Appendix B:
A review of the persistence of effects of acephate, dimethoate, methamidophos, methomyl and oxamyl

1. Executive summary

1.1. A review of the available data of the persistence of effects of acephate, dimethoate, methamidophos, methomyl and oxamyl on bees was performed in order to determine if a non treatment period should be defined prior flowering in order to protect the bees of the residual toxicity.

1.2. The conclusion of the review is as followed:

Substance	Period set in the initial control	Period set according to the available information
Acephate	7 days	7 days
Dimethoate	7 days	7 days
Methamidophos	7 days	None necessary
Methomyl	10 days	8 days
Oxamyl	10 days	10 days

2. OPC reassessment (APP201045)

2.1. Non-contact period to protect bees against residual toxicity prior re-assessment. These controls have been deleted according to the re-assessment decision (APP201045).

- Acephate: 7 days
- Dimethoate: 7 days
- Methamidophos: 7 days
- Methomyl: 10 days
- Oxamyl: 10 days

2.2. Maximum application rates controls set during the OPC re-assessment (APP201045)

- Acephate: 3500 g/ha, 3 times per season
- Dimethoate: 400 g/ha, 3 times per season
- Methamidophos: 900 g/ha

- Methomyl: 480 g/ha
- Oxamyl: 6720 g/ha

2.3. Uses evaluated during the OPC re-assessment (APP201045)

- Acephate: avocados, berry fruits, citrus, lettuce, ornamentals, potatoes, tamarillos, vegetable brassicas
- Dimethoate: berry fruits, cereals, lucerne, fodder, orchards, pasture, vegetable, peas, potatoes
- Methamidophos: beans, onions, maize/corn, potatoes, tomatoes, vegetable brassicas
- Methomyl: beans, bush/cane fruit, cereals, grapes, lettuce, pasture, strawberry, tomatoes
- Oxamyl: blackcurrant, carrots, ornamentals, pipfruit, ryegrass pasture, seed crops

3. General consideration about residual toxicity

3.1. Residual toxicity can result from several exposure routes: it can be due to contact exposure with dried residues on the leaves of the substance itself or its toxic degradation products; it can also be due to oral exposure to residues in nectar and/or pollen, honeydew or guttation fluid for systemic active ingredients.

3.2. Available information from both exposure routes is summarised below for all 5 active ingredients.

4. Available information about acephate

Aged residue studies

4.1. The information was sourced from the US EPA re-registration of acephate (ecotox appendices) (http://www.epa.gov/espp/litstatus/effects/redleg-frog/acephate/appendicies.pdf).

Species	Substance	Application rate (kg ai/ha)	Results	Reference
Apis mellifera	Formulation at 75%	0.54	Residues at 1 hr: 4.5% mortality Residues at 24 hr: 98.5% mortality Residues at 96 hr: 5.0% mortality	Atkins, 1971 cited by US EPA. Study considered as acceptable by US EPA
		1.09	Residues at 1 hr: 3.2% mortality Residues at 24 hr: 100% mortality Residues at 96 hr: 41.7% mortality	

Apis mellifera	Formulation at 75%	1.12	Residues at 0 hr: 100% mortality Residues at 2 hr: 79% mortality Residues at 8 hr: 17% mortality	Sakamoto, 1971 cited by US EPA. Study considered as acceptable by US EPA
Apis mellifera	Formulation at 75%	1.12	Residues at 2 hr: 79% mortality Residues at 8 hr: 16% mortality	Johansen, 1972 cited by US EPA. Study considered as acceptable by US EPA

Information about the degradation of acephate in/on plants

4.2. The following tables detail the dislodgeable foliar residues of acephate from different crops:

Substance	Crop	Application rates, frequency	DT50 (days)	Reference
Orthene 75 SP	Succulent beans	2 applications at 7 day-interval, 1.12 kg ai/ha	Acephate: 3.45 Methamidophos: 5.95 Combined residues: 3.66	M Huang and D Baxter (1999)
Orthene 75 SP	Tobacco	3 applications at 7 day-interval, 0.86 kg ai/ha	Acephate: 5.2 Methamidophos: 8.0 Combined residues: 5.4	A Oravetz and D Baxter (1999a)
Orthene 75 SP	Cauliflower	2 applications at 10 day-interval, 1.12 kg ai/ha	Acephate: 5.67 Methamidophos: 11.90 Combined residues: 5.92	M Huang and D Baxter (1999b)
Orthene Turf / tree and ornamental	Roses (greenhouses)	2 applications at 7 day-interval, 2.41 kg ai/ha	Acephate: 3.02 Methamidophos: 4.63 Combined residues: 3.08	T Schaeffer and D Baxter (1999)
Combination of acephate 45% and cypermethrin 5% DF	Cotton	382.5 and 765 g ai/ha	1.56 for the highest rate and 0.68 days for the lowest rate. Initial deposits: 13.45 and 27.73 mg/kg at 382.5 and	Battu *et al*., 2009

			765 g ai/ha respectively. One day after application, the residues dissipated to almost 46 and 65% in single and double dose applications, respectively. Thereafter, the residues of acephate declined slowly and after 1 week 86 and 89% of dissipation were observed.	

Conclusion about acephate

4.3. All aged residue studies were performed at lower rates than the maximum application rate approved in NZ (3.5 kg ai/ha). The results don't show a clear pattern of effects but more than 40% of mortality were observed after 4 days in a test at 1.09 kg ai/ha. The test doesn't provide information for longer periods.

4.4. The degradation rate of dislodgeable foliar residues responsible for the residual contact toxicity, observed in different crops and use patterns varies between 3.08 and 5.92 days (DT_{50}) for combined acephate and methamidophos.

4.5. No information is available to demonstrate that in the use conditions of NZ, the control for residual toxicity of 7 days should not remain on the products containing acephate.

5. Available information about methamidophos

5.1. The only relevant information was sourced from the European Commission Health & Consumer Protection Directorate-General. Review report for the active substance methamidophos. SANCO/4341/2000 - rev. 5 (14 December 2006) (http://ec.europa.eu/sanco_pesticides/public/?event=activesubstance.detail).

5.2. No laboratory data have been provided, a cage & field study is reported, conducted on Methamidophos 720 SL at 0.56 kg a.s/ha which proved the toxicity to bees exists but rapidly decreasing (reduced bee visitation for 2-3 days and a slight higher number of bees killed than observed in water-treated plots for 1 day). The overall effect considered to be a moderately low toxicity level on honeybees.

Conclusion about methamidophos

5.3. The above mentioned study has been performed at a lower application rate than the approved rate in NZ (0.9 kg ai/ha). However, the duration of the mortality effects is limited to the first day after application. Consequently, no control on residual toxicity should be applied to this substance.

6. Available information about dimethoate

GLP studies

6.1. Dimethoate is commonly used as a toxic reference in standard GLP[1] compliant studies performed to evaluate the effects of other substances. Some of these studies were recently received by EPA in the frame of an application for a new active ingredient are summarised below.

Type of Study	Semi-field test (tunnel)
Flag	Key study
Test Substance	Perfekthion EC (400 g Dimethoate/L)
Species	*Apis mellifera*
Type of exposure	Direct spray exposure and exposure via *Phacelia tanacetifolia* treated with the substance.
Endpoint	Mortality, foraging activity (flight density), sub-lethal effects, such as changes in behaviour
Value	Statistically significant increase of mortality for 4 days after treatment. Statistically significant decrease of flight intensity over the 7 days of the test. Sub-lethal effects observed the first 2 days after application.
Reference	**Confidential reference 1 (See Confidential Appendix C)**
Klimisch Score	1
GLP	Yes
Test Guideline	OEPP/EPPO (2001): Guideline for the efficacy evaluation of plant protection products - Side effects on honeybees. OEPP/EPPO, PP 11170(3) update 2000, 19 - 23.

[1] Good Laboratory Practices

No/Group	3 tunnels with 1 hive per tunnel
Dose Levels	1.5 L /ha in 400 L water /ha (600 g ai/ha)
Analytical measurements	The applied dose was 1.3% higher that the nominal dose.
Study Summary	A confidential version of this study summary will be provided to decision makers as a confidential appendix, and not be made generally available. Dimethoate was used as a toxic reference in a GLP study for another substance. Mortality and foraging activity (flight density) of the bees were assessed before and after applications. Sub-lethal effects, such as changes in behaviour, were also monitored. Colony assessments (food stores, brood status and hive populations) were made twice, 4 days before the daytime applications and at the end of the study (day + 9). After treatment by dimethoate, a distinct increase of bee mortality was observed for the first four days, which was statistically significant compared to the control (Student-t test, pairwise comparison, one-sided greater, α = 0.05). From day 0 to day 2 following the application the number of dead bees found in the reference item treatment was approximately 5 to 32 times higher compared to the control values. An overall comparison of the mortality data indicates a statistically significant difference compared to the control (Welch t-test, pairwise comparison to the control, one-sided greater, α = 0.05l). **Dead bees (* = statistically significant)**

Time	Water control	Dimethoate
5 d before app	20.0 ± 11.1	43.0 ± 24.1
4 d before app	13.3 ± 1.2	28.3 ± 15.3
3 d before app	23.0 ± 14.7	28.3 ± 21.5
2 d before app	47.0 ± 27.5	38.7 ± 31.5
1 d before app	18.0 ± 2.0	19.0 ± 12.1
Day 0 before app.	22.7 ± 5.7	36.3 ± 12.1
Daily mean from day – 5 to 0	**24.0 ± 11.8**	**32.3 ± 8.7**
mean day 0 after app.	**26.7 ± 12.1**	**865.3 ± 186.2 ***
1 d after app.	32.3 ± 9.3	357.0 ± 90.7 *

2 d after app.	40.0 ± 23.1	216.7 ± 109.9 *
3 d after app.	21.3 ± 3.8	78.7 ± 43.1 *
4 d after app.	23.3 ± 12.1	85.0 ± 49.8 *
5 d after app.	41.0 ± 14.7	35.3 ± 5.5
6 d after app.	25.7 ± 2.5	43.7 ± 41.0
7 d after app.	14.3 ± 5.1	46.7 ± 47.0
Daily mean from day 1 to 7 after app.	**28.1 ± 9.2**	**216.0 ± 285.0 ***

The foraging activity after application of dimethoate led to a clear decrease of flight intensity until the end of the experiment (7 days), which was statistically significant compared to the control (Student t-test, pairwise comparison, one-sided smaller, α =0.05)control. After treatment with dimethoate, there was a fairly rapid and significant reduction in flight intensity. Shortly after application, the bees returned to the hive so that about two hours after application, flight activity was stopped. Flight intensity remained very low for the remainder of the trial and no or only a few bees were seen foraging on the flowers over the next 7 days.

Flight density (* = statistically significant)

Time	Water control	Dimethoate
5 d before app	16.8 ± 5.7	18.7 ± 2.1
4 d before app	15.0 ± 4.1	19.3 ± 3.7
3 d before app	14.4 ±1.3	16.7 ± 2.1
2 d before app	19.8 ± 2.0	24.4 ± 3.0
1 d before app	13.3 ± 3.7	22.3 ± 0.3
Day 0 before app.	18.6 ± 6.8	29.8 ± 3.1
Daily mean from day – 5 to 0	**16.3 ± 2.5**	**21.9 ± 4.8**
mean day 0 after app.	**20.1 ± 3.9**	**0.1 ± 0.0**
1 d after app.	23.7 ± 5.8	0.0 ± 0.0
2 d after app.	18.6 ± 2.5	0.0 ± 0.0
3 (rain on this day)	0.0 ± 0.0	0.0 ± 0.0

4 d after app.	2.7 ±1.5	0.0 ± 0.8
5 d after app.	15.1 ± 5.2	0.4 ± 0.3
6 d after app.	20.6 ± 4.2	1.3 ± 0.0
7 (rain on this day)	0.0 ± 0.0	0.0 ± 0.0
Daily mean from day 1 to 7 after app.	**12.6 ± 10.0**	**0.2 ± 0.5 ***

Dimethoate treatment caused behavioural abnormalities (moving coordination problems, abnormal cleaning) at least until the first 2 days.

Conclusion	**Statistically significant increase of mortality for 4 days after treatment. Statistically significant decrease of flight intensity over the 7 days of the test. Sub-lethal effects observed the first 2 days after application.**

Type of Study	**Semi-field test (tunnel)**
Flag	Key study
Test Substance	Perfekthion EC (400 g Dimethoate/L)
Species	*Apis mellifera*
Type of exposure	Direct spray exposure and exposure via *Phacelia tanacetifolia* treated with the substance.
Endpoint	Mortality, behaviour, flight intensity, condition of the colonies and the development of the bee brood
Value	Increase mortality and decrease of the flight intensity during the 7 days of the test.
Reference	**Confidential reference 2 (See Confidential Appendix C)**
Klimisch Score	1
GLP	Yes
Test Guideline	CEB draft guideline No. 230, (2003), IVA (BEUTEL ET AL., 1992), EU (1997), and with partial integration of OEPP/EPPO guideline No 170 (3), (2001)
No/Group	1 tunnel with 1 colony

Dose Levels	1 L /ha in 300 L water /ha (400 g ai/ha)
Analytical measurements	Dose actually applied: 414.16 ai/ha
Study Summary	A confidential version of this study summary will be provided to decision makers as a confidential appendix, and not be made generally available. Dimethoate was used as a toxic reference in a GLP study for another substance. The effect of the test item was examined on small bee colonies in tunnels (approx. 100 m^2) placed on plots with *Phacelia tanacetifolia*. The mortality of the dimethoate group remained clearly higher than the control group throughout the whole post-application period (up to day 7). **Dead bees**

Time	Water control	Dimethoate
Day 3 before app.	58	118
Day 2 before app.	60	127
Day 1 before app.	46	61
Day 0 before app.	128	166
mean from day – 3 to 0 before app.	**73.0 ± 37.2**	**118.0 ± 43.3**
Day 0 after app.	64	510
Day 1 after app.	21	367
Day 2 after app.	33	515
Day 3 after app.	78	195
Day 4 after app.	108	271
Day 5 after app.	102	195
Day 6 after app.	99	211
Day 7 after app.	81	193
Daily mean from day 0 to 7 after app.	**73.3 ± 32.1**	**307.1 ± 139.7**

The foraging activity after application of dimethoate led to a clear decrease of flight intensity until the end of the experiment (7 days).

Flight density

Time	Water control	Dimethoate
3 d before app	9.5 ± 2.4	7.8 ± 2.2
2 d before app	7.5 ± 3.0	4.8 ± 1.3
1 d before app	4.3 ± 1.0	2.0 ± 0.8
Day 0 before app.	12.5 ± 0.5	10.0 ± 1.8
Daily mean from day – 3 to 0	**8.4 ± 3.6**	**6.1 ± 3.4**
mean day 0 after app.	**14.0 ± 3.6**	**0.4 ± 0.6**
1 d after app.	9.3 ± 1.0	0.0 ± 0.0
2 d after app.	9.3 ± 1.5	0.0 ± 0.0
3 d after app.	11.8 ± 4.5	0.3 ± 0.5
4 d after app.	15.0 ± 4.1	0.0 ± 0.0
5 d after app.	10.3 ± 1.3	0.5 ± 1.0
6 d after app.	15.8 ± 3.0	0.0 ± 0.0
7 d after app.	8.3 ± 2.2	0.0 ± 0.0
Daily mean from day 1 to 7 after app.	**11.7 ± 3.6**	**0.2 ± 0.4**

Dimethoate treatment caused behavioural abnormalities (Cramped bees in the dead bee trap. Forager bees approached the flowers very slowly, were disoriented or weakened) during the first 6 hours after treatment (no observation later on).

Conclusion	**Increase mortality and decrease of the flight intensity during the 7 days of the test.**

Data form overseas authority reviews

6.2. The following studies were sourced from the EFSA Draft Assessment Report - Dimethoate Volume 3, Annex B, B.9, part 4 Ecotoxicology (July 2005) (http://dar.efsa.europa.eu/dar-web/provision).

Type of Study	Semi-field test (tunnel)
Flag	Supporting study
Test Substance	Dimethoate (EC formulation at 400 g ai/L)
Species	*Apis mellifera*
Type of exposure	Bee exposure to aged residues from treated apple leaves
Endpoint	Mortality
Value	Significant mortality (above 20%) observed up to at least 120 hours for 24 h exposure and up to 12 days for 48 h exposure.
Reference	Kling, 2002 cited by the EFSA DAR (July 2004)
Klimisch Score	2, less bees than requested by the guideline
GLP	yes
Test Guideline/s	US EPA OPPTS 950.3030
Deviation	10 bees per replicates instead of 25.
No/Group	6 replicates of 10 bees
Dose Levels	720 g ai/ha in 1200 L water/ha
Analytical measurements	No data
Study Summary	In an extended laboratory study, the residual toxicity to Honey bee of 720 g ai/ha dimethoate applied as foliar spray to apple leaves was examined. Treated apple trees were covered with a UV-transparent plastic foil tunnel directly after application, to prevent any rainfall from dislodging residues until sampling of the leaves. 6 replicates of 10 bees were exposed to cut leaves samples from treated plants at 12h, 24h, 48h, 72h, 96h, 120h, 12 days and 14 days after application. Bees were maintained in environmental chambers and exposed to excised leaves on the bottom of the chamber. Bees were young, not foraging worker bees, aged between 1-7 days. During the 48-h exposure phase bees were fed with a 50% aqueous sucrose solution *ad libitum*, and were periodically observed for mortality and sub-lethal effects (after 4, 24 and 48 h exposure). Tap water was tested in parallel as control. **Mortality corrected for control mortality (never greater than 1.7%)**

Aged residues	% Mortality at 24	% Mortality at 48

		h	h
	12 hours	55.0	74.6
	24 hours	48.3	74.6
	48 hours	53.3	96.6
	72 hours	23.3	59.3
	96 hours	38.3	77.9
	120 hours	53.3	84.7
	12 days	10.0	35.0
	14 days	5.0	10.0

Mortality after 48 h exposure remained above 20% (accepted control mortality) until 12 days after treatment. The 24-h mortality remained above 20% until at least 120 hours after treatment.

Conclusion	**Significant mortality (above 20%) observed up to at least 120 hours for 24 h exposure and up to 12 days for 48 h exposure.**

Available literature

6.3. The following tables contains a summary of the available literature that provide the basis of the assessment of the extended residual toxicity of dimethoate:

Type of Study	**Semi-field test (tunnel)**
Flag	Supporting study
Test Substance	Dimethoate
Species	*Apis mellifera*
Type of exposure	Exposure to residues in treated field
Endpoint	Mortality, foraging activity, residues in nectar
Value	Increased mortality observed over 1 week after application. Decrease of foraging activity for at least 1 week after treatment. Nectar residues up to 1.40 ppm observed 4 days after treatment.
Reference	GD Waller et al. (1984): Effects of dimethoate on honey bees (Hymenoptera: Apidae) when applied to flowering lemons. Journal of Economic Entomology 77(1): 70-74

Klimisch Score	2 The control field was treated by dimethoate 12 days before the test so the foraging activity recorded in this field was very low. Consequently the foraging activity data can only be compared with record from the same field before treatment. However, the mortality data from this field can be used.
GLP	No
Test Guideline/s	None followed
No/Group	3 colonies per field
Dose Levels	1.12 kg ai/ha with 935 L/ha on lemon trees
Study Summary	2 lemon orchards were selected one for control (55 ha) and one for the treatment by dimethoate (28 ha). 3 uniform bee colonies were placed in the middle of each field and allowed 1 day of flight before the dimethoate treatment at 1.12 kg ai/ha. The hives from the treated field were removed at night just before treatment and returned the following morning. 3 additional colonies were brought to the treated field after 4 days and each week thereafter over a 3-week period. The control field was indeed treated with dimethoate 12 days before it was selected for this test. So the results of the foraging activity were not included in the table below and were generally very low (0.02 to 0.25). It is unclear if it was the consequence of dimethoate treatment. Sub-lethal effects were observed for the bees that were foraging shortly after treatment; these effects were uncontrolled movements, difficulty in flying, inability to enter flowers. **Foraging activity**

Field	Time	Bees/tree
East Grove	**7 d before**	**0.93**
	Morning before app.	**0.62**
	Afternoon before app.	**0.69**
	Day 1 (early morning)	0.40
	Day 1 (late morning)	0.09
	Day 1 (afternoon)	0.01
	Day 4	0.01
	Day 8	0.04
	Day 15	0.50

	Day 23	0.16
Bend grove	**Morning before app.**	**0.39**
	Day 1	0.16
	Day 2	0.00

Mean number of dead bees

Days	**Treated field. Colonies brought to orchard after:**				**Control**
	1 day	**4 days**	**8 days**	**15 days**	
1	1079	-	-	-	-
2	364	-	-	-	35
3	195	-	-	-	7
4	281	-	-	-	20
5	241	105	-	-	8
6	378	162	-	-	8
7-8	66	61	-	-	7
9-10	22	16	687	-	6
11	29	26	148	-	6
12	66	28	48	-	7
13	59	64	15	-	7
14	37	64	7	-	9
15	80	69	16	-	14
16	48	27	6	134	11
17	245*	196*	21	176*	18
18-20	16	42	18	36	16
21	13	20	21	7	13
22	10	34	20	14	8

* kill from an unknown cause

High mortality was observed for at least 1 week after application. The authors conclude that the reduced foraging activity over the first week was not due to repellency but to the high mortality observed in the colonies.

Concentration in nectar was also measured. The peak concentration appeared

	to be 4 days after treatment (mean value 0.482 +/- 0.474, max = 1.40 ppm) but there is no measurement on days 5 and 6. The authors conclude that the second mortality peak between days 4 and 6 can be explained by the exposure to nectar while the mortality at the beginning was probably due to direct contact with foliar residues.
Conclusion	**Increased mortality observed over 1 week after application.** **Decrease of foraging activity for at least 1 week after treatment.** **Nectar residues up to 1.40 ppm observed 4 days after treatment.**

Type of Study	**Semi-field test (tunnel)**
Flag	Disregarded study
Test Substance	Dimethoate
Species	*Apis mellifera*
Type of exposure	1) Exposure to nectar sampled from treated plants 2) Field test
Endpoint	Mortality
Value	Nectar exposure: 50% mortality after 4 days for bees fed with 0.04 mL of nectar from plants treated by 1 g ai/L. 50% mortality after 6 days for bees fed with 0.04 mL of nectar from plants treated by 2 g ai/L Field study: toxicity persists for around 3 days
Reference	ER Jaycox (1964): Effect on honey bees of nectar from systemic insecticide-treated plants. Journal of Economic Entomology 57(1): 31-35
Klimisch Score	3 not a standard test, small number of bees per group in the nectar test and application rates hardly comparable with field application rates. The control mortality values in the field tests are quite high. No statistical analysis.
GLP	No
Test Guideline/s	None followed
No/Group	Up to 6 for the nectar test 28 to 50 bees for the open area plots in the field test

	10 to 20 for the cages on plots
Dose Levels	1 or 2 g dimethoate/L applied on ca 50 cm-plants up to run off point. Nectar from these treated plants was fed to bees at amount from 0.01 to 0.04 mL Field test: 2 applications at 1.12 kg ai/ha on alfalfa
Analytical measurements	No
Study Summary	Nectar test: Foliage application of 1 or 2 g dimethoate/L was tested for its effects on honey bees, through nectar contamination in 3 species of flowering plants (*Borrago officinalis, Phacelia campanularia* and *Brassica napus*). Sprays were put on to the point of run-off on ca 50 cm-plants, 10 to 20 plants per treatment. Nectar was sampled at 24 h interval from the treated plants for at least 3 days after treatment. Bees were then fed with 0.01 to 0.04 mL of this nectar. 1 g/L of dimethoate created toxicity for at least 3 days with a peak toxicity at 24 h, 2 g/L for at least 6 days with a peak toxicity at 48 h. Field test: Dimethoate was applied twice on blooming alfalfa fields at 1.12 kg ai/ha. The first application was performed in the evening and cages containing a bee colony were placed in the field the following morning. Foraging worker bees were collected in the cages each day in the evening. After they were caught the bees were placed in cages in the laboratory and observed for mortality over a 3-day period. A second application was made after 11 days. The same observations were repeated but in addition, bees foraging in the open field were also collected in the morning, in mid-afternoon and in the evening. The evening applications of dimethoate to blooming alfalfa resulted in death of foraging bees for 3 to 4 days

Treatment	Time of collection	Mortality of bees (%) on indicated days after treatment					
		1	2	3	4	6	7
Bees collected from open areas							
Dimethoate	10-11 am	100	61	58	38	-	-
	1:30-2:30 pm	76	78	58	32	-	-
	4-5 pm	72	52	53	38	-	-
Control	10-11 am	36	29	46	52	-	-

	1:30-2:30 pm	44	30	40	46	-	-
	4-5 pm	20	49	28	36	-	-
Bees collected from cages							
Dimethoate	4-5 pm	65	55	45	35	25	10
Control	4-5 pm	25	0	5	10	20	20

This study has several weaknesses but a general conclusion can be drawn: the toxicity of dimethoate seems to persist for around 3 days.

Conclusion	**In the nectar study or the field study, the toxicity of dimethoate seems to persist for 3 days**

Type of Study	**Semi-field test (tunnel)**
Flag	Disregarded study
Test Substance	Dimethoate
Species	*Apis mellifera*
Type of exposure	Bee colonies were fed for 3 weeks with sucrose syrup containing dimethoate level similar to what was determined in nectar of onions treated by dimethoate pre-blooming or during blooming
Endpoint	Adult survival, no of eggs, no of larvae, no of pupae, pollen consumed, sugar honey stored
Value	Spraying onion plants in a pre-blossom stage resulted in harmful levels of dimethoate in nectar that lasted up to 2 weeks after treatment.
Reference	Waller & Barker (1979): Effects of dimethoate on honey bee colonies. J. Econ. Entomol. 72: 549-551.
Klimisch Score	4 (not assignable): the publication does not provide enough details about the study.
GLP	No
Test Guideline/s	None available at that time
No/Group	5 plants/ group (covered or not by plastic bag during spray, and in bloom or in pre-bloom during spray)

	3 colonies/ concentration in sucrose syrup
Dose Levels	0.3 g ai/L point of runoff on onion plants Contamination of sucrose syrup: 0, 0.2, 1, 5 mg/L dimethoate
Study Summary	One part of this study consisted in the determination of the levels of dimethoate residues in nectar of onion plants treated with 0.3 g/L of dimethoate. Plants were either in bloom or in pre-bloom stage when sprayed and umbels were either covered by a plastic bag or not. **Dimethoate residues (µg/mL) in nectar of onion** The second part of the study aimed to evaluate the effects of on bee colonies exposed to sucrose syrup contaminated by dimethoate at levels similar to the ones found in nectar of onion in the first part of the study. Bee colonies were given for 3 weeks both contaminated (at 0, 0.2, 1 or 5 µg/mL) and uncontaminated sucrose syrup and had free choice between them. The 3 colonies exposed to 5 µg/mL dies within 4 days after treatment. The 3 colonies exposed to 1 µg/mL dies within 14 days after treatment. For the first 2 weeks of treatment, there was little noticeable difference between the control and the colonies exposed to 0.2 µg/mL. However, they consumed less pollen. During the 3rd week, some spastic movements were noticed, they produce no comb and the population decreased noticeably. After 3 weeks, these colonies had only half as many surviving bees as the control. No larvae were present at the end of the 3rd week, however the pupae present indicated that brood rearing occurred earlier in the study. Egg production was seriously reduced during the 3rd week.
Conclusion	**Spraying onion plants in a pre-blossom stage resulted in harmful levels of dimethoate in nectar that lasted up to 2 weeks after treatment.**

Treatment	Days after treatment			
	4	7	11	14
Blooming and covered	1.1+/- 0.6	1.8+/- 0.4	-	0.2+/- 0.2
Blooming and uncovered	6.9+/- 2.5	1.8+/- 0.3	-	0.2+/- 0.1
Non-blooming and covered	1.0+/- 0.1	1.4+/- 0.4	0.7+/- 0.1	0.2+/- 0.1
Non-blooming and uncovered	5.0+/- 3.0	2.8+/- 0.5	0.7+/- 0.3	0.2+/- 0.2

Information about the degradation of dimethoate in/on plants.

6.4. The following tables detail the dislodgeable foliar residues of dimethoate from different crops:

Substance	Crop	Application rates, frequency	DT_{50} (days)	Reference
Dimethoate 400 g/L EC	Lettuce	2 applications at 7-8 days interval 0.28 kg ai/ha	Dimethoate: 0.43, 0.54 and 1.58 on the 3 different sites. Omethoate: level of residues too low to fit a curve	MG Bookbinder (1998a)
Dimethoate 400 g/L EC	Tomato	2 applications at 7-8 days interval 0.56 kg ai/ha	Dimethoate: 0.23, 0.34 and 1.12 on the 3 different sites. Omethoate: 0.81 and 6.4, not calculated in the 3rd site because the dissipation does not follow a first order kinetic	MG Bookbinder (1998b)
Dimethoate 400 g/L EC	Grapes	2 applications at 9 to 11 days interval 1.12 kg ai/ha	Dimethoate: 0.40, 0.85 and 1.33 on the 3 different sites. Omethoate: 5.3, 10.8 and 18.2	LM Prochaska (1999a)
Dimethoate 400 g/L EC	Apple leaves	2 applications at 8 to 10 days interval 1.12 kg ai/ha	Dimethoate: 3.12, 4.23 and 5.13 on the 3 different sites. Omethoate: 7.53, 10.3 and 34.6	LM Prochaska (1999b)

Conclusion about dimethoate

6.5. High quality data are available for dimethoate from a study performed at the same application rate as in NZ (0.4 kg ai/ha) or higher (0.6 kg ai/ha) which showed that increase mortality is observed 4 to 7 days after application.

6.6. Consequently, the residual toxicity control of 7 days which applied to dimethoate products should be kept.

7. Available information about oxamyl

7.1. The available information was sourced from the Interim Reregistration Eligibility Decision (IRED) about Oxamyl (US EPA 738-R-00-015, October 2000) (http://www.epa.gov/oppsrrd1/REDs/0253ired.pdf).

> "*Results of a residue on foliage study indicate that residues of oxamyl applied at 1.0 lb ai/acre* (1.12 kg/ha), *may remain toxic to bees for as long as 6 days after treatment (MRID 409943-01)*".

Conclusion about oxamyl

7.2. The residual toxicity control defined a period of 10 days for oxamyl products. Data show that effects are observed for 6 days but the applied dose is much lower that the application rate in NZ (6720 g/ha). Consequently, the control should remain unchanged.

8. Available information about methomyl

Available literature

8.1. The following tables provide summaries of the available literature, which was reviewed for methomyl:

Type of Study	Semi-field test (tunnel)
Flag	Key study
Test Substance	Methomyl (water dispersible powder at 90 % active ingredient)
Species	*Apis mellifera*
Type of exposure	Bees exposed to treated clover crop in field conditions for 9 days
Endpoint	Mortality (bees were collected in the fields and held in small cages, mortality was counted at 24 hr), behaviour
Value	Higher mortality than control observed up to 8 days after application. Behaviour effects observed until 6 days after application.
Reference	PG Clinch et al (1973) Effect on honey bees of dicrotophos and methomyl applied as sprays to white clover. NZ Journal of Experimental Agriculture 1: 97-99
Klimisch Score	2 Valid with restriction. Not a standard test, no statistical analysis, but results are conclusive.

GLP	No
Test Guideline/s	Not available when the study was performed
No/Group	10 hives alongside the treated field
Dose Levels	0.56 kg ai/ha
Study Summary	Methomyl was applied at a dose of 0.56 kg ai/ha on white clover in flower in a field on a 1.6 ha area of a 4.8 ha field. A similar field of 3.2 ha was used as control and was well outside bee range of the treated area. 10 hives were placed alongside the treated area, 1 day before application. The application was made in the evening when bees had ceased flying. Toxic action was studied by collecting bees from the crops with a battery-operated vacuum bee collector. The bees were held in small cages provided with sugar syrup. Mortalities were assessed after 24 hr.

Time	**% Bee mortality (number of bees collected)**	
	Methomyl	**Control**
1 day before application	2 (60)	3 (65)
Morning before application	0 (62)	2 (44)
Evening before application	0 (48)	0 (55)
Day 1 morning	65 (77)	1 (87)
Day 1 evening	77 (30)	1 (93)
Day 2 morning	100 (4)	2 (62)
Day 2 evening	92 (12)	1 (93)
Day 3 morning	80 (15)	0 (76)
Day 3 evening	56 (16)	0 (90)
Day 4 morning	63 (24)	0 (85)
Day 4 evening	86 (21)	0 (77)
Day 5 morning	100 (6)	0 (78)
Day 5 evening	38 (16)	2 (104)
Day 6 morning	46 (13)	2 (95)
Day 6 evening	42 (36)	1 (103)
Day 7 morning	15 (20)	1 (84)

Day 7 evening	28 (29)	1 (109)
Day 8 morning	35 (23)	5 (21)
Day 9 morning	0 (53)	0 (52)

Observation of the field bees indicated that the spray had no repellent effect. Small numbers of dead and dying bees were observed in front of all experimental hives on the day after application. Bees also showed irritability, some attempting to drive others out of the hives. Similar mortality was noted the next day, when the whole apiary appeared disorganised. Bees at hive entrances were irritable, challenging those returning from foraging, and biting each other. The following day the hives appeared to be recovering, but on day 4 disorganisation and mortality reached a peak. Bees were excited, flight in lanes ceased, and there was little activity. Several hundred dead and dying bees were observed in front of the four end hives, and fewer in front of the others. Disorganisation and mortality were less on day 5 and, although bees were not working the sprayed or unsprayed areas of the nearby crop, the resumption of flight in lanes showed nectar was being collected elsewhere. Although a few dead and dying bees were observed in front of hives on day 6 and, finally, on day 7, the apiary had returned to normal by day 6. Flight lanes indicated that the bees were actively working a crop some distance from the experimental one. This was confirmed by the presence of freshly collected nectar in four hives examined on day 6. Their queens and brood were normal.

Conclusion	**Higher mortality than control observed up to 8 days after application. Behaviour effects observed until 6 days after application.**

Data form overseas authority reviews

8.2. The methomyl EFSA Draft Assessment Report Vol 3, Annex B, part 2, B8-B9-appendices (April 2004) (http://dar.efsa.europa.eu/dar-web/provision) mentions 2 cage studies (semi-field test) one in apple trees and the other one in *Phacelia tanacetifolia* crop which aimed to quantify the duration of harmful effects on honey bees due to Methomyl 20 SL. The effects of spray deposits of this formulation applied at 450 g ai/ha aged for 2, 6 and 11 days (*Phacelia* test) or aged for 1, 5, and 10 days (apple trees) were evaluated. The EFSA conclusions about these tests are reported below:

"The results from the test with *Phacelia tanacetifolia* were stated to show that there was no significant effect on mortality when residues were aged for over 2 days. However, the results need to be treated with care since effects were greater for residues aged for 6 days than those aged for either 2 or 11 days. In addition, the main effect of the toxic reference was only noticeable on

evaluation day 2. No adverse effects on behaviour, flight activity or incidence of abnormal development were observed relative to control.

The results from the test with apple trees showed that there was an initial harmful effect. The study concluded that there was a temporary effect if exposed to 1 day old residues and that this effect persisted for 2 days. However, similar effects were noted in the treatment where there were 10 days aging prior to the introduction of the bees. Therefore it is considered that this statement is not supported. Most mortality seemed to occur during the first 2 days of the evaluation period yet at this time the residues in the different treatment had been aged for different periods. For instance it may have been expected that the effect from residues aged for 2 days may have persisted for longer than those aged for 10 days. However, this does not appear to be the case. Therefore these results also need to be treated with caution. It is possible that adverse effects may have been associated with disturbance to hives during their introduction into the trial as effects were seen across all treatments including the control."

Information about the degradation of methomyl in/on plants

8.3. The following tables detail the dislodgeable foliar residues of methomyl from different crops:

Substance	Crop	Application rates, frequency	DT_{50} (days)	Reference
Lannate LV 29% EC or Lannate SP 90% WP	Lettuce	2 applications at 2 days interval; 1 kg ai/ha	1.6 and 0.7 days on the 2 different sites.	DL Merricks & CJ Slaughter (1998)

Conclusion about methomyl

8.4. Information from a field study performed in NZ at a dose slightly higher (0.56 kg ai/ha) than the current approved application rate (0.48 kg ai/ha) shows persistence of mortality for 8 days after application. Consequently, the residual toxicity control of 10 days which applied to methomyl products should be kept, but could be slightly reduced to 8 days.

9. References

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