults	Free living elderly	Infants
Participants	1724	1275	488
Consumers	747	464	216
	Intake [g/kg bw/day]		
min	0	0	0
median	0.034	0.022	0.089
P97.5	0.6924	0.71	2.734
P99	0.918	0.924	4.774
max	1.751	1.649	10.936

Again the estimated median values are extremely low; the higher percentiles are not too different from the data in the study from Germany. The data for infants cannot be directly compared, since the youngest children in the German study were 4 years old; however, if one compares with figure 1, the results of the FSA survey seem to qualitatively confirm the trend calculated for the data from Germany.

Table 5d: Chronic intake of honey, in relation to body weight in the FSA study

	Consumer Group		
	Adults	Free living elderly	Infants
Participants	1724	1275	
Consumers	747	464	
	Intake [g/kg bw/day]		
min	0	0	
median	0.008	0.009	
P97.5	0.218	0.53	
P99	0.361	0.707	
max	0.889	1.099	

For the German data, the factor between 97.5[th] percentile and median is typically about 3-5, depending on the type of statistics, and for the 99th percentile and 97.5[th] percentile about 1.3. For the FSA data the 97.5[th] percentile is 14 to 62 times the median and the 99th percentile is 1.3 to 1.8 times the 97.5[th] percentile.

Data from a survey in the Netherlands (Dutch Food Consumption Survey) had been evaluated. In the Dutch Food Consumption Survey 1997/1998 (VCP3), 6250 respondents registered their food consumption on two consecutive days. 324 respondents (5%) indicated to have consumed honey on at least one of the two survey days. From this it can be concluded that at least 5% of the Dutch population eats honey on a regular basis, for it may be possible that other respondents ate honey, but just not on the survey days. A somewhat more refined estimation is as follows: 251 respondents indicated to have consumed honey on day 1, and 223 respondents on day 2. On average this would mean that on a random day 3.8% (237/6250 x 100%) of the population eats honey. From this it may be concluded that:

- Honey is consumed by about 5% of the population.

- The average intake of Dutch population is 0.7 grams per day.

- Honey consumers (5% of population) consume on average 13g honey per day;

- High consumers (95th percentile of honey consumers) have an intake of 30g of honey per day.

A recent German survey (Banasiak, et al. 2005) found that the 97.5[th] percentile of honey consumption by children of the age range of 2 up to 5 years of age was 22.1 g/child/day.

In discussing criteria for the establishing an estimate of chronic intake, the Committee concluded that such a figure should be derived from the consumption data of the "chronic" eaters only. In the study from Germany, the 97.5[th] percentile of consumption by the subgroup consuming ≥7 portions in a week was approximately 55 g per consumer per day. The data from the UK were also based on a 7 day survey. Median intakes were low; however, the highest estimated percentiles were not too different from the data from Germany. Data from a 2 day survey in the Netherlands had also been evaluated and the obtained results were significantly lower than estimates based on data from UK and

Germany. Since the data from the UK clearly indicated that on a body weight basis infants and young children have the highest consumption this finding was further investigated and the Committee concluded that this was mainly due to the lower body weight of this group and not to higher intake. In this context a recent German survey found that the 97.5[th] percentile of honey consumption by children of the age range of 2 up to 5 years of age was 22.1 g/day. The Committee concluded that a consumption figure of 50 g/person and day would be expected to protect all groups of consumers; however, further data are necessary to determine whether this figure also sufficiently covers the consumption of products containing honey.

Honey combs with their original honey content are consumed by a subgroup of consumers. Many lipophilic substances used as acaricides accumulate in wax. Therefore, the labels of certain registered products warn that wax from bees treated with the product should not be consumed. The Committee concluded that in cases where honey combs can be safely consumed it would use a ratio of 9:1 for honey and wax in the estimation of intakes.

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National Dietary and Nutrition Survey. The results of this survey are published in five volumes issued 2002-2004. Links to the reports can be found on the web site of the Food Standards Agency under: http://www.food.gov.uk/science/dietarysurveys/ndnsdocuments/
The information contained in this monograph was communicated by Dr. Jack Kay.

Dutch Food Consumption Survey. A summary of the results was prepared by Polly Boon (RIKILT) and Martine Bakker (RIVM) and communicated through Ir. Astrid S. Bulder.

Banasiak, U., Heseker, H., Sieke, C., Sommerfeld, C., and Vohmann, C. (2005). Abschätzung der Aufnahme von Pflanzenschutzmittel-Rückständen in der Nahrung mit neuen Verzehrsmengen für Kinder. Bundesgesundheitsbl - Gesundheitsforsch - Gesundheitsschutz 48, 84–98.

ANNEX 2

Supporting documentation – Residues in honey and other bee's products– selected examples

Amitraz

Amitraz is typically used as a sustained-release strip containing 500 mg of amitraz; however, other modes of application are known as well. The recommended treatment is suspension of two strips per hive for a period of 6 weeks. Other applications involve application of aerosols of emulsions. The metabolism of amitraz yields similar products in animals and plants. The 1998 JMPR evaluation proposes metabolic routes in crops and animals. Metabolism in bees has not been studied; however, the major degradation products occurring in honey are known. These are N-(2,4-dimethylphenyl)-N'-methylformamidine (DMPF) and 2,4-dimethylphenyl-formamide (DMF). DPMF may further degrade to form DMF and 2,4-dimethylaniline (DMA). The marker residue for honey in the EU is the sum of amitraz and all metabolites containing the 2,4-dimethylaniline moiety, expressed as amitraz.

According to European Medicines Agency (EMEA, 1999) a GLP compliant residue depletion study was made available in which six hives that had been treated with the product twice a year, for three successive years, were treated for a period of six weeks. Samples of honey and wax were removed at intervals and analysed using a GC method following conversion of residues to 2,4-dimethylaniline and derivatization. The LOQ was 0.05mg/kg expressed as amitraz for both honey and wax. Residues in honey were stable during storage for up to 4 months at -20°C but were not stable when stored at +25°C. Residues in wax were extremely high. The study cannot be interpreted because the EMEA summary report does not provide sufficient details. Comparably designed studies do not exist.

Table 1: Reported results (EMEA) of a residue depletion study with Amitraz in honey

Days after end of treatment	Honey	Wax
	Mean amitraz equivalents [mg/kg]	
2	0.23	44.7
4	0.10	4.7
15	0.08	72.9

Wallner (1999) has shown that simple contact with beeswax accelerates the degradation of amitraz. An interpretation of these contradictory findings is not possible.

Korta, et al. (2001) characterized the degradation products of amitraz in honey and beeswax. For spiked honey samples a multi-floral commercial honey was used fortified to contain 10 mg/kg; chopped commercial beeswax, previously analyzed to ensure that it was free of amitraz residues was used to prepare fortified wax of 100 mg/kg. The stability in vitro at room temperature was examined over 9 months. In one experiment, the half life of amitraz in honey was 55.2 hours, the half life in bees wax was 6.3 hours. After 15 days, 34 μmoles of amitraz per kg added to honey had degraded to 30 μmoles of DPMF, 27 μmoles of DMF and 3 μmoles of DMA per kg. The range of half lives determined with four different honeys was 12 to 55 hours; however, the molar ratios of DPMF and DMF were nearly constant suggesting a single hydrolysis reaction. Concentrations of DMA were always very low, even at time points of complete breakdown of the parent molecule.

Korta, et al., 2003 has published a GC-MS method for the determination of several acaricides in beeswax. Using this method they analyzed 10 samples of incurred comb wax obtained from hives treated with amitraz in different regions of Spain and France for DMPF the major hydrolysis product of amitraz (recovery of the method 90.9 ± 4.5%). The residue was found in seven samples in concentrations from 0.57 to 33.4 mg/kg. These concentrations correspond to 1.0 to 60 mg/kg of amitraz equivalents.

The above studies seem to contradict each another unless amitraz that is "naturally" incorporated into wax behaves differently from amitraz added to wax by fortification using organic solvents. The issue needs clarification. The basis for the EU MRL is not clear. Beeswax has to be included in MRL considerations. Comb wax is not only used in cosmetics; entire honey combs are offered for sale as gourmet honey. It is eaten by many people with their breakfast sandwich[1].

On the basis of 150g (20g) daily consumption of comb honey (9:1 honey to wax assumed proportions: 135 (18)g honey and 15 (2)g wax), the daily intake on day 2 after treatment according to the EMEA cited GLP study is calculated as: $0.225 \times 0.135 + 44.7 \times 0.015 = 0.7$ ($0.225 \times 0.018 + 44.7 \times 0.002 = 0.093$) mg per person per day. This corresponds to 117 (15.6) % of the JMPR ADI and 389 (51.9) % of the EMEA ADI. 95.7% of this intake results from wax consumption. The bioavailability of amitraz residues from honey and wax is not known.

The daily intake from the use of beeswax as food additive would correspond to 9.6% of the JMPR ADI or 32% of the EMEA ADI on the basis of day 2 residues of the EMEA cited GLP study and the intake estimate made by EFSA. However, use of amitraz as a pesticide can be a significant source of residues in honey and has to be taken into account as well when recommending MRLs.

The EFSA Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food has reviewed beeswax as a glazing agent and as carrier for flavours (EFSA Journal, 2007). It made a conservative intake estimate of 1290 mg beeswax per person and day. In this assessment honey sold in jars with the honeycomb is also not included.

Tylosin

Tylosin A is converted to Tylosin B (desmycosin) in acidic aqueous solutions (Paesen, et al., 1995; Kochansky, 2006) studied the stability of tylosin in samples of honey containing tylosin residues. Tylosin and desmycosin, were isolated from diluted honey samples by solid-phase extraction followed by high performance liquid chromatography. Tylosin converted to desmycosin exponentially with half-lives of about 102 days at 34°C, 9 days at 50°, 9 hours at 80°, and 48 minutes at 110°. The desmycosin then decayed to unknown products.

The stability of tylosin in sucrose solutions was also investigated (Kochansky, et al., 2006). Stability was tested at (very high) concentrations of 200 mg/L using HPLC with UV detection. When the experiments were carried out in 70% highly purified sucrose at 34 °C tylosin disappeared linearly with time. The half life for tylosin (tylosin A) was approximately 186 days. The authors measured also the concentrations of the other members of the tylosin complex and estimated a half life of approximately 287 days for the entire complex. Inhibition zones were determined using *P. larvae*. The diameter of the zones remained constant over the observation period of 50 days. This underlines that it is not possible to determine tylosin A using microbiological assays since other members of the complex also exhibit significant inhibition of *P. larvae*.

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[1] The toxicological monograph on beeswax from the 39th JECFA meeting states: "It is generally believed that waxes are not digested absorbed from the alimentary tract in most mammals, including man. Beeswax may be indigestible in mammals due to the structure of its component compounds, which are not susceptible to hydrolysis by enzymes of the alimentary tract, and due to its insolubility in water and high melting point (62 °C - 65 °C) which prevent dissolution at body temperature. There are no original research data available to support this claim". The toxicological monograph on beeswax from 65th JECFA meeting states: "There is evidence that some solubilisation of beeswax is mediated by the action of bile acids, at least in some species". On dietary exposure the Committee made very conservative assumptions (e.g., a person consumes all foods, etc., containing beeswax at the highest percentile) and arrived at a daily intake of < 650 mg/person. This estimate does not include honey sold in jars with the honeycomb. Honey combs are typically sold in portions of 340 g in Germany.

The US FDA has approved tylosin tartrate for the control of American foulbrood in honey bees. Supporting aggregated residue data are contained in the Freedom of Information (FOI) summary published by the US FDA. The marker residue is parent tylosin. The analytical method for the detection of residues of tylosin in honey used in the residue study is a microbiological assay using an oxytetracycline-resistant strain of *Paenibacillus larvae*. This assay is not selective for the marker residue, parent tylosin. The following details of the residue study are given:

- Test animals: Honey bee, *Apis mellifera*, 40,000 workers/colony
- Treatment groups:
- Untreated controls (4 colonies)
- 200 mg tylosin in 20 g confectioner sugar (1X; 4 colonies)
- 1000 mg tylosin in 20 g confectioner sugar (5X; 4 colonies)
- Duration of treatment: Once every seven days for a total of three treatments (21 days).

Honey was sampled from the honey supers (surplus honey) between the first and second treatments and from the honey supers and the brood chamber weekly for three weeks following the final treatment. The results are expressed in mg/kg and given for the mean concentrations, lower and upper 95% confidence limits of the mean. The results are summarized in table 2.

Table 2: Residues of Tylosin in honey reported in FOI Summary NADA 013-076

Treatment	Sample from:	0 days			7 days			14 days			21 days		
		Concentration of tylosin residues in mg/kg											
		L95% CL	Mean	U95% CL	L95% CL	Mean	U95% CL	L95% CL	Mean	U95% CL	L95% CL	Mean	U95% CL
200 mg	Brood chamber				0.66	1.45	3.46	0.21	0.47	1.04	0.17	0.40	0.88
1000 mg					2.20	5.55	17.46	1.85	4.52	13.39	0.87	1.98	4.90
200 mg	Surplus	0.59	1.30	3.06	0.17	0.39	0.85	0.14	0.33	0.73	0.05	0.16	0.38
1000 mg		3.21	8.73	34.27	1.50	3.57	9.90	1.07	2.46	6.33	0.72	1.61	3.85

The confidence intervals of the mean are asymmetric (see columns L95%CL, Mean and U95%CL). This observation suggests that the mean could be a mean obtained on a log-scale. As an example, the results obtained with the 200 mg treatment and in surplus honey are plotted on both a linear and a logarithmic scale. Although the plot on the right side is suggestive of a log-normal distribution as basis for the calculations, the confidence intervals still remain asymmetric. Since individual data are not given it is not clear what the data mean and how they have been calculated. The data suggest a half life of approximately 7 to 9 days for both doses.

Figure 1: Residues of tylosin in surplus honey: Left side with linear y-axe; right side with logarithmic y-axe
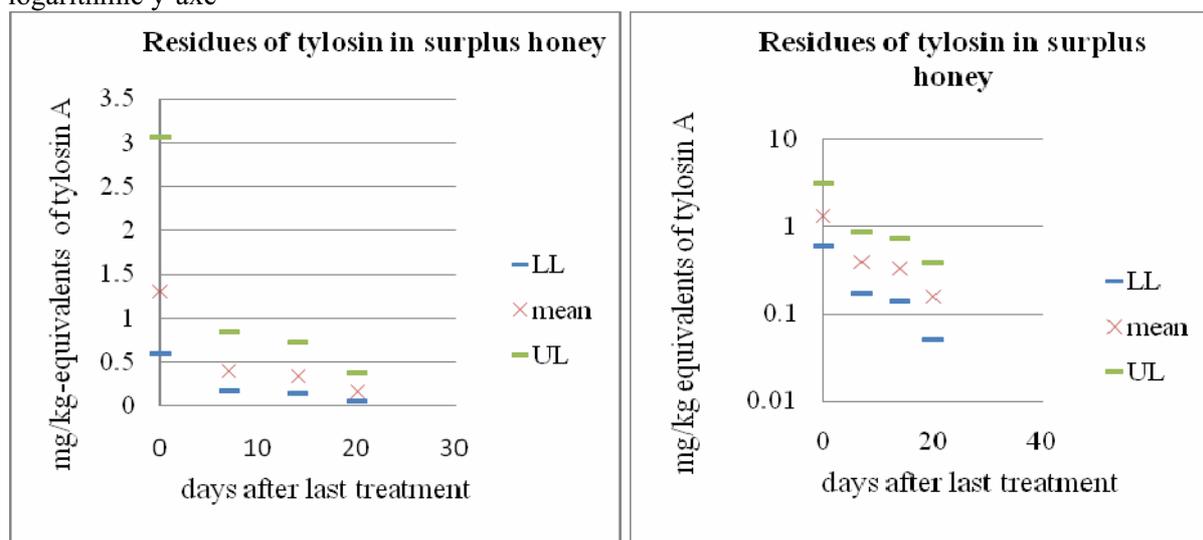
Valid instrumental methods are now available to measure the concentrations of all components of the tylosin complex simultaneously (Nozal Nalda , et. al., 2006)[2]. Desmycosin (also referred to as tylosin B) has been identified as the primary degradation product of tylosin in honey (Kochansky, 2003).

The degradation of tylosin in honey has been further investigated by Thompson et al., (2007). In their field trials, tylosin was used in single brood chamber colonies containing approximately 30,000 adult honey bees housed in Langstroth deep hive bodies. The study used higher than intended target dosages of tylosin (600 mg). Two types of formulations were used: 300 mg of tylosin tartrate mixed in 20 g of confectioner's sugar and either 300, 900 or 1500 mg tylosin tartrate incorporated into a 100 g pollen patty. Three successive treatments, spaced 7 days apart were performed in September 2004. For residue determination, 15 g samples of newly deposited honey were collected from colonies in July 2005, approximately 1 week after the start of the summer honey flow (collected representatively across several frames of the brood nest and honey super). The amount of honey in the supers was still relatively low at this time. The stability of tylosin A in honey matrices was investigated by spiking a series of replicate honey samples and storing them in the dark at −20 and 20 °C, respectively. Samples were analyzed at 2-week interval for a period of 16 weeks.

Analyses were performed using LC-electrospray MS/MS with roxithromycin as an internal standard. The method was validated using a series of antibiotic-free honey samples of varying physical appearance (i.e. color and moisture content). No appreciable degradation of tylosin A was observed when stored at −20 °C. Over the same period of time, approximately 20% of the tylosin A had degraded to desmycosin when stored at ambient temperature. The following table summarizes the results obtained in the treatment studies. In addition to the values given by the authors in units of μg/kg they are also expressed as μmoles/kg (one micromole of tylosin A is 916.1 μg; one micromole of desmycosin is 771.9 μg). The results show that it is not appropriate to use tylosin as the only residue to define MRLs for two reasons: the contribution of desmycosin may be significant and the ratio of the two molecules may vary as function of time.

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[2] This is only an example.

Table 3: Tylosin and Desmycosin in incurred honey samples (supers) of bee colonies, 294 days following the last treatment with tylosin A tartrate expressed as µg-equivalents of tylosin A / kg

Treatment	Replicate	Tylosin	Desmycosin	Tylosin	Desmycosin	Molar ratio	Total residue (*)
		µg/kg		µmoles/kg			µg/kg
Sugar dust, 3x300 mg per colony	1	179	150	0.20	0.19	1.01	357
	2	150	31	0.05	0.04	1.25	83
	3	32	32	0.04	0.04	0.84	70
	4	<5	<5				
Pollen patty, 3x900 mg per colony	1	29	33	0.03	0.04	0.74	68
	2	64	48	0.07	0.06	1.12	121
	3	<5	<5				
	4	<5	<5				
Pollen patty, 3x1500 mg per colony	1	<5	<5				
	2	<5	<5				
	3	23	19	0.03	0.03	1.02	46
	4	6	7	0.01	0.01	0.72	14

The authors, Nozal Nalda, et al., (2006) also analysed incurred multi-floral honey samples from Spain. The colonies were experimentally treated with technical grade tylosin of unknown composition administered to the animals in mixtures of sugars and food preservatives. 15 bee hives were used of which five served as controls, five received a sugar mixture with 200 mg/kg of tylosin, five other received a sugar mixture with 400 mg/kg of tylosin. The treatment was carried out in spring. Honey was collected from brood chamber combs after complete consumption of the feed plus an additional waiting time of 1 month.

No residues were found in the controls. The residue concentrations found in honey from treated bees were not dose related. For table 4 the original data have been re-calculated in µmoles/kg using the following molecular weights: tylosin A: 916.1, tylosin B: 771.9, Tylosin C: 902.1, and tylosin D: 918.1. Tylosin A usually is the major component (approximately 90% but not less than 80%).

Table 4: Residues of the tylosin family in honey obtained from bee colonies treated with tylosin of technical grade expressed as µg-equivalents Tylosin A per kg

Sample	TA	TB	TC	TD	% TA	Total residue[(*)]
	Concentration [µmoles/kg]					
201	1.59	0.10		0.12	88.0	1660
202	1.33	0.11		0.12	85.4	1430
203	0.78	0.08		0.02	88.8	800
204	1.13	0.02		0.03	95.7	1080
205	5.55	0.45	0.08	0.20	88.5	5740
401	2.01	0.25	0.01	0.09	85.2	2160
402	4.85	0.34	0.02	0.15	90.4	4910
403	0.65	0.08		0.01	88.1	670
404	2.73	0.36	0.02	0.10	85.1	2940
405	7.42	0.76	0.09	0.23	87.3	7790

Adams, et al., (2007) have studied the depletion of tylosin residues in honey from treated bee colonies. Hives (brood box) were dosed with 1.2 g of tylosin tartrate in 200-250 ml 50-60% sucrose in water (single dose) and honey was analyzed at several intervals over a 20-week period. The dosing

study was carried out from June 2005 to February 2006. During winter colonies were fed with 50% sucrose. Six colonies were treated. Baseline residue levels were determined in up to 100 g of nectar/honey before the treatment in June. Seven days after dosing and honey sampling two randomly selected colonies were shook-swarmed. Each sampling day, four comb samples (approximately 8x10 cm) were taken from each hive, two from brood chamber and two from super. The four samples were taken from different frames. On day 28 each individual sample was analyzed; on the other days brood chamber and super samples were bulked separately before analysis. The analytical method was LC-MS/MS. For the following description of the results all concentration values given by the authors were re-calculated as μmoles/kg**.**

For day 28 the authors provide individual tylosin A data for two samples per hive and type of comb taken from two different frames of either brood comb or super comb. The results are shown in table 5. Although it is immediately evident from the data that these results are not normally distributed the authors calculate mean and standard deviations for all individual eight data for a given time point. The table clearly shows that the variability within a frame can be very high (e.g., nine fold range for colony 1 and brood honey).

Table 5: **Variability of tylosin concentrations within a frame**

Colony	Type of data	Brood honey		Super honey	
		Top	Bottom	Inner	Outer
		Concentration [μmoles/kg]			
1	Individual concentration [μmoles/kg]	5.7	0.7	7.1	6.9
2		3.3	0.8	3.6	3.1
3		11.9	12.8	17.6	20.6
4		4.8	2.3	5.4	12.8
All colonies	n	8		8	
	mean	5.3		9.7	
	s.d.	4.7		6.6	

Using the average values two graphs were prepared showing the kinetic behaviour of tylosin A and tylosin B (desmycosin) in surplus honey and in brood honey. See figure 2.

Figure 2: Kinetics of tylosin residues in surplus honey and in brood honey
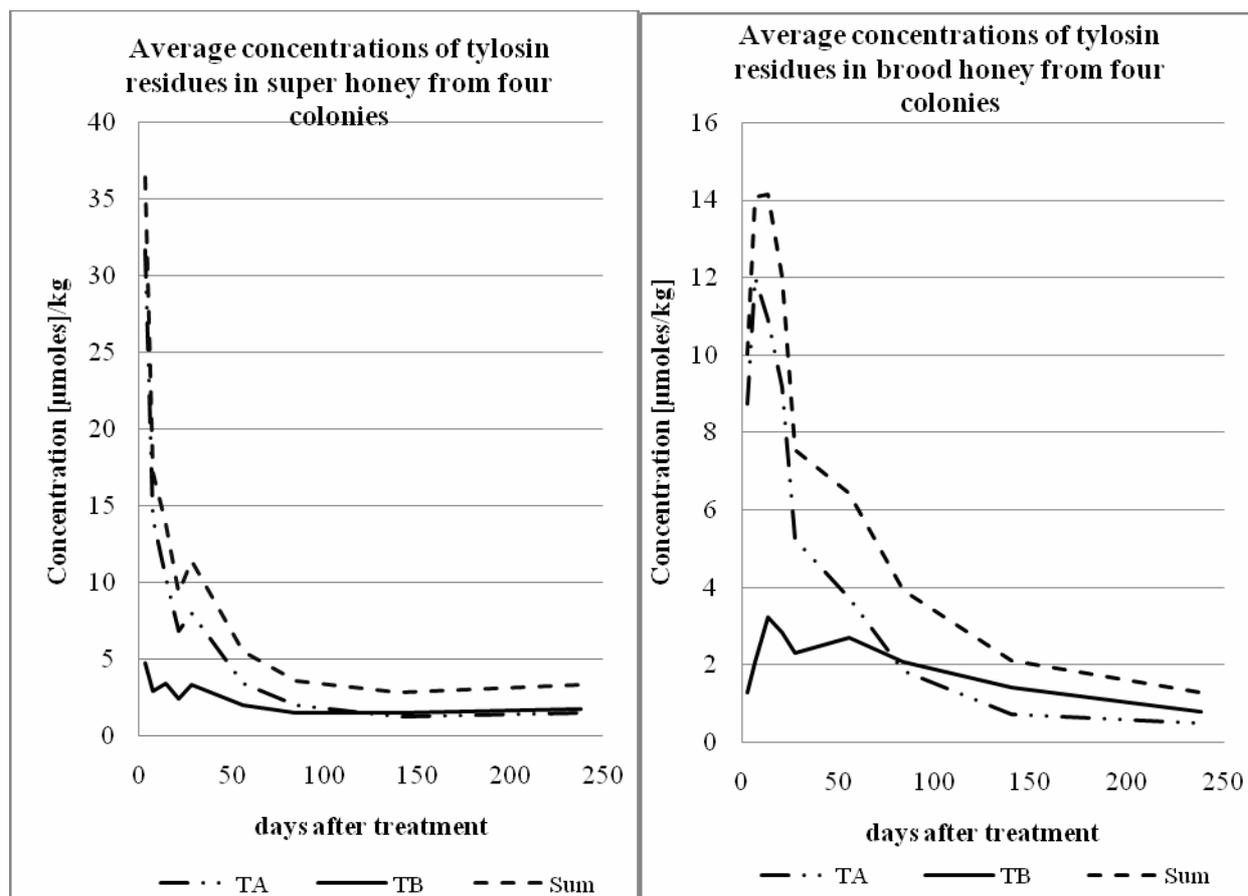
In surplus honey both tylosin A and desmycosin reach their highest concentrations shortly after the treatment. The concentrations of both compounds decrease with time; tylosin disappears more rapidly. Therefore, at the later time points desmycosin concentrations seem to be slightly higher. In brood honey tylosin A reaches its maximum concentration between 7 and 14 days after treatment and desmycosin concentrations continue increasing until after 14 days. After 84 days concentrations of desmycosin are always significantly higher compared to tylosin A.

For brood honey and for six time points the authors have provided averages for individual colonies. This permits estimates of variability between the colonies. The following figures below (figure 3and 4) show the kinetics of tylosin A and desmycosin in four individual hives.

**Figure 3: Depletion of tylosin A and B from brood honey**
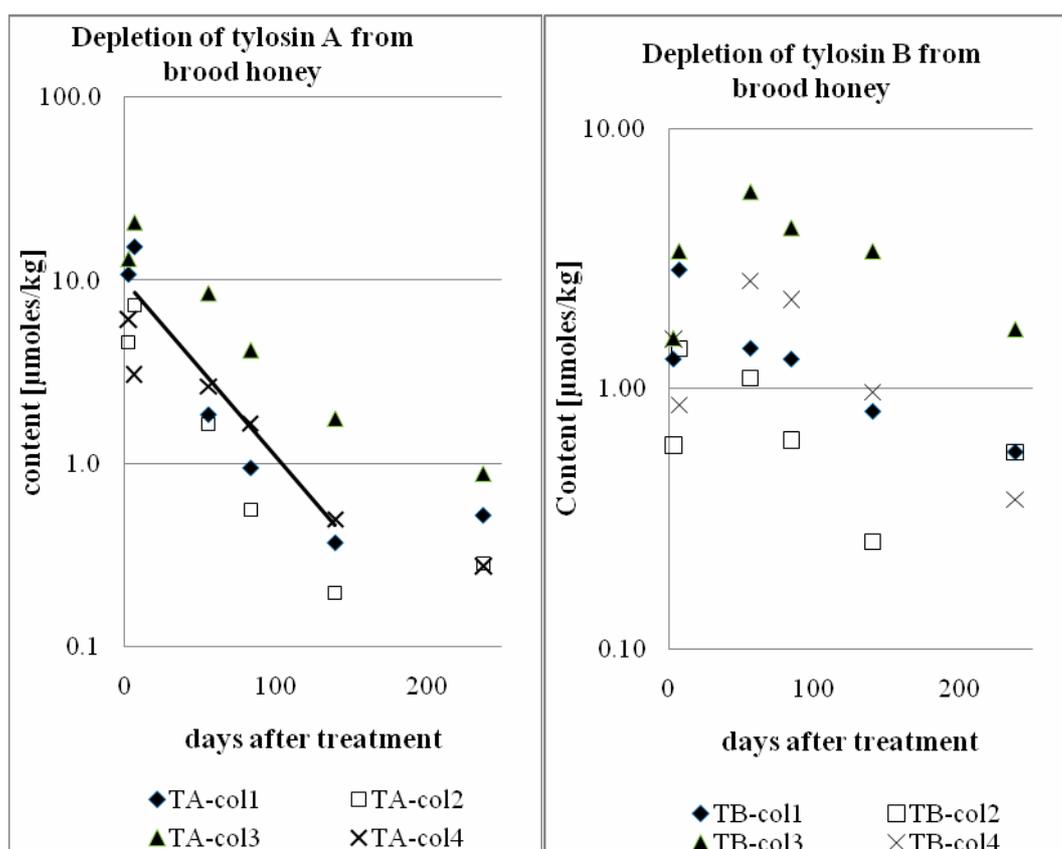
The two graphs show the great variability in the behaviour of different hives of the same apiary. The depletion curves of tylosin A suggest a log-linear part from 7 to 140 days following treatment. Linear regression analysis using the natural logarithms of the tylosin A concentrations yields the following parameters (n=16):

a	2.30294
b	-0.02200
r	-0.81989
$s_{y.x}$	0.78977

From these parameters a depletion half life of 31.5 days for tylosin A in brood honey (for the time period of 7 to 140 days after treatment) can be calculated. The ratio of the concentrations of desmycosin and tylosin A seem to reach a plateau of approximately 1.5; however, for the first approximately 100 days it can also be described by a straight line with time after treatment as the independent variable. The regression parameters using the data points from day 7 to 140 (n=16; unweighted linear regression) are:

a	0.11316
b	0.01253
r	0.95136
$s_{y.x}$	0.20865

The individual data points and the regression line are shown in the next graph (figure 4). Although the number of data points is very limited it seems that the variance of the ratio is not constant over time and weighted regression would be more appropriate.

Figure 4: Ratio of the concentrations of desmycosin and tylosin A in brood honey
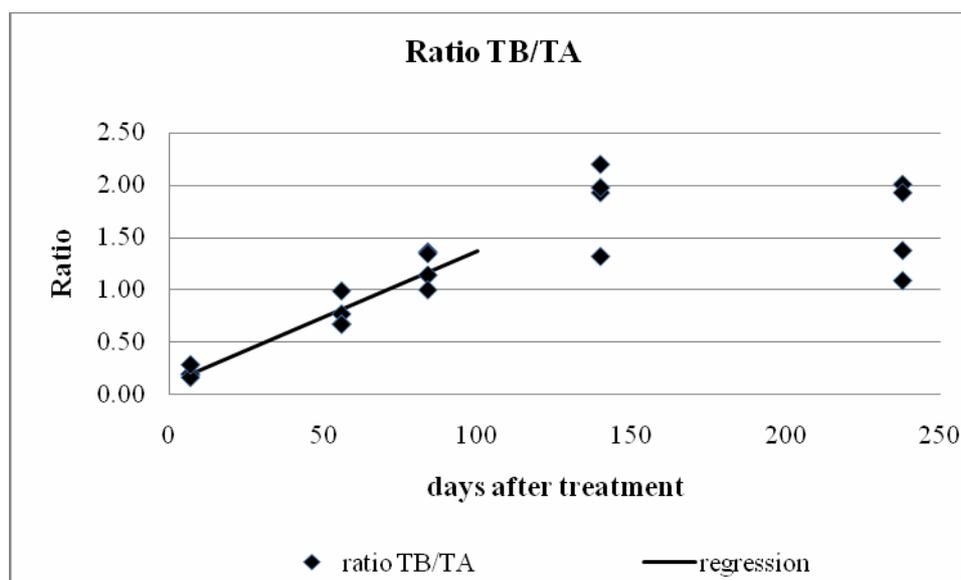
For the concentrations of tylosin A and desmycosin in surplus honey no individual data were provided; however, from the average values given for days 7 to 140 after treatment an estimated half life is 40 days.

The EMEA ADI for tylosin is 0.36 mg/kg bw/day or 21.6 mg/day for a 60 kg person. In the following table the sum of the average concentrations of tylosin A and desmycosin expressed as tylosin A has been calculated and the estimated daily intake of a 60 kg person consuming 150 (20) g of honey per day has been calculated in percent of the ADI (under the conditions of the Adams study; data on residues in wax were not included in the study).

Table 6: Theoretical consumption of "total residues" of tylosin by a 60 kg person using the conditions in the Adams et al. (2007) study

Withdrawal time [days]	% EMEA ADI equivalents consumed with 150 (20) g of Honey
3	23.2 (3.1)
7	10.9 (1.4)
14	8.8 (1.2)
21	5.9 (0.8)
28	7.3 (1.0)

The table 7 summarizes some half life estimates for tylosin A obtained in various studies, either *in vitro* (degradation) or *in vivo* (depletion). *In vivo* the actual concentration found is a complex function of uptake, degradation, metabolism and dilution. Unfortunately none of the studies cited above provide any information on the time course of the amounts of honey in the hives, supers, frames, etc. Therefore, the effect of dilution cannot be estimated. A general conclusion is the half life of tylosin A *in vivo* is much shorter than *in vitro*. The contribution of the individual factors mentioned above is not known.

Table 7: "Half life" of tylosin A *in vitro* and *in vivo*

Authors	Type of study	Initial concentration	Temperature [°C]	Half life [days]
Kochansky, J. (2006). Journal of Apicultural Research and Bee World, 45(2), 32-36.	In vitro in honey	Not given	34	102
Kochansky J., Knox D., Shimanuki H. (1999). Apidologie, 30, 321-326.	In vitro in sucrose	200 mg/L in 70% sucrose	34	186
Freedom of Information Summary, Supplemental New Animal Drug Application, NADA 013-076.	In vivo depletion in surplus honey	0.2-8.7 mg/kg of microbiologically active residues	Not given	7-9 (days 0-21)
Thompson, T. S., Pernal, S. F., Noot, D. K., Melathopoulos, A. P., van den Heever, J. P. (2007). Analytica Chimica Acta, 586 (1-2), 304-311.	In vitro in honey	Not given	Ambient	20% decay in 16 weeks
Adams, S.J., Heinrich, K., Hedmanski, M., Fussell R.J., Wilkins, W., Thompson, H.M., Sharman, M. (2007). Apidologie, 38, 315-322.	In vivo depletion in brood honey	2.8-19 mg/kg on day 7 after treatment	Not given	31.5 (days 7-140)
	In vivo depletion in surplus honey	13 mg/kg average for four hives on day 7		About 40 (days 7-140)

It has been known for long time that many antibiotics are degraded in honey. For example, microbiological inhibition assays were used to investigate the stability of certain antibiotics in sterile honey and in sugar syrup at both 34 and 4 °C. The following table is extracted from an old original publication Landerkin and Katznelson (1956). Microbiological inhibition tests are generally unable to quantify individual compounds in mixtures, but qualitative trends can be established.

Table 8: Stability of some antibiotics in honey

Month:	Start	1	2	3	4	5	6	7	8	9
Concentration [mg/kg] in honey at 4 °C										
Erythromycin	110	100	90	90	70	70	68	85	80	60
Tetracycline	110	50	40	40	35	37	36	48	54	24
Oxytetracycline	108	75	32	22	24	27	28	38	17	15
Chlorotetracycline	84	80	27	32	38	45	42	32	40	36
Streptomycin	80	65	57	50	37	32	30	42	31	35
Concentration [mg/kg] in honey at 34 °C										
Erythromycin	115	45								
Tetracycline	115	45	35	35	18	13	12	15	5	2
Oxytetracycline	90	20								
Chlorotetracycline	88	70	23	16	14	18	16	5	3	2
Streptomycin	80	60	54	20	20	21	12	31	18	10

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