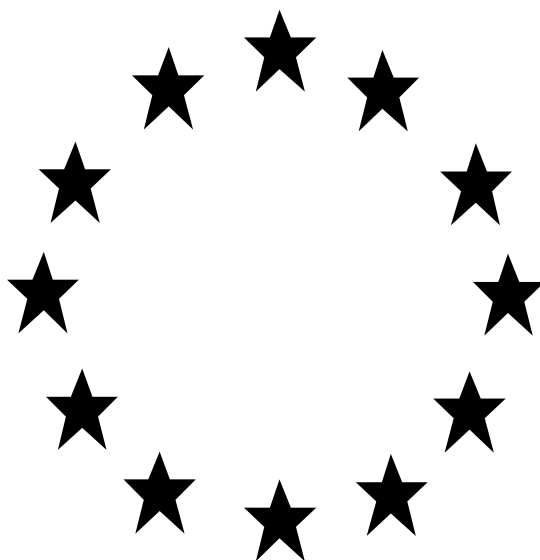




Draft Assessment Report (DAR)

- public version -

**Initial risk assessment provided by the rapporteur Member State
Austria for the existing active substance**

CYMOXANIL

**of the third stage (part B) of the review programme
referred to in Article 8(2) of Council Directive 91/414/EEC**

Volume 3, Annex B, part 5, B.9

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Volume 3

Cymoxanil

B.9

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WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

B.9 Ecotoxicology**B.9.1 Effects on birds (Annex IIA 8.1, IIIA 10.1)****B.9.1.1 Acute oral toxicity (Annex II A 8.1.1)****Active substance Cymoxanil**

Reference: Rodgers, M. H. (1996). Cymoxanil Technical. Acute oral toxicity (LD50) to the bobwhite quail. (Report no. OXN 69A/950758)

Guideline: EPA 71-1

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Cymoxanil was administered in corn oil by single oral dose to bobwhite quail.

Test substance: Cymoxanil techn., Batch Ref. 817, purity 99.1 %

Test organism: bobwhite quail (*Colinus virginianus*); both sexes, six months old, males 197- 201 g, females 181-187 g

Number of birds and replicates/group: 5 m + 5 f / treatment group and control

Test dosage levels: 0, 521, 729, 1020, 1429, 2000 mg/kg bw, corn oil for control and as vehicle

Duration: 14 d post-treatment observation period

Test conditions: Acclimation period: 15 d prior to dosing; Feeding: 21 h starvation prior to dosing, pelleted diet *ad libitum*, Mode of dose administration: oral intubation

Housing conditions: housing in pens in groups of two or three according to sex, 10 h photoperiod, 16 - 19 °C, 57 % relative humidity, ventilation as required

Biological observations: Daily observations for mortality, signs of toxicity and abnormal behaviour. Body weights and feed consumption measured at intervals prior to and during the observation period; gross necropsy at the end of the observation period

Statistical evaluation: none, LD₅₀ in excess of highest dose tested

Results:**Table B.9.1.1-1: Effect of Cymoxanil on mortality of bobwhite quail.**

Treatment (mg a.s./kg bw)	Cumulative Mortality (number of organisms)					
	day 1	day 2	day 3	day 4	day 5	day 14
carrier control	0	0	0	0	0	0
521	0	0	0	0	0	0
729	0	0	0	1	3	3
1020	0	1	1	1	1	2
1429	0	1	2	2	2	2
2000	0	2	2	2	2	2

Findings:

Regurgitation (if observed): no

Non-lethal toxicity endpoints: Subdued and unsteady behaviour on day 1 in all treated birds.

Reduced food consumption and body weight losses in all treatment groups over days 0 to 7, increasing bw losses with increasing dose level. From day 7 to 14, food consumption increased slightly and bodyweights increased in all treated groups. One and two survivors of the 1429 and 2000 mg/kg bw groups, respectively, had reduced musculature coupled (at 1429 mg/kg bw) with reduced subcutaneous fat. Similar findings were recorded for single birds of the 729 and 1020 mg/kg bw dose groups that died

during the observation period. Reddening of the intestines was noted in two other mortalities of the 729 mg/kg bw dose group, whilst dilated blood vessels throughout the intestines and a clear substance in and around the beak were found in one bird that died at 2000 mg/kg bw. The intestines of one mortality at 1429 mg/kg bw were yellow.

Conclusion:

LD₅₀: > 2000 mg/kg bw

NOEL: based on behavioural symptoms not determined

Reference: Heijink, E. (1999). Acute oral toxicity study in the mallard duck with cymoxanil technical. (Report no. 257377)

Guideline: OECD 401, EPA 71-1, SETAC (1995)

GLP: Yes

Deviations: Body weights of some females were below the minimum requirement of 900 g at start of the study. Temperature was below the range given in protocol. Because of the healthy appearance of the birds, these deviations are considered not to have affected the study integrity.

Validity: Because of vomiting limited validity with regard to risk assessment. Study acceptable in principle.

Material and methods:

Cymoxanil was administered in corn oil by single oral dose to mallard ducks.

Test substance: Cymoxanil techn., Batch 89800028, purity 98.9 %

Test organism: mallard duck (*Anas platyrhynchos*); both sexes, 34 weeks old, males 941 – 1286 g, females 727 - 1152 g

Test dosage levels: 0, 19, 61, 195, 625, 2000 mg/kg bw, corn oil for control and as vehicle

Number of birds and replicates/group: 5 m + 5 f / treatment group and control

Duration: 14 d post-treatment observation period

Test conditions: Feeding: 15-20 h starvation prior to dosing, standard commercial diet *ad libitum*, Mode of dose administration: gavage, Housing conditions: housing in pens in groups of two or three, 8 h photoperiod, 40 - 140 lux, 11.5 - 20 °C, 43 - 60 % relative humidity, 15 air changes/h

Biological observations: Daily observations for mortality, signs of toxicity and abnormal behaviour. Body weights: days 1, 8, 15; feed consumption monitored daily; gross necropsy at the end of the observation period

Statistical evaluation: Dunett-test for analysis of bodyweights

Findings:

No mortality occurred in any group.

Regurgitation: Vomiting was seen in two, five, five and six birds out of 10 at 61, 195, 625 and 2000 mg/kg bw, respectively

Non-lethal toxicity endpoints: Uncoordinated movement in the two highest dose-groups. Reduction in food consumption in males treated at 195 mg/kg bw and above and females at 2000 mg/kg bw. Body weight gain unaffected. Some isolated macroscopic findings were not considered treatment related.

Conclusion:

LD₅₀: > 2000 mg/kg bw

NOEL: 19 mg/kg bw

Reference: Campbell, S.M., Lynn, S.P. (1992). H-19,062-02: An acute oral toxicity study with the mallard (Report no. HLO 139-92)

Guideline: EPA 71-1

GLP: Yes

Deviations: Body weights of some females were below the minimum requirement of 900 g at start of the study. This deviation is considered not to have affected the study integrity.

Validity: acceptable

Material and methods:

Cymoxanil was administered in corn oil by single oral dose to mallard ducks.

Test substance: Cymoxanil techn., Batch T3217-113, purity 96.5 - 97.8 % (initial – reanalysis)

Test organism: mallard duck (*Anas platyrhynchos*); both sexes, 26 weeks old, 760 - 1290 g

Test dosage levels: 0, 292, 486, 810, 1350, 2250 mg/kg bw, distilled water for control and as vehicle

Number of birds and replicates/group: 5 m + 5 f / treatment group and control

Duration: 14 d post-treatment observation period

Test conditions: Acclimation period: 6 weeks prior to dosing; Feeding: 15 h starvation prior to dosing, standard commercial diet *ad libitum*; Mode of dose administration: oral intubation

Housing conditions: housing in pens in groups of five males or 5 females, 8 h photoperiod, approx. 130 lux, 19.5 ± 2.2 °C, 53 ± 23 % relative humidity

Biological observations: Daily observations for mortality, signs of toxicity and abnormal behaviour. Body weights: days 3, 7, 14; feed consumption monitored three times, gross necropsy at the end of the observation period

Statistical evaluation: none, LD50 in excess of highest dose tested.

Findings:

One male bird died at 486 mg/kg bw at day 3 after treatment.

Regurgitation: Regurgitation was seen in two, two and three birds out of 10 at 810, 1350 and 2250 mg/kg bw, respectively.

Non-lethal toxicity endpoints: Signs of toxicity which were observed at all dose levels were lethargy, ruffled appearance, lower limb weakness, reduced reaction to external stimuli (sound and movement), shallow and rapid respiration, loss of righting reflex, prostrate posture, depression, lower limb rigidity, staggering, head twitching, and loss of co-ordination. There was a reduction in body weight during Days 0-3 with males dosed at levels of 292 mg/kg, females at 486 and 810 mg/kg, and all birds at 1350 and 2250 mg/kg. During the same time period, a reduction in feed consumption was noted at various dose levels for males and/or females, but was particularly marked for males at 2250 mg/kg.

Conclusion:

LD₅₀: > 2250 mg/kg bw

NOEL: based on clinical symptoms not determined

Formulation: Cymoxanil 50 WP

Reference: Leuschner (2003): Avian toxicity study of Cymoxanil 50 WP oral toxicity in the Japanese quail - limit test according to SETAC guideline document, Report (Report no. 17232/03)

Guideline: SETAC guideline (1995)

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: Cymoxanil 50 WP (batch C 377), purity 500 g a.s./kg

Test organism: Japanese quail (*Coturnix coturnix japonica*); both sexes, 49 days old, males 170 - 196 g, females 174 - 193 g

Number of birds and replicates/group: 3 m + 3 f / treatment group and control

Test dosage level: 2000 mg/kg bw (limit test), dissolved in 0.5 % hydroxypropylmethylcellulose gel (5 mL/kg bw); vehicle only for control

Duration: 14 d post-treatment observation period

Test conditions: Acclimation period: 7 d prior to dosing; Feeding: 16 h starvation prior to dosing, pelleted standard diet *ad libitum*, Mode of dose administration: oral intubation

Housing conditions: housing in pens in groups of three according to sex, 12 - 16 h photoperiod, 20 - 25 °C, 40 - 80 % relative humidity

Biological observations:

Daily observations for mortality, signs of toxicity and abnormal behaviour. Body weight was determined on days 0, 5, 8 and at the end (15 d). Mean food consumption was recorded over days 0 - 3, 4 - 7 and 8 - 14; gross necropsy at the end of the observation period

Statistical evaluation: none, LD50 in excess of dose tested

Findings:

No deaths were recorded during the test period.

Regurgitation (if observed): no

Non-lethal toxicity endpoints: Reduced motility and eyelid closure was observed in all animals in the first hours after administration. No effects on bodyweight and food consumption were noted. No macroscopic post mortem findings.

Conclusion:

LD₅₀: > 2000 mg/kg bw equivalent to > 1000 mg a.s./kg bw

NOEL: based on behavioural symptoms not determined

B.9.1.2 Dietary toxicity (Annex IIA 8.1.2)

Reference: Johnson, A.J. (1997). Cymoxanil technical dietary LC50 to the bobwhite quail (Report No. OXN 215/970985)

Guideline: EPA 71-2

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: Cymoxanil techn., Lot No. DF 802/942, purity 98.9 %

Test organism: bobwhite quail (*Colinus virginianus*); 14 days old, sex not determined, bodyweights 18.6 - 19.4 g

Test dosage levels: 0, 163, 325, 650, 1300, 2600, 5200 ppm (nominal); controls received unmodified diet only

Number of birds and replicates/group: 10 birds / treatment group, 2 replicates with 10 birds each for control

Duration: 5 days exposure and 2 days post-observation period

Test conditions: Acclimation period: 3 d; Feeding: standard HRC chick diet; Environmental parameters: 27 - 28 °C, 62 % relative humidity, 14 h photoperiod, heating supplied by an infra-red lamp, ventilation as required

Analytical method: HPLC, validation performed

Biological observations: Daily observations at frequent intervals during treatment and post-treatment for mortality, health and clinical signs; bodyweights recorded on days 0, 5 and 8, food consumption over days -3 to 1, 1 to 5 (daily) and 6 to 8

Statistical evaluation: none, LC₅₀ in excess of highest concentration tested

Findings:

Table B.9.1.2-1: Effect of Cymoxanil on mortality of bobwhite quail

Treatment (mg a.s./kg diet)	cumulative mortality (number of organisms)				
	day 1	day 2	day 3	day 4	day 5-8
control rep. 1	0	0	0	0	3
control rep. 2	0	0	0	0	0
163	0	0	0	0	0
325	0	0	0	0	0
650	0	0	0	0	0
1300	0	0	0	0	0
2600	0	0	0	0	1
5200	0	0	0	0	0

All mortalities were unrelated to treatment.

Feeding: No clear food avoidance, but feed consumption was marginally reduced at 5200 ppm during the 5-day exposure phase.

Non-lethal toxicity endpoints: There were no indications of intoxication in the behaviour or posture of the birds. Slight reductions in body weight gain during the exposure period were seen among birds of the 2600 and 5200 ppm treatment groups. No treatment-related abnormalities were found in any birds during post mortem examination.

Analytical results: Mean concentrations were within 6 % of nominal, mean recovery value 96.8 %; toxicity endpoints were based on nominal concentrations.

Conclusion:

LC₅₀: > 5200 ppm

NOEC: 1300 ppm based on reduced body weight gain

Conversion of endpoints to daily dose: LD₅₀ > 999 mg a.s./kg bw based on a mean body weight of 21.35 g and a mean feed consumption of 4.1 g over the 5 day exposure period.

Reference: Freiling, W.J. and van Dreumel, I.F. (1999). 5-Day dietary toxicity study in mallard duck (Report No. 257401)

Guideline: OECD 205, EPA 71-2, EPA OPPTS 850.2200

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: Cymoxanil techn., Batch No. 89800028, purity 98.9 %

Test organism: mallard duck (*Anas platyrhynchos*); 14 days old, sex not determined, bodyweights 67.1 - 76.8 g

Test dosage levels: 0, 313, 625, 1250, 2500, 5000 ppm (nominal); controls received unmodified diet only; mean test substance intake reported as 0, 119, 257, 452, 813 and 1201 mg/kg bw

Number of birds and replicates/group: 10 ducklings / treatment group, 2 replicates with 10 birds each for control

Duration: 5 days exposure and 3 days post-observation period

Test conditions: Acclimation period: 6 d; 5 birds per cage; Feeding: standard commercial diet;

Environmental parameters: 22 - 28 °C, 56 - 58 % relative humidity, light intensity 60 - 180 lux, 14 h photoperiod, ventilation as required

Analytical method: HPLC, validation performed

Biological observations: At least daily observations during treatment and post-treatment for mortality, health and clinical signs; bodyweights recorded on days 0, 5 and 8, food consumption daily

Statistical evaluation: LC₅₀ calculated using Maximum Likelihood Method (Finney, 1971), bodyweights and food consumption analysed by Dunnett-test

Findings:

Table B.9.1.2-2: Effect of Cymoxanil on mortality of mallard duck

Treatment (mg a.s./kg diet)	cumulative mortality (number of organisms)				
	day 1	day 2	day 3	day 4	day 5-8
control (20 animals)	0	0	0	0	1*
313	0	0	0	0	0
625	0	0	0	0	0
1250	0	0	0	1	2
2500	0	0	0	0	3
5000	0	1	2	4	8

* killed *in extremis* after showing abnormal posture of the legs

Feeding: food avoidance noted in groups ≥ 1250 ppm

Non-lethal toxicity endpoints: Hunched posture and uncoordinated movements were observed at all three higher concentrations, combined with lethargy at 2500 and 5000 ppm. At 1250 ppm, symptoms were noted on days 6 and 7 and were confined to two birds with hunched posture and one with uncoordinated movement. All birds were hunched at 2500 ppm, two made uncoordinated movements, one was lethargic and effects lasted from Day 4 to Day 7. Eight birds of the 5000 ppm treatment group displayed hunched posture and lethargy from Days 4 to 7, one additionally exhibited uncoordinated movement and two showed no symptoms.

There was a treatment-related reduction of body weight during the exposure period at concentrations ≥ 625 ppm, with statistically significant effects at levels ≥ 1250 ppm. Body weight deficits were subsequently restored after the cymoxanil-treated diets were withdrawn. Food consumption was significantly reduced throughout the exposure phase at 5000 ppm and between days 1 and 6 at both 1250 and 2500 ppm.

Post mortem examination of all birds among treatment groups at ≥ 1250 ppm revealed pale and or black discolouration of the spleen, pale focus/foci on the liver and black and/or dark red discolouration of the liver. A pale spleen was found in the euthanised bird of the control group. No treatment-related abnormalities were found in survivors of any of the treatment groups terminated at test-end.

Analytical results: Mean concentrations were similar to nominal; stability and homogeneity were confirmed; toxicity endpoints were based on nominal concentrations

Conclusion:

LC₅₀: 2944.5 ppm (95 % C.I. 2140 - 5065)

NOEC: 313 ppm based on reduced body weight gain

Conversion of endpoints to daily dose: Since food consumption was reduced at dietary concentrations above and below the LC₅₀ value, it is not possible to convert the LC₅₀ to a reliable daily dose estimate expressed in terms of mg a.s./kg bw/day. The highest sub-LC₅₀ dietary concentration that caused no significant impact on food consumption (and therefore the highest concentration that permits calculation of a reliable daily dose) was 625 ppm. Based on a mean Day 1-6 body weight of 93.8 g and a mean Day 1-6 food intake rate of 39 g/bird/day, the daily cymoxanil intake of mallard ducks fed with the 625 ppm diet was 260 mg a.s./kg bw/day.

B.9.1.3 Reproductive toxicity (Annex IIA 8.1.3)

Reference: Gallagher, S.P., Foster, J.W., Beavers, J.B., Jaber, M. (1996a); DPX-T3217-113 (cymoxanil): A reproduction study with the northern bobwhite (*Colinus virginianus*). (report no. AMR 3507-95)

Guideline: OECD 206, EPA FIFRA 71-4

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: cymoxanil, batch no. T3217-113, Purity: 97.8 %

Test organism: bobwhite quail, *Colinus virginianus*; both sexes, 19 weeks of age, mean bodyweight 234 g

Test dosage concentrations: control, 100, 300, 1200 ppm ai nominal, acetone (solvent), corn oil (vehicle)

Number of birds and replicates/group: 16 pairs per treatment and one control group (1 pair per pen)

Duration: 11 weeks pre-laying exposure, 10 weeks egg-laying exposure and 6 weeks post-exposure period

Test conditions: 4 weeks acclimation period, Environmental parameters: mean temperature 16.3°C, relative humidity 37 %, 8 - 17 h photoperiod (increased to induce egg laying), lighting intensity approx. 281 lux, ventilation

Biological observations: Daily observations for abnormal behaviour and signs of toxicity. Body weights and feed consumption measured at intervals during the observation period (body weights not measured during egg laying in order to avoid any adverse effects). Eggs produced (apart from those used for egg shell thickness measurement) were incubated, with embryo viability and survival being assessed at intervals by candling. Hatchlings kept for 14 d in brooding pens. Cracked or abnormal eggs recorded and discarded. Reproductive parameters recorded: eggs laid, eggs cracked, eggs set, viable embryos, live 3-week embryos, hatchlings, body weight of hatchlings, 14-day-old survivors, body weight of 14-day-old survivors, and egg shell thickness.

Analytical methods: HPLC method; samples analysed for homogeneity and stability

Statistical evaluation: Dunett's test for differences between control group and each treatment group

Findings:

Table B.9.1.3-1: Summary of reproductive and body weight parameters in the bobwhite quail

Parameter	Control	100 ppm	300 ppm	1200 ppm
No. of replicates	15	15	14	11
Eggs laid/hen/d	0.51	0.43	0.54	0.46
Eggs laid/hen	49	42	52	45
Eggs cracked/eggs laid (%)	2	1	1	3
Egg shell thickness (mm)	0.237	0.235	0.233	0.241
Viable embryos	583	486	578	306
Viable embryos/eggs set	90	83	88	73*
Live 3-week embryos	576	481	577	301
No. of normal hatchlings	547	455	547	273
Hatchlings/eggs set	84	78	83	64*
Hatchling weight	6	6	6	6
14-day old survivors	503	426	505	235
14-day old survivors/hen	34	28	36	21

Parameter	Control	100 ppm	300 ppm	1200 ppm
14-day old survivors/eggs set	77	73	76	56*
14-day old survivors weight	30	29	30	28
Weight of females (parent) at test initiation (g)	233	234	233	235
Weight of females (parent) at termination (g)	278	275	280	284
Weight of males (parent) at test initiation (g)	231	238	235	230
Weight of males (parent) at termination (g)	250	251	260	236

* statistically significantly different ($p < 0.05$)

Feeding: at 1200 ppm in first week statistically significant reduction in feed consumption (transient aversion)

Toxicity: No treatment-related mortalities at any of the concentrations tested. Incidental mortalities: one, one, two and five in the 0, 100, 300, and 1200 ppm treatment groups, respectively. No overt signs of toxicity at any of the concentrations tested. Except for incidental clinical findings (feather loss, foot and head lesions, ventral head curl, ruffled appearance, and lethargy), all birds appeared normal throughout the study. All necropsy findings were considered to be not related to cymoxanil exposure.

Analytical results: mean recovery 95 - 98 % of nominal concentrations, homogeneity proven

Conclusions:

NOEC = 300 ppm (based on statistically significantly reduced embryo viability, no. of hatchlings and related parameters at 1200 ppm)

Conversion of endpoint to daily dose: NOED = 27.6 mg a.s./kg bw based on a mean body weight of 247.5 g and a mean feed consumption of 22.8 g/bird/d over the entire exposure period.

Reference: Gallagher, S.P., Foster, J.W., Beavers, J.B., Jaber, M. (1996b); DPX-T3217-113 (cymoxanil): A reproduction study with the mallard (*Anas platyrhynchos*). (report no. AMR 3508-95)

Guideline: OECD 206, EPA FIFRA 71-4

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: cymoxanil, batch no. T3217-113, Purity: 97.8 %

Test organism: mallard duck, *Anas platyrhynchos*; both sexes, 23 weeks of age

Test dosage concentrations: control, 100, 300, 600/450 ppm ai nominal (600 ppm for 11 weeks and 450 ppm for the remaining 10 weeks due to mortalities), acetone (solvent), corn oil (vehicle)

Number of birds and replicates/group: 16 pairs per treatment and one control group (1 pair per pen)

Duration: 11 weeks pre-laying exposure, 10 weeks egg-laying exposure and 6 weeks post-exposure period

Test conditions: 2 weeks acclimation period, Environmental parameters: mean temperature 18.9°C, relative humidity 32 %, 8 - 17 h photoperiod (increased to induce egg laying), lighting intensity approx. 313 lux, ventilation

Biological observations: Daily observations for abnormal behaviour and signs of toxicity. Body weights and feed consumption measured at intervals during the observation period (body weights not measured during egg laying in order to avoid any adverse effects). Eggs produced (apart from those used for egg shell thickness measurement) were incubated, with embryo viability and survival being assessed at intervals by candling. Hatchlings kept for 14 d in brooding pens. Cracked or abnormal eggs recorded and

discarded. Reproductive parameters recorded: eggs laid, eggs cracked, eggs set, viable embryos, live 3-week embryos, hatchlings, body weight of hatchlings, 14-day-old survivors, body weight of 14-day-old survivors, and egg shell thickness.

Analytical methods: HPLC method; samples analysed for homogeneity and stability

Statistical evaluation: Dunett's test for differences between control group and each treatment group

Findings:

Table B.9.1.3-2: Summary of reproductive and body weight parameters in the mallard duck

Parameter	Control	100 ppm	300 ppm	600/450 ppm
No. of replicates	16	16	16	10
Eggs laid/hen/d	0.56	0.62	0.39	0.34
Eggs laid/hen	50	56	35	31
Eggs cracked	7	7	7	8
Eggs cracked/eggs laid (%)	1	1	1	3
Eggs set	694	808	467	255
Egg shell thickness (mm)	0.366	0.361	0.352	0.347
Viable embryos	645	707	412	69
Viable embryos/eggs set	93	88	86	37**
Live 3-week embryos	621	696	405	66
No. of hatchlings	501	589	320	36
Hatchling weight	36	36	34	34
14-day old survivors	493	584	316	36
14-day old survivors/hen	31	37	20	4
14-day old survivors/eggs set	69	72	64	21**
14-day old survivors weight	279	292	264	253
Weight of females (parent) at test initiation (g)	1045	1047	1042	1048
Weight of females (parent) at termination (g)	1202	1147	1134	1082
Weight of males (parent) at test initiation (g)	1177	1164	1185	1160
Weight of males (parent) at termination (g)	1209	1206	1266	1155

** statistically significantly different (p<0.01)

Feeding: Significantly lower feed consumption at 600/450 ppm during week 1 (transient aversion).

Toxicity: 10 mortalities in the 600/450 ppm group were related to the treatment. Lacrimation in 4 drakes of the 300 ppm group and above. At 600/450 ppm, signs of toxicity like swollen eyes, ruffled appearance, lethargy, lower limb weakness, wing droop, loss of co-ordination, loss of body mass, and death. Necropsy findings: At 300 ppm slight increase in the incidence of regressed ovaries, correlated with a decrease in egg production that probably is related to treatment. Gross necropsies of the birds found dead typically revealed that the birds were emaciated or thin with a loss of muscle mass and prominent keel. Feather loss generally was noted around the eyes and occasionally the cheeks and neck. Internally, the spleen was commonly noted as small and/or pale and the kidneys pale.

Analytical results: mean recovery 95 - 101 % of nominal concentrations, homogeneity proven

Conclusions:

NOEC = 100 ppm (based on reduced egg production at 300 ppm)

Conversion of endpoint to daily dose: NOED = 14.9 mg a.s./kg bw based on a mean body weight of 1127 g and a mean feed consumption of 168 g/bird/d over the entire exposure period.

B.9.1.4 Supervised cage or field trials (ANNEX IIIA 10.1.2)

No study required.

B.9.1.5 Acceptance of bait, granules or treated seeds by birds (ANNEX IIIA 10.1.3)

No study required.

B.9.1.6 Effects of secondary poisoning (ANNEX IIIA 10.1.4)

No study required.

B.9.1.7 Summary of effects on birds

Table B.9.1.7-1: Summary of effects of cymoxanil on birds.

Species	Test substance	Type	NOEL/NOEC (mg a.s./kg bw)	LD ₅₀ /LC ₅₀ (mg a.s./kg bw)	Reference
bobwhite quail	cymoxanil	acute	n.d.	> 2000	Rodgers, 1996
mallard duck	cymoxanil	acute	19	> 2000	Heijink, 1999
mallard duck	cymoxanil	acute	n.d.	> 2250	Campbell & Lynn, 1992
japanese quail	Cymoxanil 50 WP	acute	n.d.	> 1000	Leuschner, 2003
bobwhite quail	cymoxanil	short-term	1300 ppm	> 999	Johnson, 1997
mallard duck	cymoxanil	short-term	313 ppm	> 260	Freiling & van Dreumel, 1999
bobwhite quail	cymoxanil	repro	27.6	-	Gallagher et al., 1996a
mallard duck	cymoxanil	repro	14.9	-	Gallagher et al., 1996b

n.d. not determined

B.9.1.8 Risk assessment for birds

Birds may be exposed to cymoxanil by eating contaminated vegetation, seeds and fruits, invertebrate prey like arthropods (i.e. insects) or earthworms or vertebrate prey.

Toxicity Exposure Ratios (TER) and Estimated Theoretical Exposures (ETE) were calculated according to the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (Sanco/4145/2000 of 25. September 2002).

Relevant toxicity figures were taken from laboratory studies with the active substance and one study on acute oral toxicity of the 50 WP formulation, which is relevant for the uses notified by Oxon only. These are listed in table 9.1.7-1 above.

The following worst case exposure scenarios were considered:

Lettuce	4 x 240 g ai/ha, interval 7 d	CYM 50 (Oxon)	Southern Europe
Potatoes	8 x 175 g ai/ha, interval 7 d	Tanos (Du Pont)	Northern + Southern Europe

B.9.1.8.1 First tier risk assessment

Exposure estimates were performed according to the standard scenarios provided in Sanco/4145/2000. In the category "leafy crops" the guidance document considers a medium herbivorous and an insectivorous bird as the relevant birds of concern. In order to calculate the estimated theoretical exposure the respective FIR/bw and RUD figures were chosen to estimate food intake rates and concentrations in the food of concern (see table 9.1.8.1-1). Up to four applications are intended in lettuce, and the respective multiple application factors (MAF) are set at 1.8 for the acute scenario and 2.2 for the short- and long-term scenarios. As up to eight applications are intended in potatoes, the respective multiple application factors (MAF) are set at 2 for the acute scenario and 2.5 for the short- and long-term scenarios. A default half-life of 10 d is assumed for estimating residue decline on herbaceous feed items. With regard to residues in the food of concern, 90th percentiles of the initial concentration are used for the acute time-scale and arithmetic means are taken for the short- and long-term scale. In the first tier worst case assessment it is assumed that birds feed on a single food type, which is not avoided and that they satisfy their entire food demand in the treated area (PD, PT = 1).

Possible other food items, for which no respective risk assessment is performed, are covered by the calculations presented because residues in these items are expected to be significantly lower.

Table B.9.1.8.1-1: TER values for acute, short-term and reproductive risk to birds, tier 1

Scenario	Bird type	AR (kg ai/ha)	FIR/bw	RUD	F _{twa}	MAF	ETE	Endpoint (mg/kg bw)	TER	trigger
lettuce / Oxon										
Acute	medium herbivorous	0.24	0.76	87	-	1.8	29	> 2000	> 70	10
Acute	insectivorous	0.24	1.04	52	-	-	13	> 2000	> 154	10
Acute/form.	medium herbivorous	0.24	0.76	87	-	1.8	29	> 1000	> 35	10
Acute/form.	insectivorous	0.24	1.04	52	-	-	13	> 1000	> 127	10
Short-term	medium herbivorous	0.24	0.76	40	-	2.2	16	260	16	10
Short-term	insectivorous	0.24	1.04	29	-	-	7	260	36	10
Long-term	medium herbivorous	0.24	0.76	40	0.53	2.2	8.6	14.9	1.7	5
Long-term	insectivorous	0.24	1.04	29	-	-	7.2	14.9	2.1	5
potatoes / DuPont										
Acute	medium herbivorous	0.175	0.76	87	-	2	23.1	> 2000	> 86	10
Acute	insectivorous	0.175	1.04	52	-	-	9.5	> 2000	> 211	10
Short-term	medium herbivorous	0.175	0.76	40	-	2.5	13.3	260	19.6	10
Short-term	insectivorous	0.175	1.04	29	-	-	5.3	260	49.3	10
Long-term	medium herbivorous	0.175	0.76	40	0.53	2.5	7.1	14.9	2.1	5
Long-term	insectivorous	0.175	1.04	29	-	-	5.3	14.9	2.8	5

FIR = Food intake rate; RUD = Residue per unit dose; F_{twa} = Time weighted average factor; MAF = Multiple application factor; AR = Application rate; ETE = Estimated theoretical exposure; TER = Toxicity exposure ratio, form. = formulation CYM 50 WP.

On the acute and short-term time scale, according to the resulting TER figures the risk can be considered low. However, a potential high risk was identified in the first tier long-term risk assessment. Hence, a refined risk assessment is considered necessary.

B.9.1.8.2 Higher tier risk assessment

The long-term risk is refined based on actual residue decline data of cymoxanil on plant foliage and by using information on focal species for refinement of the insectivorous scenario.

Risk to herbivores in lettuce:

In the six submitted residue decline trials (see B.7.6.1), which were conducted with the 50 WP formulation in Southern Europe, cymoxanil residue concentration in lettuce was found to be at about the limit of quantification within three days. Moreover it was shown that there is no accumulation of residue on lettuce plants due to repeated applications since the residue level before the 4th application was below the limit of detection. Hence, an estimated DT₅₀ of 2 days seems a more appropriate and still worst case assumption for the residue calculation in vegetation. In consequence, the MAF is set at 1 and the default time weighted average factor (f_{twa}) is calculated based on an estimated DT₅₀ of 2 d, which results in a refined f_{twa} of 0.38.

Risk to herbivores in potatoes:

This scenario is considered to be of minor relevance, as potato foliage is unpalatable to birds. Nevertheless, a refinement has been conducted and covers the risk of birds eating potential ground vegetation beneath potato plants. Results of residue decline studies in potato in Southern and Northern Europe show the same results as for lettuce (residue levels below the limit of detection, no accumulation at all after multiple applications). Therefore, based on residue information as detailed above for lettuce, MAF and f_{twa} are refined to 1 and 0.38.

Risk to insectivores in lettuce:

A reported bird species with preference for fields cropped with vegetables is the insectivorous **Yellow Wagtail** (*Motacilla flava*). In consequence, this species is considered as focal species for lettuce.

The yellow wagtail is a summer visitor in Europe from April to September. Its nesting habitat is generally associated with areas of low dense herbage either natural or managed particularly along the fringes of wetlands and where low perches are available (Buxton et al., 1998).

In a multi-annual study conducted on the nesting habitat preferences of yellow wagtails the authors revealed that the species primarily nests in spring-sown crops (Mason and Macdonald, 2000). The species was also found to nest in cereals, rape and root crops which also serve as slightly preferred feeding habitat (Buxton et al., 1998).

Data on the diet composition of yellow wagtails and on the size distribution and availability of the feed items ingested are available from Davies (1977).

The feeding behaviour of yellow wagtails in flocks or solitary was examined on non-flooded areas of a meadow, where the birds fed predominantly on flies around dung pats. Five main prey items were identified. The smallest (Staphilinidae and small Spherozeridae) were rejected and one (Sepsidae) was distasteful. The largest (Scatophagidae) were patchily distributed and difficult to capture when in swarms and so the next largest prey (large Spherozeridae) were also incorporated into the diet. The size of Scatophagidae eaten corresponded to the optimum in terms of energy intake per unit handling time. The birds' behaviour in leaving patches could also be predicted by optimal foraging theory.

According to the data of Davies (1977) yellow wagtails prefer insects of 3 - 4 mm and greater. The size of the insects and the definition of small and large feed items is essential since the estimation of the residues of plant protection products on insects as proposed by Kenaga (1973) bases on the residues on

weed seeds or wheat seeds which are considered to be comparable to small or large insects. Insects of ≥ 3 mm body length are defined as large. In consequence, and to keep consistency with the evaluation of other peer reviewed substances, the diet of yellow wagtails consists of approximately 80 % large insects and 20 % small insects. With regard to the mass of insects it is reasonable to consider that these values are rather conservative as a given number of large insects would contribute more to the daily energy intake as the same number of small insects. In line with other peer reviewed substances a FIR/bw ratio of 0.91 (17 g body weight) is used.

The extrapolation of the data derived from dung pats on a meadow to other crops may be a point of concern, as the species composition may be different. As described above, the selection of the food items is probably determined by the rate of energy content to handling time and therefore not stringently a question of species composition. This can be supposed to be the same in other cultures.

Details of the calculation and the resulting ETE and TER are presented in table 9.1.8.2-1.

Remark on the refinement proposed by the notifier:

The notifier proposed a refined PD and PT (combined) figure of 0.25 based on published general information of time birds spend feeding in different crops. The resulting TER is > 8 . The RMS thinks that a refinement for a focal species seems more appropriate and consistent with other evaluations.

Risk to insectivores in potatoes:

Focal species 1: **Yellowhammer** (*Emberiza citrinella*)

Based on information found in Buxton et al. (1998), and for reasons of consistency with the evaluation of other substances, the yellowhammer was chosen as representative insectivorous species in potato crops instead of the generic worst case bird species of the tier 1 risk assessment.

No detailed quantitative data on habitat use of the yellowhammer were found in the public domain literature. However, it can be safely assumed from the available descriptive information that the bird does not spend more than 50 % of its foraging time in potato fields, accordingly the factor PT is set to 0.5.

Considering a worst case application time, the yellowhammer can be assumed to almost exclusively feed on animal food items. According to Glutz von Blotzheim and Bauer (1997), a nestling diet in May consists of 25.7 % diptera (larvae), 2.6 % diptera, 46.2 % lepidoptera (larvae), 4.4 % lepidoptera, 7.1 % coleoptera, 3.3 % arachnida, 6.7 % cereals and 4 % other invertebrates. Assuming that diptera and lepidoptera larvae as well as about 50 % of the other invertebrate food items can be classified as "large", this can be simplified as follows: 82 % large insects, 12 % small insects, 6 % cereals. The FIR/bw ratio for the insect food items was calculated according to the recommendations given in SANCO/4145/2000, taking into account a body weight of 26.5 g, the energy expenditure parameters for passerines and an assimilation efficiency of 76 %. This calculation yielded a FIR/bw ratio of 0.78. Cereals are not treated with cymoxanil and can therefore be neglected in this exposure calculation.

Remark on the refinement details proposed by the notifier:

In the notifier's refinement for the same species a diet of 50 % large / 50 % small insects is proposed and residue decline on insects based on the foliar DT_{50} is taken into account. To be consistent with other evaluations the RMS gives preference to the approach described above.

Focal species 2: **Yellow Wagtail** (*Motacilla flava*)

Because of evidence of nesting habitat preferences in potatoes, this species was also selected as representative. In a multi-annual study conducted on the nesting habitat preferences of yellow wagtails the authors revealed that the species primarily nests in spring-sown crops, in particular in potato fields (Mason and Macdonald, 2000). Details of the refinement factors are given under "risk to insectivores in lettuce" above.

Considering all above mentioned refinement options, the refined ETE and TER calculations are shown in the table below.

Table B.9.1.8.2-1: Higher tier TER calculations for the long-term risk to birds

Type	Bird type	AR (kg ai/ha)	FIR/bw	RUD	F _{twa}	MAF	PT	PD	ETE	Endpoint (mg/kg bw)	TER	trigger
lettuce / Oxon												
Long-term	medium herbivorous	0.24	0.76	40	0.38	1	1	1	2.8	14.9	5.4	5
Long-term	insectivorous: yellow wagtail	0.24	0.91	5.1	-	-	1	0.8	0.89	14.9	6.9	5
			0.91	29	-	-	1	0.2	1.27			
			sum: 2.16									
potatoes / DuPont												
Long-term	medium herbivorous	0.175	0.76	40	0.38	1	1	1	2	14.9	7.5	5
Long-term	Insectivorous 1: yellow-hammer	0.175	0.78	5.1	-	-	0.5	0.82	0.29	14.9	28	5
			0.78	29	-	-	0.5	0.12	0.24			
			sum: 0.53									
Long-term	Insectivorous 2: yellow wagtail	0.175	0.91	5.1	-	-	1	0.8	0.65	14.9	9.5	5
			0.91	29	-	-	1	0.2	0.92			
			sum: 1.57									

PT = proportion in the treated area; PD = proportion in the diet

All resulting TER's exceed the trigger of 5 given in directive 91/414 and indicate a low long-term / reproductive risk to wild birds after the use of cymoxanil according to GAP.

B.9.1.8.3 Risk to birds from plant or soil metabolites

In lettuce cymoxanil was fast and extensively metabolised, mainly to glycine. In one of two metabolism studies in lettuce significant amounts of metabolite IN-W3595 were formed (up to 18.1 % of TRR). Other metabolites were detected only in very low amounts (< 6 % TRR). In mammalian metabolism the same metabolite IN-W3595 was the only one detected in significant amounts (up to 45.2 %). It is reasonable to assume that metabolite IN-W3595 will also occur in the avian metabolism of cymoxanil and that its toxicity is covered in toxicity studies with the parent compound.

B.9.1.8.4 Risk to birds from secondary poisoning

As the LogPow does not exceed 3 (< 1) the risk to birds from secondary poisoning can be considered as low.

B.9.1.8.5 Risk to birds from the consumption of contaminated drinking water

The water intake of birds is calculated according to SANCO/4145/2000.

Total water ingestion rate (L / day) = $0.059W^{0.67}$

where W is the body weight in kg.

As cymoxanil is of low acute toxicity, the exposure via drinking from surface water bodies is considered to be negligible. The exposure from puddles of spray liquid is assessed as a worst case. The dilution for spraying is 300(-600) L water/ha containing 175 g ai for potatoes and 500(-800) L water/ha containing 240 g ai for lettuce. When a dilution factor of 5 is applied for the concentration in the puddle, a PEC_{drinking water} of 117 / 96 mg ai/L is derived for potatoes and lettuce, respectively.

The total water ingestion rate (daily water intake DWI) of a small bird (15 g) is calculated as 3.5 mL/d. Figures for acute toxicity are the same as above used for the dietary risk assessment. The Toxicity Exposure Ratios (TER) for the acute time scale is calculated as LD_{50}/ETE . The ETE is calculated as $PEC_{drinking\ water} \times DWI$ (daily water intake).

Table B.9.1.8.5-1: Acute TERs for birds drinking contaminated water from puddles

Scenario		ETE	TER
Acute	lettuce / Oxon	23	> 88
Acute / formulation	lettuce / Oxon	23	> 44
Acute	potatoes / DuPont	27.5	> 72.7

All TERs for acute exposure indicate a low risk to birds via drinking water. The risk from consumption of drinking water contaminated with cymoxanil applied according to the GAP to wild bird populations is considered low.

B.9.1.8.6 Overall conclusion

Bird populations are unlikely to be affected by cymoxanil after its use according to GAP.

References:

Buxton, Crocker and Pascual (1998): Birds and farming: information for risk assessment. Update Contract PN0919 Milestone Report CSL Project No M37.
 Davies (1977): Prey selection and social behaviour in wagtails. J. Anim. Ecol. 46: 37-57.
 Glutz von Blotzheim (Edt., 2001): Handbuch der Vögel Mitteleuropas, Band 13/I, Passeriformes. Aula Verlag Wiesbaden.
 Mason and Macdonald (2000): Influence of landscape and land-use on the distribution of breeding birds in farmland in eastern England. Journal of Zoology, 251: 339-348

B.9.2 Effects on aquatic organisms (Annex IIA 8.2, Annex IIIA 10.2)

B.9.2.1 Acute toxicity to fish (Annex IIA 8.2.1; Annex IIA 10.2.1)

Active substance

Reference: Baer, K.N. (1993a) Static, acute 96-hour LC_{50} of DPX-73217-113 (Cymoxanil) to rainbow trout, *Oncorhynchus mykiss*. Report/Doc no.: Du pont HLR 735-92

Guidelines: OECD 203, EPA 72-1

GLP: Yes

Deviations: The pH of the dilution water was adjusted to a value of 6 to enhance the stability of the test substance.

Validity: The study is considered acceptable.

Material and methods:

Test substance: Cymoxanil technical, purity: 96.5 % (initial analysis), 97.8 % (reanalysis), batch no.: DPX-T3217-113

Test species: Rainbow trout (*Oncorhynchus mykiss*), weight: 0.71 - 1.7 g (mean: 1.1 g), length: 3.6 – 4.6 cm (mean: 4.1 cm)

Treatments: Dilution water control, pH adjusted control, 19, 32, 54, 90 and 150 mg/L

No. of organisms: One replicate with 10 fish per control and test substance treatment

Type of test and duration: Static test system, 96 hours

Test medium:

Dilution water originated from the [REDACTED] well. The water was buffered with 4 mM sodium phosphate and the pH then adjusted to 6.0 using phosphoric acid in order to enhance the stability of the test substance. Analytical results of the used well water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 6.3 – 10.6 mg/L (≥ 60 % saturation)

pH: Dilution water control: 6.8 – 7.2, pH-adjusted control and treatments: 6.0 – 6.3

Total hardness: 92 mg/L as CaCO_3

Test conditions:

Temperature: 12 – 13.3 °C

Photoperiod: 16 hours light and 8 hours darkness with 30 minutes transition periods

Feeding: No feeding from approximately 24 hours prior to and during the test.

Observations: Sublethal effects and mortalities were recorded once daily.

Analytical measurements: For chemical analysis of the test substance samples of the control, the pH adjusted control and the test substance treatments were taken on days 0 and 4.

Method of analysis: HPLC

Statistical evaluation: LC_{50} and respective 95 % confidence limits (CL) were estimated using the moving average angle method.

Findings:

Analytical results: Mean measured concentrations over time were 17, 28, 47, 79 and 135 mg/L. Measured concentrations ranged from 86 to 88 % of nominal for individual sampling dates.

Mortality:

Water control, pH-adjusted control, 17, 28 and 47 mg/L: No mortalities

79 mg/L: 10 % and 100 % after 72 hours and 96 hours, respectively

135 mg/L: 40 % and 100 % after 48 hours and 72 hours, respectively

Sublethal effects: At 47 mg/L, 79 mg/L and 135 mg/L fish exhibited dark colouration. At lower test concentrations no effects were observed.

Conclusion:

LC_{50} (96 h): 61 mg/L (95 % CL: 49 – 76 mg/L)

NOEC (96 h): 28 mg/L

Values are based on mean measured concentrations.

Comments (RMS):

The pH of the dilution water was adjusted to a value of 6 to enhance the stability of the test substance.

Since no effects were observed in fish of the pH adjusted control the pH adjustment is not considered to have significantly influenced the outcome of the test. Therefore the study is considered acceptable.

Reference: Baer, K.N. (1993b) Static, acute, 96-hour LC_{50} of DPX-T3217-113 (cymoxanil) to bluegill sunfish, *Lepomis macrochirus*. Report/Doc no.: Du Pont HLR 834-92

Guidelines: OECD 203, US EPA 72-1

GLP: Yes

Deviations: The pH of the dilution water was adjusted to a value of 6 to enhance the stability of the test substance.

Validity: The study is considered acceptable.

Material and methods:

Test substance: Cymoxanil technical, purity: 96.5 % (initial analysis), 97.8 % (reanalysis), batch no.: DPX-T3217-113

Test species: Bluegill sunfish (*Lepomis macrochirus*), weight: 0.058 – 0.14 g (mean: 0.10 g) at the end of the test, length: 1.5 – 2.0 cm (mean: 1.6 cm) at the end of the test

Treatments: Dilution water control, pH adjusted control, 19, 32, 54, 90 and 150 mg/L

No. of organisms: One replicate with 10 fish per control and test substance treatment

Type of test / duration: Static test system, 96 hours

Test medium:

Dilution water originated from the [REDACTED] well. The water was buffered with 4 mM sodium phosphate and the pH adjusted to 6.0 using phosphoric acid in order to enhance the stability of the test substance. Analytical results of the used well water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 7.7 – 8.7 mg/L (≥ 60 % saturation)

pH: Dilution water control: 7.3 – 7.5, pH-adjusted control and treatments: 6.0 – 6.3

Total hardness: 78 mg/L as CaCO_3

Test conditions:

Temperature: 20.4 – 21.0 °C

Photoperiod: 16 hours light and 8 hours darkness with 30 minutes transition periods

Feeding: No feeding from approximately 48 hours prior to and during the test.

Observations: Sublethal effects and mortalities were recorded once daily.

Analytical measurements: For chemical analysis of the test substance both controls and all treatment vessels were sampled before fish were inserted and after 4 days or when all fish had died.

Method of analysis: HPLC

Statistical evaluation: The 96 hour LC_{50} values and respective 95 % confidence limits were estimated using the moving average angle method.

Findings:

Analytical results: Mean measured concentrations over time were 17, 29, 50, 82 and 150 mg/L. Measured concentrations ranged from 88 to 108 % of nominal for individual sampling dates.

Mortality:

Dilution water control, pH-adjusted control, 17 mg/L: No mortalities

29 mg/L: 50 % after 96 hours

50 mg/L: 80 % after 72 hours and 100 % after 96 hours

82 mg/L: 40 % after 48 hours and 100 % after 72 hours

150 mg/L: 100 % after 48 hours

Sublethal effects:

29 mg/L: All surviving fish exhibited dark colouration and were lying on the bottom

50 mg/L: After 72 hours surviving fish were lying on the bottom

82 mg/L: After 48 hours surviving fish showed erratic swimming

150 mg/L: After 24 hours all fish exhibited erratic swimming and dark colouration

Conclusion:

LC_{50} (96 h): 29 mg/L (95 % CL: 22 – 36 mg/L)

NOEC (96 h): 17 mg/L

Values are based on mean measured concentrations.

Comments (RMS):

The pH of the dilution water was adjusted to a value of 6 to enhance the stability of the test substance. Since no effects were observed in fish of the pH adjusted control, the pH adjustment is not considered to have significantly influenced the outcome of the test. Therefore the study is considered acceptable.

Reference: Boeri, R. L., Kowalski, P. L., Ward, T. J. (1996a) Acute toxicity of DPX-T3217-113 (cymoxanil) to the sheepshead minnow, *Cyprinodon variegatus*. Report/Doc no.: DuPont HLO 634-96

Guidelines: US EPA 72-3: Acute toxicity test for estuarine and marine organisms

GLP: Yes

Deviations: None of relevance

Validity: The study is considered acceptable.

In two screening tests concentrations up to 50 mg/L were tested. After 96 hours of exposure at least 80 % survival was found at all treatment levels.

Material and methods:

Test substance: Cymoxanil technical, purity: 96.5 % (initial analysis), 97.8 % (reanalysis), batch no.: DPX-T3217-113

Test species: Sheepshead minnow (*Cyprinodon variegatus*), mean weight: 0.17 g at the end of the test, mean length: 18 mm at the end of the test, for weight and length no raw data or any measure of statistical spread of data is stated in the study report.

Treatments: Dilution water control, solvent control (0.5 mL solvent/L), 7.5, 13, 21, 30 and 50 mg/L
Solvent: dimethylformamide (DMF)

No. of organisms: 2 replicates with 10 fish each per control and test substance treatment

Test system / duration: Flow-through system, 22 volume additions per 24 hours (this high rate was performed to maintain steady test concentrations), duration: 96 hours

Test medium:

Dilution water: Natural sea water collected at [REDACTED]

Water was carbon filtered and adjusted to a salinity of 11 to 17 parts per thousand with deionised water, passed through particle filters, activated carbon and an ultraviolet sterilizer. Analytical results of the used dilution water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 7.0 – 8.0 mg/L

pH: 7.0 – 8.1

Salinity: 15 – 16 ppt

Test conditions:

Temperature: 21.6 – 22.1 °C

Photoperiod: 16 hours light and 8 hours darkness with 15 minutes transition periods

Feeding: No feeding from approximately 48 hours prior to and during the test

Observations: Mortality and sublethal effects were recorded daily.

Analytical measurements: For chemical analysis of the test substance samples were taken from each test vessels after 0 and 96 hours.

Method of analysis: HPLC

Statistical evaluation: Mortality was less than 50 % at all treatment levels. Therefore no statistical analysis of mortality data was performed.

Findings:

Analytical results: Mean measured concentrations over time were 6.6, 11.3, 18.8, 29.0 and 47.5 mg/L.

Measured concentrations ranged from 82 – 104 % of nominal for individual sampling dates.

Mortality: Dilution water control, solvent control, 6.6 and 11.3 mg/L: No mortalities

18.8 and 29.0 mg/L: 5 % after 96 hours

47.5 mg/L: 10 % after 96 hours

Sublethal effects: 18.9 up to 47.5 mg/L: Lethargic fish at the surface of the test media

Conclusion:

LC₅₀ (96 h): > 47.5 mg/L

NOEC (96 h): 11.3 mg/L

Values are based on mean measured concentrations.

Metabolites

Reference: Boeri, R. L., Wyskiel D. C., Ward, T. J. (2002a) IN-T4226: Static-renewal, acute, 96-hour limit test to rainbow trout (*Oncorhynchus mykiss*). Report/Doc no.: DuPont-9386

Guidelines: OECD 203, US EPA 72-1

GLP: Yes

Deviations: The dilution water was buffered and the pH adjusted to about 6.6 – 6.7 (control) and 6.2 - 6.6 (test substance vessels). Only a buffered control was set up.

Validity: The study is considered acceptable.

In a range finding study 5 fish per treatment were exposed to nominal concentrations of 0, 1.0, 5.0, 10, 50 and 130 mg/L under static conditions. Up to and including the highest treatment level no fish had died after 96 hours.

Material and methods:

Test substance: IN-T4226, purity: 99.1 %, batch no.: IN-T4226-1

Test species: Rainbow trout (*Oncorhynchus mykiss*), wet weight: 0.32 – 0.61 g (mean: 0.45 g) at the end of the test, length: 3.5 – 4.5 cm (mean: 3.9 cm) at the end of the test

Treatments: HEPES buffered control and 130 mg/L in HEPES buffered dilution water

No. of organisms: 3 replicates with 10 fish each per control and treatment

Test system / duration: Static-renewal test, renewal of test media after 48 hours, duration: 96 hours

Test medium:

Dilution water: Buffered reconstituted water, HEPES (N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) was used as buffer. Analysis data of the dilution water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 8.9 – 10.8 mg/L

pH: HEPES-buffered control: 6.5 – 7.0, HEPES-buffered test substance treatment: 6.3 – 6.9

Total hardness: 44 – 48 mg/L as CaCO₃

Test conditions:

Temperature: 10.1 – 11.0 °C

Photoperiod: 16 hours light and 8 hours darkness with 15 minutes transition periods

Feeding: No feeding from approximately 48 hours prior to and during the test

Observations: Mortality and sublethal effects were recorded daily.

Analytical measurements: For chemical analysis of the test substance samples were taken from each test chamber at the start of the test, after 48 hours from old and new media and after 96 hours (old media).

Method of analysis: HPLC

Statistical evaluation: Since no mortalities were observed, no statistical analysis was performed.

Findings:

Analytical results: The mean measured concentration over time was 111 mg/L. Measured concentrations were 72 – 98 % of nominal for individual sampling times.

Mortality: No mortalities were observed.

Sublethal effects: No effects were observed.

Conclusion:

LC₅₀ (96 h): > 111 mg/L

NOEC (96 h): 111 mg/L

Values are based on mean measured limit concentration.

Comment (RMS):

The dilution water was buffered using HEPES (N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) to enhance the stability of the test substance and the pH adjusted to about 6.6 – 6.7 (control) and 6.2 - 6.6 (test substance vessels). Only a buffered control group was set up, a pure dilution water control was not

run. However, no effects were observed in fish of the buffered control and hence the buffer is not considered to have significantly influenced the outcome of the test. Therefore the study is considered acceptable.

Reference: Samel, A. M. S. (2002a) IN-KQ960: Static, acute, 96-hours limit test to rainbow trout (*Oncorhynchus mykiss*). Report/Doc no.: DuPont-9560

Guidelines: OECD 203, US EPA 72-1

GLP: Yes

Deviations: None of relevance

Validity: The study is considered acceptable.

In a range finding test five fish per treatment were exposed to nominal concentrations of 0, 60 and 120 mg/L under static conditions. Up to and including the highest treatment level no fish had died after 96 hours. The 60 and 120 mg/L test solutions had precipitate present.

Material and methods:

Test substance: IN-KQ960, purity: 94.6 %, batch no.: IN-KQ960-002

Test species: Rainbow trout (*Oncorhynchus mykiss*), wet weight: 0.46 – 0.95 g (mean: 0.69 g) at test termination, length: 3.6 – 4.3 cm (mean: 4 cm) at test termination

Treatments: Dilution water control and 120 mg/L (adjusted for 94.6 % purity)

No. of organisms: One replicate with 10 fish for the control and 3 replicates with 10 fish each for the test substance treatments

Test system / duration: Static limit test, 96 hours

Test medium:

Dilution water originated from the [REDACTED] well. Analysis data of the used dilution water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 8.0 – 11.0 mg/L

pH: 7.0 – 7.7

Total hardness: 90 mg/L as CaCO₃

Test conditions:

Temperature: 13.2 – 13.4 °C

Photoperiod: 16 hours light and 8 hours darkness with 30 minutes transition periods

Feeding: No feeding from approximately 46 hours prior to and during the test

Observations: Mortalities and sublethal effects were recorded daily.

Analytical measurements: For chemical analysis of the test substance samples of each test chamber were taken on days 0 and 4. A small amount of undissolved test material was present on the bottom of each tank.

Method of analysis: HPLC

Statistical evaluation: Since no mortalities were observed no statistical analysis was performed.

Findings:

Analytical results: All measurements of the test substance yielded a concentration of 120 mg/L (100 % of nominal). A small amount of undissolved test material was present on the bottom of each tank.

Mortality: No mortalities were observed.

Sublethal effects: No effects were observed.

Conclusion:

LC₅₀ (96 h): > 120 mg/L

NOEC (96 h): 120 mg/L

Values are based on a nominal limit concentration.

Comment (RMS):

Although it is stated in the study report that a small amount of precipitate was present on the bottom of each tank measured concentrations were found to be 100 % of nominal for each sampling time and each sampled test vessel. No explanation is given in the study report for this finding and no information is provided on how samples were taken from the test vessels (e.g. was the test solution stirred and the precipitate suspended before samples were taken or was just the supernatant water taken?). However, the RMS considers the study to be of sufficient quality to demonstrate that the metabolite IN-KQ960 is of low acute toxicity to fish.

Reference: Samel, A. M. S. (2002b) IN-U3204: Static-renewal, acute, 96-hour limit test to rainbow trout, *Oncorhynchus mykiss*. Report/Doc no.: DuPont-9558

Guidelines: OECD 203, US EPA 72-1

GLP: Yes

Deviations: The dilution water was buffered and the pH of test solutions was adjusted to a value of 6.5.

Validity: The study is considered acceptable.

Material and methods:

Test substance: IN-U3204, purity: 94.4 %, batch no.: IN-U3204-009

Test species: Rainbow trout (*Oncorhynchus mykiss*), wet weight: 0.34 – 0.6 g (mean: 0.49 g), length: 3.3 – 3.9 cm (mean: 3.6 cm)

Treatment: Unbuffered control, HEPES buffered control and 120 mg/L in HEPES buffered dilution water, not corrected for the purity of the test substance (corrected for purity 113 mg/L). The pH of test solutions was adjusted to 6.5 by adding appropriate amounts of 1.0 N HCl.

No. of organisms: One replicate with 10 fish each for the HEPES buffered control and the unbuffered control and 3 replicates with 10 fish each for the test substance treatment group

Test type / duration: Static-renewal, renewal after 48 hours, duration: 96 hours

Test medium:

Dilution water originated from the [REDACTED] well. Analytical data of the used well water indicate adequate quality for the purpose of this study. The dilution water was buffered with HEPES (N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]).

Dissolved oxygen: 8.5 – 11.4 mg/L

pH: Dilution water control: 7.5 – 7.9, HEPES-buffered control and test substance treatment: 6.7 – 6.9

Total hardness: 126 - 131 mg/L as CaCO₃

Test conditions:

Temperature: 11.4 – 12.0 °C

Photoperiod: 16 hours light and 8 hours darkness with 30 minutes transition periods

Feeding: No feeding from approximately 28 hours prior to and during the test.

Observations: Mortalities and sublethal effects were recorded daily.

Analytical measurements: For chemical analysis of the test substance samples were taken from each replicate test chamber on days 0, 2 and 4.

Method of analysis: HPLC

Statistical evaluation: No mortalities were observed and hence no statistical analysis was performed.

Findings:

Analytical results: The mean measured concentration over time was 97 mg/L. For individual sampling dates measured concentrations were in the range of 76 – 96 % of nominal (corrected for purity).

Mortality: No mortalities were observed.

Sublethal effects: No sublethal effects were observed

Conclusion:

LC₅₀ (96 h): > 97 mg/L

NOEC (96 h): 97 mg/L

Values are based on a mean measured limit concentration.

Comment (RMS):

The dilution water was buffered using HEPES (N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) and the pH of test solutions was adjusted to 6.5 by adding appropriate amounts of 1.0 N HCl to enhance the stability of the test substance. No effects were observed in fish of the buffered and pH adjusted control, hence the pH adjustment is not considered to have significantly influenced the outcome of the test. Therefore the study is considered acceptable.

Reference: Boeri, R. L., Wyskiel, D. C., Ward, T. J. (2002b) IN-W3595: Static, acute, 96-hour limit test to rainbow trout, *Oncorhynchus mykiss*. Report/Doc no.: DuPont-9384

Guidelines: OECD 203, US EPA 72-1

GLP: Yes

Deviations: The pH of the test media containing test substance was 2.9 – 3.1. It was adjusted to 7.5 – 7.6 with 0.1 N sodium hydroxide.

Validity: The study is considered acceptable.

In a range finding test five fish per treatment were exposed to nominal concentrations of 0, 0.1, 1.0, 10, 50 and 130 mg/L under static conditions. Up to and including the highest treatment level no fish had died after 96 hours.

Material and methods:

Test substance: IN-W3595, purity: 98.8 %, batch no.: IN-W3595-004

Test species: Rainbow trout (*Oncorhynchus mykiss*), wet weight: 0.46 – 0.99 g (mean: 0.69 g), length: 3.6 – 5.0 cm (mean: 4.2 cm)

Treatment: Dilution water control and 130 mg/L (corrected for purity)

No. of organisms: 3 replicates with 10 fish each per control and treatment group

Test type / duration: Static test system, 96 hours

Test medium:

Dilution water: Reconstituted water, analysis data of the dilution water indicate adequate quality for the purpose of this study. The pH of the test media containing test substance was 2.9 – 3.1. It was adjusted to 7.5 – 7.6 with 0.1 N sodium hydroxide. The pH of the control vessels was not adjusted.

Dissolved oxygen: 8.1 – 9.7 mg/L

pH: Dilution water control: 6.6 – 7.5, pH in the test substance treatment: 6.7 – 7.6

Total hardness: 44 – 48 mg/L as CaCO₃

Test conditions:

Temperature: 11.9 – 12.8 °C

Photoperiod: 16 hours light and 8 hours darkness with 15 minutes transition periods.

Feeding: No feeding from approximately 48 hours prior to and during the test

Observations: Mortalities and sublethal effects were recorded daily.

Analytical measurements: For chemical analysis of the test substance samples were taken from each replicate test chamber of all treatment groups at 0 and 96 hours.

Method of analysis: HPLC

Findings:

Analytical results: The mean measured test substance concentration was 130 mg/L. For individual sampling dates measured concentrations in individual replicates were in the range of 98 – 101 % of nominal.

Mortality: No mortalities were observed.

Sublethal effects: No sublethal effects were observed.

Conclusion:

LC₅₀ (96 h): > 130 mg/L

NOEC (96 h): 130 mg/L

Values are based on a measured limit concentration.

Comment (RMS)

The pH of the test solutions was rather low after test substance addition (2.9 – 3.1) and hence adjusted to 7.5 – 7.6 with 0.1 N sodium hydroxide to prevent toxic effects on fish due to the low pH. The pH of the control vessels was not adjusted. With FOCUS step 2 estimated PEC_{sw} values for the metabolite IN-W3595 are below 1 µg/L. Such low values will not lead to a significant shift in the pH of surface waters due to introduction of the metabolite IN-W3595. Therefore the RMS considers the study acceptable to demonstrate that IN-W3595 is not acutely toxic to fish provided that it is not changing the pH of the water to a degree which would lead to toxic effects on fish. In conclusion the study is only considered acceptable in relation to the low expected environmental PEC_{sw} values for the test substance.

Reference: Migchielsen, M. H. J., van de Waart, E. J. (2003) Demonstrating exposure of rainbow trout to cymoxanil degradation products during a 96-hour acute toxicity study in rainbow trout (limit test under static conditions). Report/Doc no.: 363904

Guidelines: Based on OECD 203

Deviations: None of relevance for the final limit test. For the first limit test conditions are not reported in detail, it is just stated in the study report that test conditions are the same as for the final limit test.

GLP: Yes

Validity: The study is considered acceptable.

The purpose of this study was to evaluate and demonstrate exposure of rainbow trout (*Oncorhynchus mykiss*) to degradation products of cymoxanil (basically hydrolysis products). In this summary the RMS has concentrated on presenting data for metabolites that were considered relevant in the fate and behaviour section, but were not tested individually in other studies of aquatic toxicity. Information on other metabolites identified in the study are not considered relevant for risk assessment and hence are not presented here.

In a range finding test 3 fish per concentration were exposed to 10, 50 and 100 mg/L unlabelled cymoxanil. Respective mortalities were 0, 100 and 100 % after 96 hours.

Following the range finding test two limit tests were performed:

In a first limit test fish were exposed to a high test substance concentration of 200 mg/L unlabelled cymoxanil. Parallel a test solution with 200 mg/L unlabelled plus 4.8 mg/L radiolabelled cymoxanil (in total ≈ 205 mg/L) was exposed without fish under the same test conditions as the solution with fish to get information on the degradation of the active substance in the test medium and the amounts of resulting degradation products.

In the final limit test fish were exposed to 10 mg/L unlabelled cymoxanil under the same test conditions as for the first limit test.

Material and methods:

Test substance: Cymoxanil technical (purity: 98.8 %, batch no.: 89800028) and Cymoxanil-[cyanoacetamide-2-¹⁴C] (purity: 97.7 %, batch no.: 3362-236)

Test species: Rainbow trout (*Oncorhynchus mykiss*),

Mean wet weight and length of fish:

First limit test (200 mg/L): 5.53 ± 1.28 g, 7.0 ± 0.6 cm

Final limit test: (10 mg/L): mean wet weight: 2.54 ± 0.56 g, mean length: 5.2 ± 0.49 cm

Treatments:

First limit test: Dilution water control and 200 mg/L unlabelled cymoxanil with fish and

parallel 200 mg/L unlabelled cymoxanil plus 4.8 mg/L radiolabelled cymoxanil (in total $\approx$ 205 mg/L) without fish

Final limit test: Dilution water control and 10 mg/L unlabelled cymoxanil with fish

No. of organisms: One replicate with 7 fish per control and treatment for each test

Test type / duration: Static test systems, duration: 96 hours

Test medium: Tap water was used as dilution water. Analysis data of the dilution water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 8.4 – 9.4 mg/L (final limit test, treatment: 10 mg/L)

pH: Dilution water control and test substance treatment: 7.8 – 8.4 (final limit test, treatment: 10 mg/L)

Total hardness: 220 mg/L as CaCO₃ (used tap water)

Test conditions:

Temperature: 13.8 – 14.5 (final limit test, treatment: 10 mg/L)

Photoperiod: 16 hours light and 8 hours darkness

Feeding: No feeding from 48 hours prior to and during the test

Observations: Mortalities and sublethal effects were recorded shortly after test start and thereafter once daily.

Analytical measurements:

First limit test: The degradation of the test substance and the occurrence of resulting metabolites were monitored in the test solution without fish (200 mg/L unlabelled plus 4.8 mg/L labelled cymoxanil).

Samples were taken after 5, 24, 48, 72 and 96 hours of exposure. All samples were analysed by an initial HPLC, an additional HPLC (in study report called HPLC1), TLC and LSC.

Final limit test (10 mg/L unlabelled cymoxanil): Samples were taken after 0, 6, 24, 48, 72 and 96 hours to measure cymoxanil concentrations. Resulting metabolites were not monitored in the respective test solutions. Samples were analysed by HPLC.

Statistical evaluation: Both tests were limit tests with only one replicate and hence no statistical evaluation of mortality data was performed.

Results and discussion:

Analytical results:

With the initial HPLC analysis of samples from the test solution with 205 mg/L without fish (unlabelled plus radiolabelled a.s.) the metabolites IN-W3595 and IN-KP533 could not be separated. Also the metabolites IN-R3273 and IN-JX915 could not be quantified individually. Additionally no peak could be assigned to the metabolite fraction M5.

With TLC it was possible to separate IN-W3595 and IN-KP533. With the additional HPLC (called HPLC1 in the study report) the metabolites IN-R3273 and IN-JX915 could be separated and also the metabolite fraction M5 could be assigned to a peak.

Table B.9.2.1-1: Characterisation of radioactivity [% of AR] in rainbow trout test media. Relative values in the table are derived from the test solutions with 200 mg/L unlabelled cymoxanil + 4.8 mg/L radiolabelled cymoxanil (first limit test without fish).

Hours of exposure	Sum of IN-W3595 & IN-KP533	IN-KP533	IN-W3595	Sum of IN-R3273 & IN-JX915	IN-R3273	IN-JX915	Metabolite fraction M 5	Cymoxanil
0	3.1	0.4	2.7	5.0	n.d.	3.5	0	84.1
5	5.9	0.8	5.1	5.4	n.d.	4.2	0	72.0
24	16.4	1.2	15.2	8.7	1.0	8.9	1.3	32.9
48	27.2	2.5	24.7	10.0	1.9	8.3	22.8	5.7
72	33.0	5.0	28.0	11.1	2.5	8.7	22.5	0.64
96	33.7	9.8	23.9	9.6	2.9	7.8	29.0	n.d.
Method ^b	HPLC	TLC	TLC	HPLC	HPLC1 ^a	HPLC1 ^a	HPLC1	HPLC

^a Additional information submitted by the notifiers (see comments of RMS below)

^b Method used for the analysis of the test substance.

For the final limit test (10 mg/L, with fish) measured cymoxanil concentrations were 99, 83, 37, 9.5, 2.3 and 0.45 % of nominal after 0, 6, 24, 48, 72 and 96 hours.

Mortality:

First limit test (unlabelled & radiolabelled cymoxanil, 205 mg/L): All fish had died within 24 hours

Second limit test (unlabelled cymoxanil, 10 mg/L): No mortalities observed.

Sublethal effects:

Second limit test: After 48 hours some discoloured fish were observed.

Conclusion:

An initial concentration of 10 mg cymoxanil/L and resulting degradation products (basically hydrolysis products) did not cause any mortalities during a 96 hour exposition period.

Comments (RMS):

Based on analytical results for the 205 mg/L test solution without fish and the 10 mg/L test solution with fish the RMS calculated half-lives for cymoxanil. Respective DT50 values were 10.3 and 12.3 hours (single first order kinetics). The half-lives of the systems are comparable and therefore it can be assumed that the hydrolytic degradation pattern and the relative amounts of metabolites formed was very similar in these two test systems. This assumption is supported by the fact, that under slightly alkaline conditions the degradation of cymoxanil is mainly driven by hydrolysis which is well understood (see section fate and behaviour). For these reasons it seems to be justified that relative amounts (percentages) of metabolites found in the first limit test (205 mg/L) can be extrapolated to approximate concentrations that have been present in the test solution of the second limit test with a high likelihood.

The RMS performed such an extrapolation to get a rough estimate on metabolite concentrations during the final limit test (10 mg/L, no mortalities observed). The estimated concentrations for the metabolites IN-R3273, IN-JX915, IN-KP533 and the metabolite fraction M5 is given in Table B.9.2.1-2.

Table B.9.2.1-2: Relative and absolute amounts of degradation products of cymoxanil assumed to have been present in the final limit test with 10 mg/L unlabelled cymoxanil.

Hours of exposure	Metabolite fraction M5		IN-R3273		IN-JX915		IN-KP533	
	[%] ^a	[mg/L] ^b	[%] ^a	[mg/L] ^b	[%] ^a	[mg/L] ^b	[%] ^a	[mg/L] ^b
0	n.d.	n.d.	n.d.	n.d.	3.5	0.35	0.4	0.03
5	n.d.	n.d.	n.d.	n.d.	4.2	0.42	0.8	0.06
24	1.3	0.12	1.0	0.09	8.9	0.89	1.2	0.10
48	22.8	2.16	1.9	0.16	8.3	0.83	2.5	0.20
72	22.5	2.14	2.5	0.22	8.7	0.87	5.0	0.41
96	29.0	2.75	2.9	0.25	7.8	0.78	9.8	0.79

^a Relative values derived from the test solutions with 205 mg/L cymoxanil without fish (plus radiolabelled cymoxanil).

^b Absolute values for the test solution set up with 10 mg/L unlabelled cymoxanil under the assumption that relative values are the same as derived for the 205 mg/L test solution. Molar ratios between metabolite and cymoxanil weights were considered. For the unknown metabolite fraction M5 it was assumed that the molar weight equals the molar weight of one chemical substance within the fraction that could be identified (see Table B.9.2.9.2-1 under point B.9.2.9 Risk assessment)

The information given here includes the information provided in the document Willems, H (2005)

"Additional information related to Notox Project 363904". In this document chromatograms for the HPLC1 (additional HPLC) are given.

Metabolite identification was performed in accordance with section fate and behaviour and the report of Brands & Noorloos (2006), Notox project 457818 which is referred to in section fate and behaviour.

Formulation Tanos

Reference: Brown, M. R. (1997a) DPX-KP481-25 50WG: Flow-through, acute, 96-hour LC₅₀ to rainbow trout, *Oncorhynchus mykiss*. Report/Doc no.: DuPont HL-1997-00440

Guidelines: OECD 203, US EPA 72-1

GLP: Yes

Deviations: Measured concentrations for the second active ingredient (famoxadone) were unexpectedly high (126 – 209 % of nominal) whereas measured concentrations for cymoxanil were 100 – 124 % of nominal.

Validity: Study is considered acceptable. See also comment of RMS below.

Material and methods:

Test substance: DPX-KP481-25 50WG, purity: 26.6 % DPX-T3217 (cymoxanil) and 25.4 % DPX-JE874 (famoxadone), batch no.: not stated in the study report

Test species: Rainbow trout (*Oncorhynchus mykiss*), wet weight: 0.22 – 0.92 g (mean: 0.48 g), length: 2.6 – 4.0 cm (mean: 3.3 cm)

Treatments: Dilution water control, solvent control, 8, 14, 25, 43 and 75 µg formulation/L

Nominal cymoxanil concentrations: Control, solvent control, 2.1, 3.7, 6.7, 11 and 20 µg/L

Nominal famoxadone concentrations: Control, solvent control, 2.0, 3.6, 6.4, 10.9 and 19.1 mg/L

Solvent: Dimethylformamide (DMF), maximum DMF concentration in the solvent control and test solutions was 0.1 mL/L

No. of organisms: 2 replicates with 5 fish each per treatment and control

Test type / duration: Flow-through system, 12 turnovers per day, duration: 96 hours

Test medium: Dilution water originated from the [REDACTED] well. Analytical data of the used well water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 10.0 – 10.7 mg/L

pH: 7.3 – 7.7

Total hardness: 81 – 83 mg/L as CaCO₃

Test conditions:

Temperature: 11.2 – 11.9 °C

Photoperiod: 16 hours light and 8 hours darkness with 30 minutes transition periods

Feeding: No feeding from approximately 24 hours prior to and during the test.

Observations: Mortalities and sublethal effects were recorded daily.

Analytical measurements: For chemical analysis of the active substances cymoxanil and famoxadone samples were taken from all replicates of all treatment groups at day 0 and day 4. Additionally samples from test chambers were taken when all fish had died in a chamber.

Method of analysis: HPLC

Statistical evaluation: LC₅₀ values and their 95 % confidence limits were estimated using the moving average angle method.

Findings:

Analytical results: Mean measured cymoxanil concentrations (over time) were 2.3, 4.3, 7.3, 16 and 23 µg/L which is 110 – 140 % of nominal. For individual sampling times and replicates measured concentrations were in the range of 100 – 124 %. Just for the 11.4 µg/L treatment group measured concentrations were 132 – 140 % of nominal concentrations.

Mean measured concentrations of famoxadone were 3.5, 5.7, 9.2, 18 and 27 µg/L which is 142 – 175 % of nominal. For individual sampling times and replicates measured concentrations were in the range of 126 – 209 %.

Mortality: Dilution water control, solvent control, 8 µg/L and 14 µg/L: No mortalities

25 µg/L: 20 % after 72 h and 96 h

43 µg/L: 30 % after 24 hours and 100 % after 48 hours

75 µg/L: 100 % after 24 hours

Sublethal effects: Up to and including 14 µg/L no sublethal effects were detected. From 25 µg/L onwards effects such as fish at the surface or lying on the bottom were observed already after 24 hours. At 25 µg/L some of surviving fish were found lethargic or with dark colouration after 96 hours.

Conclusion:

LC₅₀ (96 h): 28.7 µg formulation/L (95 % CL: 22.4 – 35.6 µg/L)

NOEC (96 h): 14 µg formulation/L

Values are based on nominal concentrations.

Comment (RMS)

Measured famoxadone concentrations were 126 - 209 % of nominal for individual sampling times and replicates. However, measured cymoxanil concentrations were 100 – 124 % of nominal. The measured famoxadone values of the stock solutions correlated to the higher than expected values in the test solutions. For days -1, 0 and 2 measured famoxadone concentrations were 133 – 192 % of nominal in stock solutions. No explanation for these findings is given in the study report. The LC₅₀ is based on nominal concentrations, which are clearly lower than measured concentrations for the highly toxic substance famoxadone (LC₅₀ fish = 0.011 mg/L) and so the resulting LC₅₀ is rather overestimating the toxicity of the test substance Tanos. Therefore the study can be accepted as being a rough estimate of the acute toxicity of the formulation Tanos to fish.

Reference: Hoke, R. A. (1998a) DPX-KP481-25 50WG: Static, acute, 96-hour LC₅₀ to rainbow trout, *Oncorhynchus mykiss*. Report/Doc no.: DuPont-1148

Guidelines: OECD 203, US EPA 72-1

GLP: Yes

Deviations: Concentrations of cymoxanil were not analytically verified. Only concentrations of the second active ingredient (famoxadone) were measured at test start.

Validity: The study is only considered as additional information (see comment of RMS below).

Material and methods:

Test substance: DPX-KP481-25 50WG, purity: 25.63 % DPX-T3217 (cymoxanil) and 25.08 % DPX-JE874 (famoxadone), batch no.: not stated in the study report

Test species: Rainbow trout (*Oncorhynchus mykiss*), wet weight: 0.102 – 0.234 g (mean: 0.139 g), length: 2.2 – 2.9 cm (mean: 2.5 cm)

Treatments: Dilution water control, 8, 14, 25, 43 and 75 µg formulation/L

Nominal cymoxanil concentrations: 2.1, 3.6, 6.4, 11.0 and 19.2 µg/L

Nominal famoxadone concentrations: 2.0, 3.5, 6.3, 10.8 and 18.8 µg/L

No. of organisms: One replicate with 10 fish per test concentration and control

Test type / duration: Static test system, duration: 96 hours

Test medium:

Dilution water originated from the [REDACTED] well. Analytical data of the used well water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 9.3 – 11.0 mg/L

pH: 7.1 – 7.5

Total hardness: 118 mg/L as CaCO₃

Test conditions:

Temperature: 11.7 – 12.8 °C

Photoperiod: 16 hours light and 8 hours darkness with 30 minutes transition periods

Feeding: No feeding from approximately 48 hours prior to and during the test

Observations: Mortalities and sublethal effects were recorded after 0, 2, 4, 8, 16, 24, 48, 72 and 96 hours.

Analytical measurements: Cymoxanil concentrations were not analytically verified. For analysis of famoxadone samples of each test solution were taken at test start. At test end famoxadone concentrations were not measured.

Method of analysis (famoxadone): HPLC.

Statistical evaluation: LC₅₀ values and their 95 % confidence limits were estimated by probit analysis.

Findings:

Analytical results: In samples taken at test start measured concentrations of famoxadone were in the range of 80 to 85 % of nominal concentrations. For cymoxanil no measurements were performed.

Sublethal effects: In the water control and at 8 µg/L no sublethal effect were observed. From 14 µg onwards effects such as fish at the surface or lying on the bottom, erratic swimming, laboured respiration, haemorrhages, hyperactivity, lethargy, partial loss of equilibrium or rapid respiration were recorded.

Mortality:

Table B.9.2.1-3: Cumulative mortality of rainbow trout (*Oncorhynchus mykiss*) exposed to the formulation DPX-KP481-25 50 WG in a static, acute, 96-hour test.

Test concentration [µg formulation/L] ^a	Cumulative mortality [%]						
	8 h	16 h	24 h	36 h	48 h	72 h	96 h
Control	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0
14	0	0	0	0	0	10	10
25	0	0	0	10	10	10	10
43	0	50	80	90	90	90	90
75	50	100	100	100	100	100	100

^a nominal concentrations

Conclusion

LC₅₀ (96 h): 30 µg formulation/L (95 % CL: 24 – 39 µg/L)

NOEC (96 h): 8 µg formulation/L

Values are based on nominal concentrations.

Comments (RMS):

Concentrations of cymoxanil were not analytically verified. Only concentrations of the second active ingredient (famoxadone) were measured at test start. These measurements confirm that the intended initial concentrations of test substance Tanos were achieved. However, no analytical measurements of the active ingredients were performed at test end and therefore no information on the exposure pattern over the test duration is available. For this reason the test is only considered as additional information.

Formulation Cymoxanil 50 % WP

Reference: Bogers, M. (1999a) 96-hour acute toxicity study in rainbow trout with Cymoxanil 50 % WP (semi-static). Report/Doc no.: [REDACTED] Project 262428

Guidelines: OECD 203

GLP: Yes

Deviations: The pH of test solutions was adjusted to 6.5 ± 0.1 to enhance the stability of cymoxanil.

Validity: The study is considered acceptable.

In a range finding test 3 fish per treatment were exposed to a nominal concentration ranging from 0.2 to 200 mg formulation/L with an increasing factor of 10 under static conditions. After 96 hours no fish had died at 20 mg formulation/L and all fish had died at 200 mg formulation/L.

Material and methods:

Test substance: Cymoxanil 50 % WP, purity: 505 g cymoxanil/kg, batch no.: V343

Test species: Rainbow trout (*Oncorhynchus mykiss*), mean wet weight ± s.d.: 2.69 ± 0.32 g, mean length ± s.d.: 5.33 ± 0.36 cm

Treatments: Dilution water control, 0, 20, 36, 65, 120 and 200 mg formulation/L

Nominal cymoxanil concentrations: 10.1, 18.2, 32.8, 60.6 and 101 mg/L

The pH of test solutions was adjusted to 6.5 ± 0.1 to enhance the stability of cymoxanil.

No. of organisms: 7 fish per concentration and control

Test type / duration: Semi-static test system with daily renewal of test solutions,
duration: 96 hours

Test medium:

Dilution water: Tap-water, analytical data indicate adequate quality for the purpose of this study.

Dissolved oxygen: 10.1 – 10.7 mg/L in fresh solutions, 8.2 – 9.8 mg/L in old solutions

pH: 6.4 – 6.6 in fresh solutions, 6.6 – 7.3 in old solutions

Total hardness: 118 mg/L as CaCO₃

Test conditions:

Temperature: 13.3 – 14.3 °C

Photoperiod: 16 hours light and 8 hours darkness

Feeding: No feeding from approximately 48 hours prior to and during the test.

Observations: Mortalities and sublethal effects were recorded after 3, 24, 48, 72 and 96 hours.

Analytical measurements: For chemical analysis of cymoxanil samples were taken from freshly prepared solutions after 0 and 48 hours and from old test solutions after 24 and 72 hours.

Method of analysis: HPLC

Statistical evaluation: LC₅₀ values and their 95 % confidence limits were estimated by probit analysis.

Findings:

Analytical results: In fresh test solutions measured concentrations were in the range of 99 to 110 % of nominal. In old test solutions measured concentrations were in the range of 92 to 104 % of nominal cymoxanil concentrations.

Mortality: At 0, 20, 36 and 65 mg/L: No mortalities

120 mg/L: After 72 hours 1 fish out of 7 had died and after 96 hours 3 fish had died (43 %).

200 mg/L: After 72 hours all fish had died.

Sublethal effects: From 65 mg/L onwards sublethal effects such as hypoactive swimming and discolouration were observed.

Conclusion:

LC₅₀ (96 h): 120 mg formulation/L (95 % CL: 100 – 160 mg/L)

60.6 mg a.s./L (95 % CL: 51 – 81 mg/L)

NOEC (96 h): 36 mg formulation/L

18.2 mg a.s./L

Values are based on nominal concentrations.

Comments (RMS):

The pH of test solutions was adjusted to 6.5 ± 0.1 to enhance the stability of cymoxanil. However, no effects were observed in fish of the buffered and pH adjusted control. Hence the pH adjustment is not considered to have significantly influenced the outcome of the test. Therefore the study is considered acceptable.

The LC₅₀ was estimated by probit analysis which is strictly speaking not an appropriate statistical tool for results with less than two test concentrations with partial mortalities (between 0 and 100 %). However, taking the raw data into consideration the derived LC₅₀ seems reasonable and the study is considered acceptable, especially to demonstrate the low toxicity of the formulation Cymoxanil 50 % WP to fish.

B.9.2.2 Long-term and chronic toxicity to fish (Annex IIA 8.2.2)

B.9.2.2.1 Chronic toxicity test on juvenile fish (Annex IIA 8.2.2.1)

Reference: Baer, K. N. (1992a) Flow-through, 21-day toxicity of DPX-T3217-113 (cymoxanil) to rainbow trout, *Oncorhynchus mykiss*. Report/Doc no.: Du Pont HLR 545-92

Guidelines: OECD 204

GLP: Yes

Deviations: None of relevance.

Validity: The study is considered acceptable.

In a range finding test 5 fish per treatment were exposed to nominal concentrations of 0, 1, 25 and 50 mg/L under static conditions. Respective mortalities were 0, 0, 80 and 100 %. The test duration is not stated in the study report.

Material and methods:

Test substance: Cymoxanil technical, purity: 96.5 % (initial analysis), 97.8 % (reanalysis), batch no.: DPX-T3217-113

Test species: Rainbow trout (*Oncorhynchus mykiss*)

Mean wet weight (dilution water control at test end): 3.0 g (2.4 – 4.1 g)

Mean length (dilution water control at test end): 5.5 cm (5.1 – 6.2 cm)

Treatments: Dilution water control, solvent control, 0.26, 0.64, 1.6, 4.0 and 10 mg/L

Solvent: Dimethylformamide (DMF), concentrations of DMF in the solvent control and treatments are not stated in the study report

No. of organisms: 2 replicates with 5 fish each per test concentration and control, replicate A of the 0.22 mg/L treatment group contained 6 fish

Test type / duration: Flow-through system, 6 volume exchanges per day, duration: 21 days

Test medium:

Dilution water originated from the [REDACTED] well. Analytical data of the used well water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 8.6 – 9.1 mg/L

pH: 7.0 – 7.6

Total hardness: 76 – 86 mg/L as CaCO₃

Test conditions:

Temperature: 12.5 – 14.9 °C

Photoperiod: 16 hours light and 8 hours dark with 25 minutes transition periods

Feeding: Fish were fed Purina Trout Chow once daily.

Observations: Mortalities were recorded once daily. Body weights and lengths of fish were determined at the end of the test (21 d).

Analytical measurements: For chemical analysis of the test substance samples were taken from each test vessel on days 0, 7, 14 and 21.

Method of analysis: HPLC

Statistical evaluation: LC₅₀ and its 95 % confidence limits: Probit analysis; NOECs: ANOVA followed by Dunnett's test and Jonckheere's trend test

Findings:

Analytical results: Mean measured concentrations were 0.22, 0.5, 1.2, 2.6, 6.8 mg/L (65 – 85 % of nominal). For individual sampling dates and replicates measured concentrations were in the range of 46 – 113 % of nominal

Mortality:

Table B.9.2.2.1-1: Cumulative Mortality of juvenile rainbow trouts exposed to cymoxanil for 21 days. Test concentrations are mean measured concentrations.

Day of exposure	Cumulative mortality [%]						
	Control	DMF control	0.22 mg/L	0.5 mg/L	1.2 mg/L	2.6 mg/L	6.8 mg/L
0 – 6	0	0	0	0	0	0	0
7	0	0	17	0	0	0	0
8	0	0	17	0	0	0	20
9	0	0	17	0	0	10	40
10	0	0	17	0	0	10	50

Day of exposure	Cumulative mortality [%]						
	Control	DMF control	0.22 mg/L	0.5 mg/L	1.2 mg/L	2.6 mg/L	6.8 mg/L
11	0	0	17	0	0	10	60
12	0	0	17	0	30	20	70
13	0	0	17	0	30	20	70
14	0	0	17	0	30	20	70
15	0	0	17	0	30	20	70
16	0	0	17	0	40	20	70
17	0	0	17	0	40	30	80
18	0	0	17	0	40	40	90
19	0	0	17	0	40	40	90
20	0	0	17	0	50	60	90
21	0	0	17	0	50	70	90

Table B.9.2.2.1-2: Effects of cymoxanil on growth and survival of juvenile rainbow trouts after 21 days of exposure. Test concentrations are mean measured concentrations.

Test parameter	Control	DMF control	0.22 mg/L	0.5 mg/L	1.2 mg/L	2.6 mg/L	6.8 mg/L
Mean length [cm]	5.5	5.4	5.6	5.0*	4.8*	5.0	5.2
Mean wet weight [g]	3.03	2.88	3.40	2.20*	1.86*	2.33	1.92
Mortality [%]	0	0	9	0	50	70	90

Statistically significant from control at $p < 0.05$

Conclusion:

LC₅₀ (14 d): 5 mg/L (95 % CL: 3.6 – 7.8 mg/L)

LC₅₀ (21 d): 1.5 mg/L (95 % CL: 0.94 – 2.7 mg/L)

NOEC (21 d): 0.22 mg/L

Values are based on mean measured concentrations.

B.9.2.2.2 Early life-stage toxicity test (Annex IIA 8.2.2.2)

Reference: Boeri, R. L., Magazu, J. P., Ward T. J. (1997) DPX-T3217-113 (Cymoxanil): Early life-stage toxicity to rainbow trout, *Oncorhynchus mykiss*. Report/Doc no.: DuPont HLO 1013-96

Guidelines: OECD 210, US EPA 72-4

GLP: Yes

Deviations: None of relevance.

Validity: The study is considered acceptable.

Material and methods:

Test substance: Cymoxanil technical, purity: 97.8 % (initial analysis), 97.3 % (reanalysis), batch no.: DPX-T3217-113

Test species: Rainbow trout (*Oncorhynchus mykiss*), embryos, approximately 1 hour post fertilisation

Treatments: Dilution water control, 1.0, 2.5, 6.5, 16, 40 and 120 µg/L, stock solutions were adjusted to a pH of ≤ 5 with 0.4 % phosphoric acid to increase the stability of the test substance.

No. of organisms: 2 replicate test vessels per treatment, initially 40 embryos per replicate vessel (2 embryo cups with 20 embryos each per replicate vessel), thinned to 15 fish per replicate vessel at hatch (35 d). At test initiation total of 80 embryos and post hatch 30 fish per treatment.

Test type / duration: Flow-through system, average of 7.6 volume additions per 24 hours, duration: 97 days (62 days post hatch)

Test medium:

Dilution water: Carbon-filtered deionised tap water adjusted to a hardness of 40 to 48 mg/L as CaCO₃ and to a pH of approximately 7 with phosphoric acid. The water was filtered through a 5 µm filter and ultraviolet steriliser prior to use in the study. Analytical results of the dilution water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 9.2 – 11.2 mg/L

pH: 6.9 – 7.2

Total hardness: 40 – 48 mg/L as CaCO₃

Test conditions:

Temperature: 9.0 – 11.8 °C

Photoperiod: Embryos were initially kept in darkness except for a short period of observation and taking of water quality data. One week following hatch 16 hours light and 8 hours darkness with 15 minutes transition periods were applied.

After hatch (35 days of exposure) fish were released from the exposure cups and randomly selected and thinned to 15 live fish per replicate vessel.

Feeding: Following swim up, fish were fed live, newly hatched *Artemia salina* nauplii ad libitum 3 times per day except during the final 28 hours of the test.

Endpoints: Number and per cent of healthy embryos hatched, time to hatch (start and end of hatch), time to swim up, time to first feeding, survival and sublethal effects at test end, total length and wet weight (blotted) of surviving fish at the end of the test. Mortalities and sublethal effects were recorded daily.

Analytical measurements: For chemical analysis of the test substance samples were collected from each replicate test vessel at test initiation, every 7 days after test initiation and at test termination.

Method of Analysis: HPLC

Statistical evaluation: Survival at hatch, length and weight data: If data were normally distributed and variances were homogenous, a one-way analysis of variance (ANOVA) and a Dunnett's test were used to compare treatment and control data. In other cases, a non-parametric analysis (Kruskal and Wallis test) was used to compare treatment and control data.

Findings:

Analytical results: Mean measured concentrations over time were 0.98, 2.4, 5.7, 15, 38 and 120 µg/L.

Individual measurements were within the range of 80 to 120 % of their nominal values with few exceptions in the three lowest test concentrations.

Mortality and sublethal effects:

Table B.9.2.2.2-1: Effects of cymoxanil on hatch, survival at hatch, swim-up, juvenile survival, and growth of *Oncorhynchus mykiss* after 97 days of exposure (62 days post hatch).

Treatment [µg/L] ^a	Start - end of hatch [d]	First day of swim-up & feeding	Survival at hatch [%]	Juvenile survival ^b [%]	Length ^b [mm]	Weight ^{bc} [g]
Control	32 - 35	44	67.5	100	50.4 ± 2.5	1.41 ± 0.25
0.98	33 - 35	44	66.3	100	49.0 ± 2.6	1.45 ± 0.21
2.4	32 - 35	44	62.5	100	47.2 ± 1.9 *	1.44 ± 0.16
5.7	32 - 35	44	66.3	100	47.2 ± 2.1 *	1.45 ± 0.19
15	32 - 35	44	61.3	100	48.6 ± 2.8 *	1.41 ± 0.22
38	32 - 35	44	63.8	100	48.4 ± 3.6 *	1.55 ± 0.34
120	32 - 33	44	66.3	100	48.4 ± 3.0 *	1.54 ± 0.28

^a Mean measured concentrations

^b At test end

^c Blotted wet weight

* Statistically significantly different from the control (p < 0.05)

No other sublethal effects except some reduction in length (see Table B.9.2.2.2-1) were observed at any treatment during the test.

Conclusion:

NOEC (97 d): 0.98 µg/L (based on mean measured concentrations)

At 2.4 µg/L total length of surviving fish was reduced.

Comments (RMS):

The LOEC of 2.4 µg/L and the NOEC of 0.98 µg/L are based on a statistically significant difference in length compared to control fish. However, no dose response relationship was found for the decrease in body lengths of fish (see Figure B.9.2.2.2-1). At 2.4 µg/L body length was reduced by 6.3 % relative to the control. At 120 µg/L the reduction was only 4 %. Since no dose-response relationship was found, this small reduction in length is not considered ecologically relevant. Therefore the RMS considers a NOAEC of 120 µg/L acceptable for risk assessment.

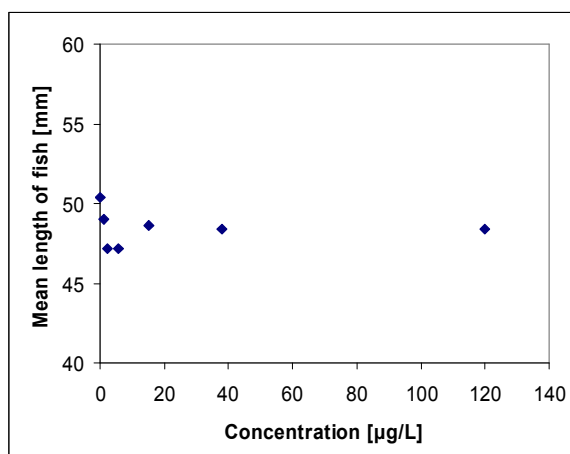


Figure B.9.2.2.2-1: Mean length of fish at the end of the early life-stage toxicity test (92 d) with cymoxanil. No clear dose-response relationship is given.

Reference: Boeri, R. L., Kowalski, P. L., Ward T. J. (1996b) Early life-stage toxicity of DPX-T3217-113 (cymoxanil) to sheepshead minnow, *Cyprinodon variegatus*. Report/Doc no.: DuPont HLO 913-96

Guidelines: OECD 210, US EPA 72-4

GLP: Yes

Deviations: The pH of the dilution water was adjusted to approximately 7.0 with hydrochloric acid to increase the stability of the test substance.

Validity: The study is considered acceptable.

In a range finding test juvenile sheepshead minnows were exposed to a dilution water control, a solvent control and nominal concentrations of 0.0050, 0.050, 0.50, 5.0 and 50 mg/L under semi static conditions for 11 days. Survival for these treatments was 100, 100, 80, 100, 100, 0 and 0 %.

In a second range finding study embryos of sheepshead minnows (less than 24 hours old) were exposed to a control and nominal concentrations of 0.36, 0.6, 1.3, 2.6 and 5.0 mg/L under flow-through conditions for 20 days.

At the end of the test survival for these treatments was: > 80, > 80, 30, 0, 0 and 0 %

Material and methods:

Test substance: Cymoxanil technical, purity: 97.8 % (initial analysis), 96.5 % (reanalysis), batch no.: DPX-T3217-113

Test species: Sheepshead minnow (*Cyprinodon variegatus*), embryos less than 24 hours old at test initiation

Treatments: Dilution water control, solvent control, 0.058, 0.1, 0.2, 0.4 and 0.8 mg/L

No. of organisms: 2 replicate test vessels per treatment, initially 40 embryos per replicate vessel (2 embryo cups with 20 embryos each per replicate vessel), thinned to 15 fish per replicate vessel at hatch (day 4). At test initiation a total of 80 embryos at test start and 30 fish per treatment post hatch.

Test type / duration: Flow-through system, average of 22 volume additions per 24 hours. This relatively high turnover rate was employed to maintain the concentrations of the test substance in the dilution water. Test duration: 36 days (32 days post hatch)

Test medium:

Dilution water: Natural sea water collected from the Atlantic Ocean at [REDACTED] in [REDACTED]

The water was adjusted to a salinity of 15 - 17 parts per thousand with deionised water, was passed through particle filters including an activated carbon filter and UV sterilised prior to use. The pH of the dilution water was adjusted to approximately 7.0 with hydrochloric acid to increase the stability of the test substance. Analytical results of the used sea water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 6.5 – 7.6 mg/L

pH: 6.9 – 7.6

Salinity: 15 – 17 parts per thousand

Test conditions:

Temperature: 29.2 – 31.0 °C

Photoperiod: 16 hours light and 8 hours darkness with 15 minutes transition periods

Feeding: Beginning on day 4, fish were fed newly hatched *Artemia salina* nauplii two or three times each day except during the final 24 hours of the test. Fish were fed in excess of requirements.

Endpoints: Number and per cent of healthy embryos after 48 hours and at hatch, time to start and end of hatch, time to first feeding, survival and sublethal effects of embryos, larvae and juveniles, total length and wet weight (blotted) of surviving fish at the end of the test. Mortalities and behavioural observations were recorded daily.

Analytical measurements: For chemical analysis of the test substance samples from each replicate test vessel were collected on days 0, 7, 14, 21, 28, 35 and 36.

Method of Analysis: HPLC

Statistical evaluation: A one-way analysis of variance (ANOVA) and a Dunnett's test were used to compare treatment and control means.

Findings:

Analytical results:

Mean measured concentrations over time were 0.0581, 0.0942, 0.178, 0.364 and 0.767 mg/L. For individual sampling dates measured concentrations were in the range of 80 – 109 % of nominal concentrations.

Hatch: Hatching started on day 3 and was completed on day 4 in all treatment groups.

Time to feeding: All fish fed when first presented with food.

Sublethal effects: Sublethal effects such as erratic swimming, loss of equilibrium and lethargy were observed in the 0.364 mg/L treatment group on day 36 and in the 0.767 mg/L treatment group on day 4 of exposure in some fish.

Table B.9.2.2.2-2: Effects of cymoxanil on egg hatching, swim-up, survival at hatch, juvenile survival, and growth of *Cyprinodon variegatus*.

Treatment ^c [µg/L]	Survival at hatch [%]	Juvenile survival [%]	Length ^a [mm]	Weight ^b [g]
Control	98	93	20.5 ± 1.4	159 ± 36
0.0581	100	90	19.5 ± 1.6	140 ± 34
0.0942	96	80	19.1 ± 3.3	141 ± 71
0.178	100	60	20.6 ± 2.8	186 ± 71
0.364	96	23	20.7 ± 4.2	196 ± 124

Treatment ^c [µg/L]	Survival at hatch [%]	Juvenile survival [%]	Length ^a [mm]	Weight ^b [g]
0.767	94	7	12.6 ± 2.9	205 ± 90

^a At test end, mean ± S.D.

^b Blotted wet weight at test end, mean ± S.D.

^c Mean measured concentrations

Conclusion:

NOEC (36 d): 0.0581 mg/L (set by the RMS, see comments below)

At the next higher concentration of 0.0942 mg/L survival of fish was reduced.

Values are based on mean measured concentrations.

Comments (RMS):

The notifier used a one-way analysis of variance (ANOVA) followed by a Dunnett's test to compare treatment and control means if data were normally distributed. However, for the parameters survival at hatch and juvenile survival for each treatment group only two values (from the two replicate test chambers per treatment) were available as input data for the statistical procedures. Generally with two values no statement on the distribution of data can be made and no sound statistical analysis is possible. Therefore the statistics for these parameters was not considered acceptable by the RMS.

In the study report the notifier stated separate NOEC values for the different endpoints and observation times of the study. The lowest NOEC set by the notifier was 0.0942 mg/L based on juvenile survival. The RMS does not agree with this setting of the NOEC because the statistics on which it is based is not considered acceptable and it can be seen from figure B.9.2.2.2-2 that the test concentration of 0.0942 mg/L is already part of the dose-response relationship for the endpoint juvenile survival. In conclusion the RMS is of the opinion that a more appropriate NOEC would be 0.0581 mg/L based on survival of juvenile fish after 32 days post hatch exposure (36 days of total exposure).

The notifier submitted a detailed comment and a revaluation of the data of this study to demonstrate that the correct NOEC from this study would be 0.0942. This comment was received only a few days before finalising the DAR. Therefore it was not taken into account by the RMS and for risk assessment a NOEC of 0.0581 was used by the RMS. However, the RMS will consider the additional information provided during the peer review of cymoxanil.

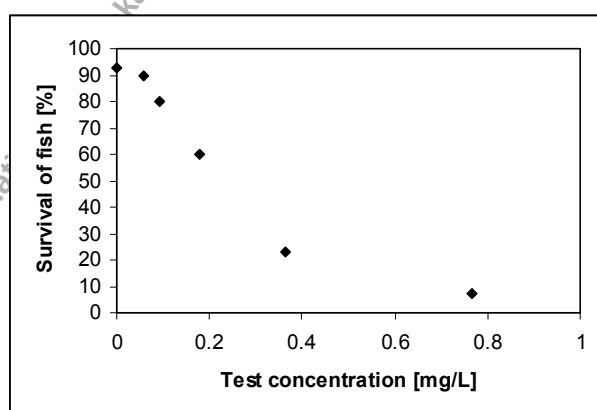


Figure B.9.2.2.2-2: Survival of fish after 32 days of exposure (post hatch) to five concentrations of cymoxanil and a dilution water control.

B.9.2.2.3 Fish full life cycle test (Annex IIA 8.2.2.3)

No study submitted, not required.

B.9.2.3 Bioconcentration in fish (Annex IIA 8.2.3)

Active substance:

The log Pow of cymoxanil was found to be 0.67 - 0.59 at 20 °C. Hence no bioconcentration study is demanded.

Metabolites:

No experimentally derived log Pow values are available for metabolites of cymoxanil. The RMS estimated log Pow values with the software KOWWIN v1.67 (software developed by US Environmental Protection Agency) for the metabolites IN-KQ960, IN-U3204, IN-T4226, IN-JX915, IN-R3273 and IN-KP533 and IN-W3595 (see table B.9.2.3-1).

Table B.9.2.3-1: Log Pow values for metabolites estimated with the software KOWWIN v1.67

Metabolite	Log Pow
IN-KQ960	-1.64
IN-U3204	0.39
IN-T4226	0.16
IN-JX915	-0.37
IN-R3273	2.07
IN-KP533	-1.29
IN-W3595	4.27

For the metabolites IN-KQ960, IN-U3204, IN-T4226, IN-JX915, IN-R3273 and IN-KP533 modelled log Pow values were below the trigger of 3 and hence no fish bioconcentration studies are demanded for these degradation products.

For IN-W3595 a log Pow of 4.27 was derived with KOWWIN. IN-W3595 is an organic acid and the software estimates the log Pow for the non dissociated form of the acid. However, in aquatic environments the substance is partly dissociated and it was shown to be highly water soluble. Taking this information into account it is unlikely that it will accumulate in fat tissue (for which the log Pow is an indication). Therefore no bioconcentration study in fish with the metabolite IN-W3595 is considered necessary.

Comment (RMS):

In the report "Assessment of the Environmental Properties and Effects of Pesticide Transformation Products" (Sinclair & Boxall, 2002) an evaluation of the relationship between measured log K_{ow} (=P_{ow}) values and K_{ow} values derived with KOWWIN is given. For KOWWIN it was found that 99 % of transformation products in the test set were predicted to within one log K_{ow} unit. See also figure B.9.2.3-1.

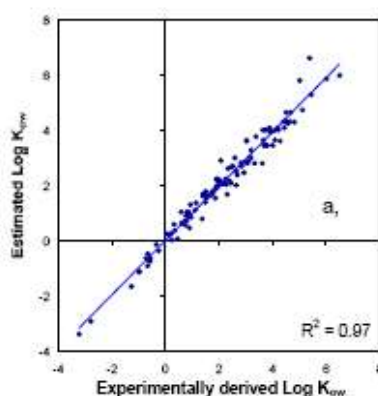


Figure B.9.2.3-1: Relationship between experimentally derived log Kow values for a range of pesticide transformation products and log Kow values predicted using KOWWIN (from Sinclair & Boxall, 2002).

Reference:

Sinclair, C.J. & Boxall, A. (2002) Assessment of the Environmental Properties and Effects of Pesticide Transformation Products. Report to DEFRA, No.PN-0930, Cranfield Centre for EcoChemistry, Cranfield University, Silsoe.
http://www.silsoe.cranfield.ac.uk/ecochemistry/publications/papers/final_pn0930.pdf

B.9.2.4 Acute toxicity to aquatic invertebrates (Annex IIA 8.2.4, Annex IIIA 10.2.1)

Active substance

Reference: Baer, K.N. (1993c) Static, acute, 48-hour EC₅₀ of DPX-T3217-113 (cymoxanil) to *Daphnia magna*. Report/Doc no.: Du Pont HLR 736-92

Guidelines: OECD 202, US EPA 72-2

GLP: Yes

Deviations: The dilution water flowed through aquaria with fathead minnows prior to its use for the daphnid test. Then the water was buffered with 4 mM sodium phosphate and the pH was adjusted to 6.0.

Validity: The study is considered acceptable.

Material and methods:

Test substance: Cymoxanil technical, purity: 96.5 % (initial analysis), 97.8 % (reanalysis), batch no.: DPX-T3217-113

Test species: *Daphnia magna*, neonates less than 24 hours old

Treatments: Dilution water control, buffer control, 19, 32, 54, 90 and 150 mg/L

No. of organisms: Per treatment 4 replicates with 5 daphnids each (20 daphnids per treatment)

Type of test / duration: Static test system, 48 hours

Test medium:

Dilution water originated from the Haskell Laboratory well which flowed through aquaria with fathead minnows prior to use for the daphnid test. Then the water was buffered with 4 mM sodium phosphate and the pH was adjusted to 6.0 with phosphoric acid. Analytical results of the used well water prior to the flow through fish aquaria indicate adequate quality for the purpose of this study.

Dissolved oxygen: 8.4 – 9.0 mg/L

pH dilution water control: 7.6, pH buffer control and cymoxanil treatments: 6.0 – 6.3

Total hardness: 86 mg/L as CaCO₃

Test conditions:

Temperature: 20.6 – 21.0 °C

Photoperiod: 16 hours light and 8 hours darkness with 25 minutes transition periods

Feeding: Daphnids were not fed during the test.

Observations: Observations for immobility were made after 24 and 48 hours.

Analytical measurements: For chemical analysis of the test substance samples from all test concentrations were taken at test start and at the end of the test.

Method of analysis: HPLC

Statistical evaluation: EC₅₀ was estimated by the moving average angle method.

Findings:

Analytical results: Mean measured concentrations over time were 15, 26, 49, 84 and 140 mg/L. Measured concentrations for individual replicates and sampling dates were in the range of 80 - 100 % of nominal concentrations with one exception (76 %).

Sublethal effects: None reported

Immobility:

Table B.9.2.4-1: Immobility of *Daphnia magna* after exposure to cymoxanil

Treatment [mg/L] ^a	Cumulative immobility [%]	
	24 hours	48 hours
Control	0	0
Buffer control	0	0
15	0	0
26	0	65
49	40	90
84	20	80
140	40	100

^a Mean measured concentrations

Conclusion:

EC₅₀ (48 h): 27 mg/L (95 % CL: 20 – 34 mg/L)

NOEC (48 h): 15 mg/L

Values are based on mean measured concentrations.

Comment (RMS):

Dilution water flowed through aquaria with fathead minnows prior to its use for the daphnid test. Then the water was buffered with 4 mM sodium phosphate and the pH was adjusted to 6.0 with phosphoric acid. Since no effects were observed in daphnids of the dilution water control and the pH adjusted control, the buffer and the pH adjustment is not considered to have significantly influenced the outcome of the test. Therefore the study is considered acceptable.

Reference: Boeri, R. R., Kowalski, P. L., Ward, T. J. (1996c) Acute toxicity of DPX-T3217-113 (Cymoxanil) to the mysid, *Mysidopsis bahia*. Report/Doc no.: DuPont HLO 632-96

Guidelines: US EPA 72-3(c)

GLP: Yes

Deviations: None of relevance.

Validity: The study is considered acceptable.

In a range finding test mysids were exposed under flow-through conditions to nominal concentrations of 7, 13, 21, 31 and 50 mg/L as well as to a dilution water and a solvent control for 96 hours. Survival was at least 80 % at all tested concentrations.

Material and methods:

Test substance: Cymoxanil technical, purity: 96.5 % (initial analysis), 97.8 % (reanalysis), batch no.: DPX-T3217-113

Test species: *Mysidopsis bahia*, less than 24 hours old at test initiation

Mean wet weight (blotted): 0.18 mg for control mysids at the end of the test (no measure of statistical spread is provided in the study report)

Treatments: Dilution water control, solvent control (0.5 mL/L DMF), 7.5, 13, 21, 31 and 50 mg/L

Solvent: Dimethylformamide (DMF), maximum of 0.5 mL/L in test solutions

No. of organisms: Per treatment 2 replicates with 10 mysids each (20 mysids per treatment)

Type of test / duration: Flow-through test system, 14 volume additions per 24 hours, duration: 96 hours

Test medium:

Dilution water: Natural seawater collected at T.R. Wilbury Laboratories in Marblehead Massachusetts.

Water was adjusted to a salinity of 11 – 17 parts per thousand and passed through particle filters, activated carbon and an ultraviolet steriliser. Analytical results of the used water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 6.2 – 8.3 mg/L (> 60 % saturation)

pH: 7.4 – 8.0

Salinity: 15 – 16 parts per thousand

Test conditions:

Temperature: 21.0 – 22.8 °C

Photoperiod: 16 hours light and 8 hours darkness with 15 minutes transition periods

Feeding: Mysids were fed live *Artemia salina* nauplii at least once each day.

Observations: Mortalities and the occurrence of sublethal effects were recorded daily.

Analytical measurements: For chemical analysis of the test substance samples of each replicate test vessels were collected after 0 and 96 hours.

Method of analysis: HPLC

Statistical evaluation: Since mortality was less than 50 % in the highest concentration tested no statistical evaluation of data was performed.

Findings:

Analytical results: Measured concentrations were in the range of 88 – 95 % of nominal at test start and between 71 and 82 % of nominal at test end (96 h). Mean measured concentrations over time were 6.09, 10.4, 17.6, 26.5 and 44.4 mg/L.

Sublethal effects:

No sublethal effects were noted at any treatment level.

Mortality:

Table B.9.2.4-2: Mortality of *Mysidopsis bahia* after exposure to cymoxanil

Treatment [mg/L] ^a	Cumulative mortality [%]			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
Solvent control	0	0	0	0
6.09	0	0	0	0
10.4	0	0	0	0
17.6	0	0	0	0
26.5	0	5	5	20
44.4	0	10	10	25

^a Mean measured concentrations

Conclusion:

EC₅₀ (96 h): > 44.4 mg/L

NOEC (96 h): 17.6 mg/L

Values are based on mean measured concentrations.

Reference: Boeri, R. R., Kowalski, P. L., Ward, T. J. (1996d) Acute Flow-through mollusc shell deposition test with DPX-T3217-113 (Cymoxanil). Report/Doc no.: DuPont HLO 633-96

Guidelines: US EPA 72-3(b)

GLP: Yes

Deviations: None of relevance.

Validity: The study is considered acceptable.

A range finding test under static conditions was conducted with a dilution water control, a solvent control and nominal concentrations of 0.005, 0.05, 0.5, 5.0 and 50 mg/L for 96 hours. New shell growth was 2.9, 2.6, 2.1, 3.1, 3.4, 0.8 and 0 mm. Survival was 100 % at all tested concentrations.

In a second screening test a dilution water control, a solvent control and nominal concentrations of 7.5, 13, 21, 31 and 50 mg/L resulted in average new shell growth of 2.9, 3.4, 3.5, 3.0, 3.0, 1.4 and 1.0 mm after 96 hours. Survival was 100 % at all tested concentrations.

Material and methods:

Test substance: Cymoxanil technical, purity: 96.5 % (initial analysis), 97.8 % (reanalysis), batch no.: DPX-T3217-113

Test species: *Crassostrea virginica*, oysters were 25 – 50 mm in height (measured along the long axis). At test initiation from each oyster approximately 3 – 5 mm of shell was removed with a rotary grinder.

Treatments: Dilution water control, solvent control (0.5 mL/L DMF), 7.0, 12, 20, 31 and 50 mg/L.

Solvent: Dimethylformamide (DMF), max. of 0.05 mL/L in all test solutions

No. of organisms: Per treatment 2 replicates with 10 oysters each (20 oysters per treatment)

Type of test / duration: Flow-through test system, 19 volume additions per 24 hours and 0.56 litres per oyster per hour, duration: 96 hours

Test medium:

Dilution water: Unfiltered, natural seawater collected at T.R. Wilbury Laboratories in Marblehead Massachusetts.

Dissolved oxygen: 5.0 – 7.6 mg/L (≥ 56 % saturation)

pH: 7.7 – 8.3

Salinity: 32 – 33 parts per thousand

Test conditions:

Temperature: 20.5 – 21.4 °C

Photoperiod: 16 hours light and 8 hours darkness with a 15 minutes transition period

Feeding: Live marine phytoplankton to supplement the existing food in the unfiltered, natural seawater that was used as dilution water

Observations: Mortalities and sublethal effects were recorded daily. At the end of the study oysters were removed from test vessels and the longest finger of new shell growth was measured to the nearest 0.1 mm.

Analytical measurements: For chemical analysis of the test substance samples were taken from each replicate test vessel after 0 and 96 hours.

Method of analysis: HPLC

Statistical evaluation: Control and solvent control new shell growth data were compared with a t-test and found to be statistically significantly different for a alpha of 0.05. Therefore new shell growth data in the treatments were compared to the solvent control data using analysis of variance and Dunnett's test to set a NOEC. Since effects on shell growth were less than 50 % at the highest treatment level, no statistical estimate for the EC50 was obtained.

Findings:

Analytical results: Measured concentrations for individual replicates and the two sampling dates (0 and 96 h) were in the range of 79 – 102 % of nominal concentrations. Mean measured concentrations over time were 6.32, 9.87, 18.6, 28.2 and 46.9 mg/L.

Shell deposition:

Table B.9.2.4-3: Effects of cymoxanil on shell deposition in *Crassostrea virginica*

Treatment [mg/L] ^a	Shell deposition after 96 hours [mm]	
	Mean	Standard deviation
Control	2.9	0.8
Solvent control	3.4	0.6
6.32	3.3	1.1
9.87	3.3	1.2
18.6	3.0	1.5
28.2	3.0	0.9
46.9	2.2 *	0.9

^a Mean measured concentrations

* Significantly different from solvent control (p = 0.05)

Sublethal effects:

No other sublethal effects apart from effects on shell growth were noted at any treatment level.

Conclusion:

EC₅₀ (96 h): > 46.9 mg/L

NOEC (96 h): 28.2 mg/L

Values are based on mean measured concentrations.

Metabolites

Reference: Boeri, R. L., Wyskiel, D. C., Ward, T. J. (2002c) IN-T4226: Acute, 48-hours EC₅₀ to *Daphnia magna*. Report/Doc no.: DuPont-9385

Guidelines: OECD 202, US EPA OPPTS 850.1010

GLP: Yes

Deviations: No dilution water control, only a HEPES buffered control. The pH of test solutions was adjusted to 6.6.

Validity: The study is considered acceptable.

In a range finding test 10 daphnids per treatment were exposed under static conditions to a dilution water control, 0.99, 5.0, 10, 50 and 130 mg/L for 48 hours. Respective immobilities were 0, 0, 10, 10, 100 and 100 %.

Material and methods:

Test substance: IN-T4226, purity: 99.1 %, batch no.: IN-T4226-1

Test species: *Daphnia magna*, neonates less than 24 hours old

Treatments: HEPES-buffered control, 17, 28, 47, 78 and 130 mg/L. The pH of test solutions was adjusted to 6.6 with 0.1 N hydrochloric acid and 0.1 N sodium hydroxide.

No. of organisms: Per treatment 4 replicates with 5 daphnids each (20 daphnids per treatment)

Type of test / duration: Static-renewal system, renewal of test solutions after 24 hours, duration: 48 hours

Test medium:

Dilution water: Deionised water, hardness was adjusted to 160 - 180 mg/L as CaCO₃, the pH was automatically adjusted to 7.7 with 5 % phosphoric acid, the water was passed through a particle filter.

Data of semi-annual analysis of the dilution water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 8.0 – 8.7 mg/L

pH: 6.6 – 6.8

Total hardness: 164 – 172 mg/L as CaCO₃

Test conditions:

Temperature: 19 – 20.8 °C

Photoperiod: 16 hours light and 8 hours darkness with 15 minutes transition periods

Feeding: Daphnids were not fed during the test.

Observations: Immobility and behavioural observations were made every 24 hours.

Analytical measurements: For chemical analysis of the test substance samples of new test solutions prior to use were taken at 0 and 24 h and samples of old test solutions were taken from two randomly selected replicate test vessels of each treatment group.

Method of analysis: HPLC

Statistical evaluation: No statistical evaluation of immobility data was performed because immobility was less than 50 % in all tested concentrations.

Findings:

Analytical results: Mean measured concentrations over time were 16.3, 26.8, 42.7, 69.3 and 116 mg/L. Measured concentrations for individual sampling dates were in the range of 76 - 117 % of nominal concentrations.

Immobility:

Test concentrations of 16.3, 26.8, 42.7, 69.3 and 116 mg/L resulted in 0, 0, 5, 0 and 0 % immobility, respectively.

Sublethal effects:

No sublethal effects were observed in surviving daphnids.

Conclusion:

EC₅₀ (48 h): > 116 mg/L

NOEC (48 h): 116 mg/L

Values are based on mean measured concentrations.

Comment (RMS):

No dilution water control was set up. However in the HEPES buffered and pH adjusted control no effects on daphnids were observed. Therefore the buffer and the pH adjustment are not considered to have significantly influenced the results of the test and hence the study is considered acceptable.

Reference: Boeri, R. L., Wyskiel, D. C., Ward, T. J. (2002d) IN-W3595: Acute, 48-hours EC₅₀ to *Daphnia magna*. Report/Doc no.: DuPont-9383

Guidelines: OECD 202, US EPA OPPTS 850.1010

GLP: Yes

Deviations: None of relevance

Validity: The study is considered acceptable.

In a range finding test 5 daphnids per treatment were exposed to a dilution water control, 0.098, 0.98, 10, 50 and 130 mg/L under static conditions for 48 hours. Respective immobilities were 10, 0, 10, 0 and 10 %.

Material and methods:

Test substance: IN-W3595, purity: 98.8 %, batch no.: IN-W3595-004

Test species: *Daphnia magna*, neonates less than 24 hours old

Treatments: Dilution water control, 17, 29, 47, 78 and 130 mg/L

No. of organisms: Per treatment 4 replicates with 5 daphnids each (20 daphnids per treatment)

Type of test / duration: Static system, 48 hours

Test medium:

Dilution water: Deionised water, hardness was adjusted to 160 to 180 mg/L as CaCO₃/L, pH was adjusted to 7.2 – 7.3 with 5 % phosphoric acid, the water was passed through a particle filter. Data of semi-annual analysis of the dilution water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 8.7 – 9.0 mg/L

pH: 7.4 – 7.9

Total hardness: 160 mg/L as CaCO₃

Test conditions:

Temperature: 19.1 – 20.1 °C

Photoperiod: 16 hours light and 8 hours darkness with 15 minutes transition periods

Feeding: Daphnids were not fed during the test.

Observations: Immobility and behavioural observations were made every 24 hours.

Analytical measurements: For chemical analysis of the test substance samples of each test solution prior to distribution to the test chambers were taken at test start. On day 2 samples from two randomly selected replicates of each treatment level were taken.

Method of analysis: HPLC

Statistical evaluation: No statistical evaluation of the immobility data was performed because immobilities were less than 50 % in all tested concentrations.

Findings:

Analytical results: Measured concentrations for individual sampling dates were in the range of 92 – 98 % of nominal concentrations. Mean measured concentrations over time were 16.2, 27.3, 44.1, 74.2 and 126 mg/L.

Sublethal effects:

No sublethal effects were observed in surviving daphnids.

Immobility:

No immobilities were observed in test substance treatments. In the dilution water control one daphnid died (5 % immobility).

Conclusion:

EC₅₀ (48 h): > 126 mg/L

NOEC (48 h): 126 mg/L

Values are based on mean measured concentrations.

Reference: Samel A. (2002c) IN-U3204 Static-renewal, acute, 48-hours EC₅₀ to *Daphnia magna*.

Report/Doc no.: DuPont-9557

Guidelines: OECD 202, US EPA 72-2

GLP: Yes

Deviations: Test solutions were buffered with HEPES and the pH was adjusted to 6.5

Validity: The study is considered acceptable.

In a range finding test 10 daphnids per treatment were exposed to a HEPES buffered control, 1, 60 and 120 mg/L under static-renewal conditions for 48 hours. Respective immobilities were 0, 0, 0 and 50 %.

Material and methods:

Test substance: IN-U3204, purity: 97.1 %, batch no.: IN-U3204-6

Test species: *Daphnia magna*, neonates less than 24 hours old

Treatments: Dilution water control, HEPES-buffered control, 7.5, 15, 30, 60 and 120 mg/L

Test solutions were buffered with HEPES (N-[2*-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) and the pH was adjusted to 6.5 with 0.1 N hydrochloric acid.

No. of organisms: Per treatment 4 replicates with 5 daphnids each (20 daphnids per treatment)

Type of test / duration: Static-renewal system, renewal of test solutions after 24 hours, duration: 48 hours

Test medium:

Dilution water: Haskell Laboratory well water, analysis data of the dilution water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 8.5 – 8.9 mg/L

pH: 6.7 – 7.9

Total hardness: 98 – 100 mg/L as CaCO₃

Test conditions:

Temperature: 19.9 – 20.4 °C

Photoperiod: 16 hours light and 8 hours darkness with 30 minutes transition periods

Feeding: Daphnids were not fed during the test.

Observations: Immobility and behavioural observations were made every 24 hours.

Analytical measurements: For chemical analysis of the test substance samples of new test solutions were collected prior to their distribution to replicate test vessels at the beginning of the test and at the 24-hour media renewal. After 24 hours and 48 hours samples of old test solutions from two randomly selected replicate test vessels of each treatment group were taken.

Method of analysis: HPLC

Statistical evaluation: The 48-hour EC₅₀ was estimated by the moving average angle method.

Findings:

Analytical results: Mean measured concentrations over time were 6.7, 13, 27, 53 and 105 mg/L.

Measured concentrations were in the range of 74 – 103 % of nominal concentrations for individual sampling times.

Sublethal effects:

No sublethal effects were observed in surviving daphnids.

Immobility:

No immobility was observed in the dilution water and HEPES-buffered control. Mean measured concentrations of 6.7, 13, 27, 53 and 105 mg/L resulted in 0, 0, 0, 0 and 60 % immobility after 48 hours, respectively.

Conclusion:

EC₅₀ (48 h): 100 mg/L (95 % CL: 84 – 123 mg/L)

NOEC (48 h): 53 mg/L

Values are based on mean measured concentrations

Comment (RMS):

Test solutions were buffered with HEPES (N-[2*-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) and the pH was adjusted to 6.5 with 0.1 N hydrochloric acid. However, no effects were found in the buffer control and hence this treatment of test solutions is not considered to have significantly influenced the results of the test. Therefore the study is considered acceptable.

Reference: Samel, A. (2002d) IN-KQ960: Static, acute, 48-hours EC₅₀ to *Daphnia magna*. Report/Doc no.: DuPont-9559

Guidelines: OECD 202, US EPA 72-2

GLP: Yes

Deviations: None of relevance

Validity: The study is considered acceptable.

Two range finding tests were conducted under static conditions. In the first test 10 daphnids per treatment level were exposed to nominal concentrations of 0 (dilution water control), 1, 60 and 120 mg/L. After 48 hours respective immobilities were 30, 60, 100 and 100 %. In the second test 10 daphnids per treatment level were exposed to 0 (dilution water control), 0.6, 6.0, 60 and 120 mg/L. After 48 hours respective immobilities were 0, 40, 60, 80 and 100 %.

Material and methods:

Test substance: IN-KQ960, purity: 94.6 %, batch no.: IN-KQ960-002

Test species: *Daphnia magna*, neonates less than 24 hours old

Treatments: Dilution water control, 0.5, 1, 2, 4 and 8 mg/L (not adjusted for purity).

No. of organisms: Per treatment 4 replicates with 5 daphnids each (20 daphnids per treatment)

Type of test / duration: Static system, duration: 48 hours

Test medium:

Dilution water: Haskell Laboratory well water, analysis data of the dilution water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 8.8 – 8.9 mg/L

pH: 7.5 – 8.0

Total hardness: 99 – 101 mg/L as CaCO₃

Test conditions:

Temperature: 19.9 – 20.1 °C

Photoperiod: 16 hours light and 8 hours darkness with 30 minutes transition periods

Feeding: Daphnids were not fed during the test.

Observations: Immobility and behavioural observations were made every 24 hours.

Analytical measurements: For chemical analysis of the test substance samples of test solutions were taken at the start of the test before the test solutions were distributed to the test chambers and at the end of the test from two replicate test chambers from each test concentration.

Method of analysis: HPLC

Statistical evaluation: The 48-hour EC₅₀ was estimated by the moving average angle method.

Findings:

Analytical results: Mean measured concentrations over time were 0.56, 1.1, 2.1, 4.3 and 8.7 mg/L. For individual sampling times measured concentrations were in the range of 111 – 121 % of nominal concentrations (corrected for purity).

Sublethal effects:

At all test concentrations the majority of surviving daphnids exhibited lethargy after 24 and 48 hours of exposure. Some daphnids were floating on the surface.

Immobility:

No immobility was observed in the dilution water control. Mean measured concentrations of 0.56, 1.1, 2.1, 4.3 and 8.7 mg/L resulted in 0, 20, 30, 70, 50 and 50 % immobility after 24 hours and 45, 70, 90, 75 and 75 % immobility after 48 hours, respectively.

Conclusion:

EC₅₀ (48 h): 0.8 mg/L (95 % CL: 0.6 – 0.9 mg/L)

NOEC (48 h): Not determined, effects were observed at all tested concentrations

Values are based on mean measured concentrations.

Plant Protection product Cymoxanil 50 % WP

Reference: Migchielsen, M. H. J. (1999) Acute toxicity study in *Daphnia magna* with cymoxanil 50 % WP (semi-static). Report/Doc no.: NOTOX Project 262439

Guidelines: OECD 202

GLP: Yes

Deviations: The pH of the test solutions was adjusted to 6.5 ± 0.1 to enhance the stability of the test substance.

Validity: The study is considered acceptable.

In a range finding test daphnids were exposed to concentrations of 0.2, 2, 20 and 200 mg/L for 48 hours. The pH of test solutions was adjusted to 6.5. After 48 hours no immobilities were observed.

Material and methods:

Test substance: Cymoxanil 50 % WP (505 g cymoxanil/kg), batch no.: V343

Test species: *Daphnia magna*, neonates less than 24 hours old

Treatments: Dilution water control and 200 mg/L (101 mg cymoxanil/L), the pH of test solutions was adjusted to 6.5 ± 0.1

No. of organisms: Per treatment 2 replicates with 10 daphnids each (20 daphnids per treatment)

Type of test / duration: Semi-static system, renewal of test solutions after 24 hours, duration: 48 hours

Test medium:

Dilution water: Reconstituted water (ISO 6341)

Dissolved oxygen: 8.8 – 9.2 mg/L

pH: 6.5 – 7.3

Total hardness: 250 mg/L as CaCO_3

Test conditions:

Temperature: 20.2 – 21.8 °C

Photoperiod: 16 hours light and 8 hours darkness

Feeding: Daphnids were not fed during the test.

Observations: Immobility was recorded after 24 and 48 hours.

Analytical measurements: For chemical analysis of the test substance samples of test solutions were taken at the start of the test (new solutions) and after 24 hours (old and new test solutions). No samples were taken at test end.

Method of analysis: HPLC

Statistical evaluation: Since no mortalities were observed, no statistics was applied.

Findings:

Analytical results: Measured concentrations were 103 mg/L at test start, 80.3 mg/L in old solutions after 24 hours and 99.7 mg/L in fresh solutions at 24 hours. The mean measured concentration was 92 mg/L for the first 24 hours of the test period.

Sublethal effects:

No observations reported.

Immobility:

No immobilities were observed during the 48 hour test period.

Conclusion:

EC₅₀ (48 h): > 200 mg formulation/L
> 101 mg a.s./L

NOEC (48 h): 200 mg formulation/L
101 mg a.s./L

The NOEC is only based on immobility, sublethal effects were not investigated.

Values are based on a nominal limit concentration.

Comment (RMS):

Cymoxanil concentrations were not measured at test end. However, sufficient stability of the active ingredient was demonstrated during the first 24 hours of the test. The RMS considers the study acceptable because it can be assumed that cymoxanil concentrations were also at an acceptable level for the second half of the test.

Plant Protection product Tanos

Reference: Brown, M. R. (1997b) DPX-KP481-25 50 WG: Flow-through, Acute, 48 hour EC₅₀ to *Daphnia magna*. Report/Doc no.: DuPont HL-1997-00437

Guidelines: OECD 202, US EPA 72-21

GLP: Yes

Deviations: Mean measured concentrations for the second active ingredient (famoxadone) were rather high (128 – 169 % of nominal, only for one treatment level 103 % of nominal). Mean measured concentrations for cymoxanil were 75– 127 % of nominal.

Validity: Study is considered acceptable. See comment of RMS below.

Material and methods:

Test substance: DPX-KP481-25 50WG, purity: 26.6 % DPX-T3217 (cymoxanil) and 25.4 % DPX-JE874 (famoxadone), batch no.: not stated in the study report

Test species: *Daphnia magna*, neonates less than 24 hours old

Treatments: Dilution water control, solvent control, 14, 27, 51, 96 and 182 µg formulation/L

Cymoxanil: 3.7, 7.2, 14, 26 and 48 µg/L

Famoxadone: 3.6, 6.9, 13, 24 and 46 µg/L

Solvent: Dimethylformamide (DMF), the maximum DMF concentration in the solvent control and test solutions was 0.1 mL/L.

No. of organisms: Per treatment 4 replicates with 5 daphnids each (2 test chambers suspended in a fixed position in an aquarium, 2 aquaria, a total of 20 daphnids per treatment)

Type of test / duration: Flow-through system, turnover rate is not clear from the information provided in the study report, duration: 48 hours

Test medium:

Dilution water originated from the Haskell Laboratory well. Analytical data of the used well water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 8.4 – 8.9 mg/L

pH: 7.7 – 7.9

Total hardness: 82 – 85 mg/L as CaCO₃

Test conditions:

Temperature: 20.0 – 21.4 °C

Photoperiod: 16 hours light and 8 hours darkness with 30 minutes transition periods

Feeding: Daphnids were not fed during the test.

Observations: Sublethal effects and immobility were recorded every 24 hours.

Analytical measurements: For chemical analysis of the test substance samples of test solutions were taken from all replicate test chambers at test start and at test end.

Method of analysis: HPLC

Statistical evaluation: The 24 hour and 48 hour EC₅₀ and their 95 % confidence limits were calculated by probit analysis.

Findings:

Analytical results: Mean measured cymoxanil concentrations were 4.7, 5.4, 13.0, 25 and 48 µg/L which is 127, 75, 93, 96 and 100 % of nominal. Mean measured famoxadone concentrations were found to be 6.1, 7.1, 19.0, 31 and 59 µg/L which is 169, 103, 146, 129 and 128 % of nominal concentrations.

Sublethal effects:

All surviving daphnids at 27, 51, and 96 µg/L were lethargic.

Immobility:

Exposure to nominal concentrations of 0 (dilution water control), 0 (solvent control), 14, 27, 51, 96 and 182 µg formulation/L resulted in 0, 0, 0, 10, 20, 45 and 60 % immobility after 24 hours and 0, 0, 0, 30, 35, 45 and 100 % immobility after 48 hours.

Conclusion:

EC₅₀ (48 h): 55.5 µg/L (95 % CL: 43.8 – 70.9)

NOEC (48 h): 14 µg/L

Values are based on nominal concentrations.

Comment (RMS):

The RMS is of the opinion that the EC₅₀ based on nominal concentrations is acceptable in the light of the high toxicity of famoxadone (EC₅₀ daphnids: 0.012 mg/L) and the high measured concentrations for famoxadone. In such a situation the resulting EC₅₀ would be rather an overestimate of the toxicity of the formulation.

Reference: Hoke, R. A. (1998b) DPX-KP481-25 50 WG: Static, acute, 48-hour EC₅₀ to *Daphnia magna*. Report/Doc no.: DuPont-1076

Guidelines: OECD 202, US EPA 72-21

GLP: Yes

Deviations: Concentrations of cymoxanil were not analytically verified. Only concentrations of the second active ingredient (famoxadone) were measured at test start.

Validity: The study is considered as additional information only. See also comments of RMS below.

Material and methods:

Test substance: DPX-KP481-25 50WG, purity: 25.63 % DPX-T3217 (cymoxanil) and 25.08 % DPX-JE874 (famoxadone), batch no.: DPX-KP481-25 50WG

Test species: *Daphnia magna*, neonates less than 24 hours old

Treatments: Dilution water control, 16, 26, 43, 72, 120 and 200 µg formulation/L

Cymoxanil: 4.1, 6.7, 11.0, 18.5, 30.8 and 51.3 µg/L

Famoxadone: 4.01, 6.52, 10.8, 18.1, 30.1 and 50.2 µg/L

No. of organisms: Per treatment 4 replicates with 5 daphnids each (20 daphnids per treatment)

Type of test / duration: Static test system, duration: 48 hours

Test medium:

Dilution water originated from the Haskell Laboratory well. Analytical data of the used well water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 8.2 – 8.5 mg/L

pH: 7.3 – 7.9

Total hardness: 111 mg/L as CaCO₃

Test conditions:

Temperature: 19.9 – 20.9 °C

Photoperiod: 16 hours light and 8 hours darkness with 30 minutes transition periods

Feeding: Daphnids were not fed during the test.

Observations: Observations of test organisms were made at 0, 2, 4, 8, 16, 24, 36 and 48 hours.

Analytical measurements: For chemical analysis of the test substance samples of test solutions were taken from each replicate test chamber at all treatment levels at test start. Only the concentration of famoxadone was analysed, for cymoxanil no analysis was performed.

Method of analysis for famoxadone: HPLC

Statistical evaluation: Since immobility was less than 50 % at all tested concentrations no statistics was applied.

Findings:

Analytical results: Measured concentrations of famoxadone were in the range of 77 to 90 % of nominal concentrations at test start. For cymoxanil no analytical measurements are available.

Sublethal effects:

120 µg/L: Lethargy

200 µg/L: Lethargy and pale colouration

Immobility:

Dilution water control, 16, 26, 43 and 72 µg formulation/L: 0 % after 48 hours

120 µg formulation/L: 0 % after 24 hours and 25 % after 48 hours

200 µg formulation/L: 20 % after 24 hours and 45 % after 48 hours

Conclusion:

EC₅₀ (48 h): > 200 µg formulation/L

NOEC (48 h): 72 µg formulation/L

Values are based on nominal concentrations.

Comment (RMS):

Concentrations of cymoxanil were not analytically verified. Only concentrations of the second active ingredient (famoxadone) were measured at test start. Therefore the RMS considers the study as additional information only.

B.9.2.5 Chronic toxicity to aquatic invertebrates (Annex IIA 8.2.5, Annex IIIA 10.2.1)

Reference: Baer K. N. (1993d) Chronic toxicity of DPX-T3217-113 (cymoxanil) to *Daphnia magna*: 24-hour renewal. Report/Doc no.: DuPont HLR 354-93

Guidelines: OECD 202, US EPA 72-4

GLP: Yes

Deviations: The validity criterion of ≥ 60 young per surviving female in the control was not met (it was 58 ± 4.1 young/surviving female).

Validity: The study is considered acceptable although the validity criterion is not fully met (see comment of RMS below).

Material and methods:

Test substance: Cymoxanil, purity: 96.5 % (initial analysis), 97.8 % (reanalysis),
batch no.: DPX-T3217-113

Test species: *Daphnia magna*, neonates less than 24 hours old

Treatments: Dilution water control, 0.039, 0.078, 0.16, 0.31, 0.63, 1.3 and 2.5 mg/L

No. of organisms: Per treatment 10 replicates with 4 daphnids each (40 daphnids per treatment)

Type of test / duration: Semi-static system, renewal every 24 hours, duration: 21 days

Test medium:

Dilution water originated from the Haskell Laboratory well. The water passed through aquaria with fathead minnows prior to its use for the daphnid test. Then the water was buffered with 4 mM sodium phosphate and the pH was adjusted to 7.0 with phosphoric acid. Analytical results of the used well water prior to the flow through fish aquaria indicate adequate quality for the purpose of this study.

Dissolved oxygen: 7.9 – 9.1 mg/L

pH of dilution water control: 7.9 – 8.8, pH of buffer control and cymoxanil treatments: 6.9 – 7.3

Total hardness: 72 – 87 mg/L as CaCO₃

Test conditions:

Temperature: 19.5 – 20.3 °C

Photoperiod: 16 hours light and 8 hours darkness with 25 minutes transition periods

Feeding: Daphnids were fed *Ankistrodesmus falcatus* and *Selenastrum capricornutum* at a final concentration of 100000 cells/mL of test solution (for both algal species) daily after test solution renewal.

Endpoints: First day of reproduction, total live young produced, total live young produced per surviving adult, total immobile young produced, length of surviving adults at test end, behavioural observations were made daily

Analytical measurements: For chemical analysis of the test substance samples of two replicates of all treatment levels were collected at day 0 (new solutions), day 7 (new solutions), day 8 (old solutions), day 14 (new solutions), day 15 (old solutions) and day 21 (old solutions).

Method of analysis: HPLC

Statistical evaluation: Size data: Analysis of variance followed by Dunnett's multiple comparison procedure. If prerequisites of these tests were violated appropriate modifications (e.g. Tamhane Dunnett)

or non parametric tests such as Steel's test or Kruskal Wallis test as appropriate followed by Dunn's test, Mann-Whitney with Bonferroni correction were applied. As a supplement to analysis of variance or the Kruskal-Wallis test, a Jonckheere-Terpstra trend test was performed.

Survival data was analysed by the Cochran-Armitage trend test applied in a sequential manner. If the Cochran-Armitage test revealed significant lack of fit a Fisher's exact test was performed.

Findings:

Analytical results: Measured concentrations of new test solutions were 85 – 128 % of nominal and of old test solutions 54 – 94 % of nominal. Mean measured concentrations over time were 0.034, 0.067, 0.15, 0.27, 0.53, 1.1 and 2.0 mg/L (80 – 94 % of nominal). These mean concentrations were calculated from the first sample replicate only, however, they were generally lower than the means of pooled replicate measurements.

Effects on survival, growth and reproduction:

Table B.9.2.5-1: Effects of cymoxanil on survival, reproduction and growth of *Daphnia magna* after 21 days of exposure.

Treatment [mg/L] ^a	Adult survival [%]	1 st day of reproduction	Young / surviv. parent	Total immobile young/replicate ^b	Adult length [mm]
Water control	95	8 ± 0.3	58 ± 4.1	0.30 ± 0.95	3.8 ± 0.10
Buffer Control	93	8 ± 0.3	64 ± 11	5.2 ± 4.0	3.8 ± 0.10
0.034	93	7 ± 0.5	63 ± 9.2	2.4 ± 2.5	3.8 ± 0.10
0.067	88	7 ± 0.5	67 ± 14	3.3 ± 3.2	3.8 ± 0.09
0.15	70 *	9 ± 0.3 *	65 ± 20	2.8 ± 2.9	3.8 ± 0.12
0.27	50 *	10 ± 2 *	61 ± 13	3.8 ± 4.5	3.7 ± 0.16
0.53	13 *	12 ± 2 *	7.0 ± 13 *	14 ± 26	3.9 ± 0.19
1.1	33 *	13 ± 3 *	5.1 ± 7.5 *	8.1 ± 8.9	3.4 ± 0.28 *
2.0	15 *	16 ± 0.0	0.0 ± 0.0 *	0.4 ± 1.3 *	2.8 ± 0.17 *

^a Mean measured concentrations

^b 4 adult daphnids as parents per replicate

Conclusion:

NOEC (21 d): 0.067 mg/L, based on adult survival and first day of reproduction

The value is based on mean measured concentrations.

Comment (RMS):

Validity criteria according to OECD 211 were met for the buffer control (parent immobility ≤ 20 % at the end of the test and mean number of offspring per surviving parent at the end of the test ≥ 60). For the dilution water control the immobility criterion was met, however, the number of young per surviving female was slightly below the demanded value of 60 (it was 58 ± 4).

The mean number of total immobile young was higher in the buffer control than in the dilution water control (5.2 ± 4.0 per replicate with 4 daphnids in relation to 0.3 ± 0.94 immobile young per replicate). However, these values have to be viewed in the light of the total number of live young produced. From the raw data it became evident that even in the buffer control only a small number of produced young was immobile (less than 2 %).

In conclusion the RMS is of the opinion that there are some deviations from the test guideline and there might have been some influence of the buffer on reproduction. However, if the results are considered comprehensively the study can be considered acceptable. Additionally the RMS thinks that a new study would not significantly alter the toxicity picture of cymoxanil for daphnids. Therefore the study is considered acceptable.

Reference: Samel, A. (2003) IN-KQ960: 21-day chronic toxicity to *Daphnia magna*. Report/Doc no.: DuPont-11971

Guidelines: OECD 211, US EPA OPPTS 850.1300 (1996)

GLP: Yes

Deviations: None of relevance

Validity: The study is considered acceptable.

Material and methods:

Test substance: IN-KQ960, purity: 96.2 %, batch no.: IN-KQ960-003

Test species: *Daphnia magna*, neonates less than 24 hours old

Treatments: Dilution water control, 19, 38, 75, 150 and 300 µg/L

No. of organisms: Per treatment 10 replicates with 1 daphnid each (10 daphnids per treatment)

Type of test / duration: Static-renewal system, renewal of test solutions was every Monday, Wednesday and Friday, duration: 21 days

Test medium:

Dilution water originated from the Haskell Laboratory well. Analysis data of the dilution water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 5.9 – 8.8 mg/L (≥ 65 % saturation)

pH: 7.3 – 8.0

Total hardness: 115 – 128 mg/L as CaCO₃

Test conditions:

Temperature: 19.9 – 20.5 °C

Photoperiod: 16 hours light and 8 hours darkness with 30 minutes transition periods

Feeding: Daphnids were fed *Selenastrum capricornutum* at a rate of 62000 cells/mL of test solution and 2 mL/L of a yeast, cereal leaves and trout chow mixture (YCT) daily. The combination of alga and YCT was equivalent to approximately 0.1 - 0.2 mg total organic carbon per daphnid based on a routine analysis of TOC.

Endpoints: Immobility of adults, total number of live young produced per surviving adult at 21 days, total number of immobile young produced per surviving adult at 21 days, length and dry weight of surviving adults at test end, behavioural observations were made daily

Analytical measurements: For chemical analysis of the test substance samples of newly prepared test solutions were taken on days 0, 2, 9, 16 and 19. Samples from old test solutions were taken on days 5, 12, 19 and 21 from three randomly assigned replicates at each treatment level. The same replicates were sampled throughout the test.

Method of analysis: HPLC

Statistical evaluation: A multitude of statistical methods were applied on the data. Relevant for the setting of a NOEC were: Kruskal-Wallis followed by Dunn's multiple comparison and Jonckheere-Terpstra trend test

Findings:

Analytical results: For individual sampling dates measured concentrations were in the range of 95 – 123 % of nominal concentrations corrected for purity. Mean measured concentrations over time were 19.9, 40.0, 78.5, 155 and 302 µg/L (104 – 109 % of nominal concentrations corrected for purity of 96.2 %).

Effects on survival, growth and reproduction:

Table B.9.2.5-2: Effects of the metabolite IN-KQ960 on survival, reproduction and growth of *Daphnia magna* after 21 days of exposure.

Treatment [µg/L] ^a	Adult survival [%]	1 st day of reproduction	Live young / surviving adult	Total immobile young	Adult dry weight [mg]	Adult length [mm]
Water control	90	9.4 ± 1.0	162 ± 25	0 ± 0	0.6 ± 0.1	4.67 ± 0.10
19.9	80	10.1 ± 1.2	134 ± 10 *	0 ± 0	0.5 ± 0.1	4.58 ± 0.13

Treatment [µg/L] ^a	Adult survival [%]	1 st day of reproduction	Live young / surviving adult	Total immobile young	Adult dry weight [mg]	Adult length [mm]
40.0	100	9.8 ± 0.7	138 ± 50	0 ± 0	0.5 ± 0.2	4.33 ± 0.60
78.5	100	9.6 ± 0.9	131 ± 50	0 ± 0	0.6 ± 0.2	4.50 ± 0.54
155	90	9.4 ± 1.0	147 ± 25	0 ± 0	0.5 ± 0.1	4.62 ± 0.11
302	100	9.8 ± 0.6	153 ± 15	0 ± 0	0.6 ± 0.1	4.86 ± 0.11

^a Mean measured concentrations

* Statistically significantly different from the control at p<0.05

Conclusion:

NOEC (21 d): 302 µg/L (highest concentration tested), based on mean measured concentrations

Comment (RMS):

The statistical significant (p<0.05) decrease relative to the control in the number of live young per surviving female at the treatment level of 19.9 mg/L is not considered relevant due to the lack of a dose response relationship. Therefore the RMS accepts a NOEC of 302 µg/L.

B.9.2.6 Effects on algae growth (Annex IIA 8.2.6, Annex IIIA 10.2.1)

Active substance

Reference: Boeri, R. L., Magazu, J. P., Ward, T. J. (1999). Cymoxanil technical: Growth and reproduction test with the freshwater alga *Selenastrum capricornutum*. Report/Doc. Number: DuPont-2498

Guideline: OECD 201, US EPA 123-2

GLP: Yes

Deviations: Initial loading was lower than recommended in the test guidelines (only 3000 cells/mL instead of 10000 cells/mL).

Validity: The study is considered acceptable.

In a range finding test nominal treatments of 0.50, 1.0, 5.0, 10 and 50 mg/L cymoxanil resulted in growth rates of 89 % and 61 % relative to the control at 0.50 and 1.0 mg/L and growth rates of < 1 % relative to the control at test concentrations of 5.0 mg/L and higher.

Material and methods:

Test substance: Cymoxanil technical, batch no.: DPX-T3217-113, purity: 97.2 %

Test species: *Selenastrum capricornutum* (*Pseudokirchneriella subcapitata*)

Test concentrations: Nutrient medium control, 0.64, 1.3, 2.5, 5.2 and 10 mg/L

Number of replicates / loading: 3 replicates per treatment, initial loading: 3000 cells/mL

Test type / duration: Static test system, 120 hours

Test medium: AAP medium, adjusted to a target pH of 7.5 ± 0.1

Test conditions:

Continuous illumination at approximately 3700 – 3800 lux

Temperature: 23.7 – 24.0 °C

Shaking rate: 100 rpm

pH: 7.5 at test start and 7.5 – 10.0 at test end (due to decreased algal growth in higher test concentrations a lower pH was found).

Observations: Cell counts were performed using a haemocytometer and a microscope after 24, 48, 72, 96 and 120 hours. Morphological observations were also recorded at these time intervals.

Analytical measurements: For chemical analysis of the test substance samples from all treatment levels were taken at test start, after 72 and 120 hours.

Method of analysis: HPLC

Statistical evaluation: EC₅₀ values for average specific growth rate, cell concentration and area under the growth curve were estimated by a weighted least squares non-linear regression technique (Bruce, R. D. and J. D. Versteeg, 1992: "A Statistical procedure for Modelling Continuous Toxicity Data. Environmental Toxicology and Chemistry". Vol. 11, No. 10, pp 1485 -1494). The NOEC was determined by ANOVA followed by a Dunnett's test.

Recovery test:

After 120 hours of testing, 0.5 mL of solution from each replicate of the 10 mg/L test chambers were pooled and brought up to 50 mL with fresh nutrient medium and incubated under test conditions for another 96 hours.

Findings:

Analytical results: Initial measured concentrations were 0.662, 1.38, 2.47, 5.10 and 9.56 mg/L (96 – 106 % of nominal concentrations corrected for a purity of 97.2 %). After 72 and 120 hours measured concentrations had decreased to < 15 % of nominal concentrations.

Morphological effects: No effects observed.

Growth inhibition:

Table B.9.2.6-1: Effects of cymoxanil on biomass (area under growth curve, AUC) and growth rate of *Pseudokirchneriella subcapitata* after 72, 96 and 120 hours of exposure.

Initial measured concentration [mg/L]	Inhibition of biomass (AUC) [%]			Inhibition of growth rate [%]		
	72 h	96 h	120 h	72 h	96 h	120 h
0.662	40	45	28	10	9	2
1.38	80	88	75	39	33	11
2.47	89	96	95	50	54	34
5.10	93	98	98	71	76	52
9.56	94	99	99	76	81	84

Table B.9.2.6-2: Toxicity of cymoxanil to the freshwater alga *Pseudokirchneriella subcapitata*. Toxicity values are based on initial measured concentrations.

Endpoint	Time scale	NOEC [mg/L]	EC ₅₀ [mg/L]	95 % CL [mg/L]
Biomass (AUC)	72 h	Not derived	< 0.662	-
	96 h	Not derived	< 0.662	-
	120 h	0.662	0.794	0.692 – 1.02
Growth rate	72 h	Not derived	2.39	1.85 – 3.10
	96 h	Not derived	2.47	2.03 – 3.01
	120 h	0.662	4.22	3.72 – 4.79

Results of recovery test:

Within 96 hours cell concentrations increased from < 300 cells/mL to 480 000 cells/mL. These data indicate that cymoxanil is algistatic rather than algicidal.

Conclusion:

The following toxicity values are regarded acceptable for risk assessment (see comment below):

E_bC₅₀ (96 h): < 0.662 mg/L

E_rC₅₀ (96 h): 2.47 mg/L

NOEC (96 h) : Not derived

Toxicity values are based on initial measured concentrations.

Comment (RMS):

Derived EC₅₀ values for 120 hours are higher than those for 72 and 96 hours. This is a result of decreased growth rates in the control between 96 and 120 hours due to already high population densities

in the control cultures. Between 96 and 120 hours the average growth rate was 0.02/hour in control cultures, between 0 and 96 hours the average growth rate in control cultures was 0.07/hour. Therefore toxicity values derived for a 120 hour exposure period are not regarded acceptable.

Due to inappropriate test concentrations no EC₅₀ for biomass inhibition could be derived from the obtained data relating to 72 and 96 hours of exposure.

Reference: Bell, G., Thirkettle, K. M., Smith, B. (1996) Cymoxanil technical Algal growth inhibition.

Report/Doc. Number: OXN 107A(a)950955

Guideline: OECD 201

GLP: Yes

Deviations: None of relevance

Validity: The study is considered acceptable.

Material and methods:

Test substance: Cymoxanil technical, batch no.: 805, purity: 98.8 %

Test species: *Selenastrum capricornutum* (*Pseudokirchneriella subcapitata*)

Test concentrations: Nutrient medium control, 1.0, 2.2, 4.6, 10 and 22 mg/L

Number of replicates / loading: Per treatment 3 replicates, initial loading: $\approx 1.3 \times 10^4$ cells/mL

Test type / duration: Static test system, 72 hours

Test medium: Sterile nutrient medium according to the "Official Journal No. L383A Part C.3"

Test conditions:

Continuous illumination at approx. 7000 lux

Temperature: 24 ± 1 °C (raw data not provided in the study report)

Shaking rate: 120 rpm

pH: 7.4 – 7.6 at test start and 7.6 – 9.7 at test end

Observations: Cell densities were counted at test initiation, at 24, 48 and 72 hours by direct counting with a Clulter® Multisizer II particle counter. All test and control cultures were inspected microscopically at 72 hours.

Analytical measurements: For chemical analysis of the test substance samples were taken from all treatment levels at 0 and 72 hours.

Method of analysis: HPLC

Statistical evaluation: EC₅₀ values were estimated by logistic regression, the NOEC was determined employing Williams's test.

Findings:

Analytical results: Measured concentrations ranged from 87 – 95 % of nominal at test start but were below the limit of detection (0.05 mg/L) after 72 hours. In the study report mean measured concentrations of 0.22, 0.31, 0.45, 0.67, and 1.0 mg/L are stated. However, the derivation of these values is unclear to the RMS (see comment below).

Morphological effects: No effects observed.

Growth inhibition:

Table B.9.2.6-3: Effects of cymoxanil on biomass (area under growth curve, AUC) and growth rate of *Pseudokirchneriella subcapitata* after 72 hours of exposure.

Mean measured concentration [mg/L]	Inhibition of biomass (AUC) [%]	Inhibition of growth rate [%]
0.22	18 *	5 *
0.31	45 *	14 *
0.45	64 *	25 *
0.67	85 *	56 *

Mean measured concentration [mg/L]	Inhibition of biomass (AUC) [%]	Inhibition of growth rate [%]
1.0	97 *	83 *

* Statistically significant difference from the control (p<0.05)

Table B.9.2.6-4: Toxicity of cymoxanil to the freshwater alga *Pseudokirchneriella subcapitata*. Toxicity values are based on mean measured concentrations.

Endpoint	Time scale	NOEC [mg/L]	EC ₅₀ [mg/L]	95 % CL [mg/L]
Biomass (AUC)	72 h	Not derived	0.35	0.34 – 0.37
Growth rate	72 h	Not derived	0.63	0.61 – 0.66

Conclusion:

The following toxicity values are regarded acceptable for risk assessment:

E_bC₅₀ (72 h): 0.35 mg/L

E_rC₅₀ (72 h): 0.63 mg/L

NOEC (72 h) : Not derived

Toxicity values are based on mean measured concentrations (see comment below).

Comment (RMS):

In the study report mean measured concentrations of 0.22, 0.31, 0.45, 0.67 and 1.0 mg/L are stated.

From the information provided in the study report it is not clear how mean measured concentrations were derived from measurement data. However the stated mean measured concentrations are lower than time weighted average concentrations over the study period (estimated by the RMS). Therefore the RMS considered the stated mean measured concentrations acceptable as basis for EC50 estimates.

Reference: Hughes, J. S., Williams, T. L., Leigh, A. C. (1996a) DPX-T3217-113 (Cymoxanil): Influence on growth and reproduction of *Anabaena flos-aquae*. Report/Doc. Number: AMR 4109-96

Guideline: US EPA 122-2 and 123-2

GLP: Yes

Deviations: Initial loading was lower than recommended in the test guidelines.

Validity: The study is considered acceptable.

In a range finding test concentrations of 0.113, 1.13, 11.3, 113 and 1130 µg/L resulted in slight inhibition or stimulation at the three lowest concentrations, 44.7 % inhibition at 113 µg/L and 99 % inhibition at 1130 µg/L. The time scale of this range finding test was not stated in the study report.

Material and methods:

Test substance: Cymoxanil technical, batch no.: DPX-T3217-113, purity: 97.3 %

Test species: *Anabaena flos-aquae*

Test concentrations: Nutrient medium control, 38, 76, 150, 300 and 600 µg/L.

Number of replicates / loading: Per treatment 3 replicates, initial loading: 3000 cells/mL

Test type / duration: Static test system, 120 hours

Dilution water: AAP medium, adjusted to a pH of 7.5 ± 0.1

Test conditions:

Continuous illumination at approximately 2152 ± 323 lux

Temperature: 23.1 – 25.1 °C,

Manual shaking of test vessels each day

pH: 7.3 – 7.6 at test start and 7.9 – 8.5 at test end

Observations: On days 3, 4 and 5 cell densities were counted with a Coulter Counter after sonication of samples to break up filaments.

Analytical measurements: For chemical analysis of the test substance samples were from each treatment level were taken at test start and test end (120 h)

Method of analysis: HPLC

Statistical evaluation: EC₅₀ values were estimated using a weighted least squares non linear regression. The NOEC was determined by ANOVA followed by Dunnett's test. Since EC₅₀ values and a NOEC were only derived for 120 h cell count data (biomass), the RMS estimated EC₅₀ and NOEC values for growth rate and biomass (cell counts) for 72 and 96 hours of exposure by probit analysis and employing ANOVA followed by Dunnett-C test (variances not homogenous).

Findings:

Analytical results: Initial measured concentrations were 34.0, 65.2, 138, 281 and 563 µg/L (86 – 94 % of nominal). No test substance was detectable at any treatment level on day 5 (detection limit: 3.99 µg/L).

Morphological effects: Morphological observations were not performed.

Table B.9.2.6-5: Effects of cymoxanil on biomass (cell counts) and growth rate of *Anabaena flos-aquae* after 72, 96 and 120 hours of exposure.

Initial measured concentration [µg/L]	Inhibition of biomass (cell counts) [%]			Inhibition of growth rate ^a [%]		
	72 h ^a	96 h ^a	120 h	72 h ^a	96 h ^a	120 h
34.0	9	15	1	4	4	0.3
65.2	15	28*	13	6	8	3
138	50*	59*	33*	29*	21*	8
281	72*	74*	54*	53*	32*	16*
563	85*	88*	83*	79*	50*	36*

^a Per cent inhibition and statistical significance compared to the control calculated by RMS from cell count data provided in the study report

* Statistically significant difference from the control (p<0.05)

Table B.9.2.6-6: Toxicity of cymoxanil to the freshwater alga *Anabaena flos-aquae*. Toxicity values are based on initial measured concentrations.

Endpoint	Time scale	NOEC [mg/L]	EC ₅₀ [µg/L]	95 % CL [µg/L]
Biomass (cell counts)	72 h ^a	65.2	160	139 – 184
	96 h ^a	34.0	122	104 – 142
	120 h ^a	34.0	231	182 – 294
Growth rate	72 h ^a	65.2	254	221 – 296
	96 h ^a	65.2	564	428 – 838
	120 h ^a	138	949	681 – 1615

^a Setting of NOEC and EC50 estimate derived by RMS.

Conclusion:

The following toxicity values are regarded acceptable for risk assessment:

E_bC₅₀ (96 h): 122 µg/L

E_rC₅₀ (72 h): 254 µg/L

NOEC (96 h) : 34 µg/L for biomass inhibition, 65.2 µg/L for growth rate inhibition

Toxicity values are based on initial measured concentrations.

Comment (RMS):

In the OECD 201 (draft 2002) and the US EPA guideline (OPPTS 850.5400, 1996) for *Anabaena flos-aquae* an initial loading of 10⁴ cells/mL is recommended. Here a lower loading of 3000 cells/mL was used. The RMS considers the lower loading acceptable since appropriate growth in control cultures was given.

The study authors provided only an EC₅₀ and a NOEC for cell counts after 120 hours. However, these values are not acceptable because growth rates in control replicates decreased clearly from 96 – 120 hours (0.88 d⁻¹) compared to the growth rates from 72 to 96 hours (1.66 d⁻¹). Therefore the RMS calculated EC₅₀ values for growth rate and biomass (cell counts) 72 and 96 hours of exposure to derive sound toxicity estimates.

Reference: Hughes, J. S., Williams, T. L., Leigh, A. C. (1996b) DPX-T3217-113 (Cymoxanil): Influence on growth and reproduction of *Navicula pelliculosa*. Report/Doc. Number: AMR 4112-96

Guideline: US EPA 122-2 and 123-2

GLP: Yes

Deviations: Initial cell concentrations in cultures were lower than recommended in the test guideline, only 3 replicates/treatment level were set up (recommended are 4 replicates per treatment level for this species and 8 for the control), EC₅₀ values were only estimated for inhibition rates relative to the control after 120 hours of exposure, however, for the last day of exposure (96 – 120 h) no exponential growth was given.

Validity: The study is not considered acceptable due to poor data analysis and the deviations from the test guidelines.

Metabolites

Reference: Sloman, T. L. (2001a) IN-T4226: Influence on growth and growth rate of the blue-green alga *Anabaena flos-aquae*. Report/Doc. Number: DuPont-3747

Guideline: US EPA OPPTS 850.5400

GLP: Yes

Deviations: Test concentrations were not arranged in a geometric series.

Validity: The study is considered acceptable

Material and methods:

Test substance: IN-T4226, batch no.: IN-T4226-1, purity: 99.1 %,

Test species: *Anabaena flos-aquae*

Test concentrations: Nutrient medium control, 20, 30, 40, 50 and 60 mg/L

Number of replicates / loading: Per treatment 3 replicates, initial loading: 10000 cells/mL

Test type / duration: Static test system, 96 hours for the definitive test and 216 hours for the recovery test

Dilution water: AAP medium, adjusted to a pH of 7.5 ± 0.1

Test conditions:

Continuous illumination at approximately 1963 – 2242 lux (definitive test) and 1998 – 2320 (recovery test)

Temperature: 24.2 – 24.7 °C

Shaking rate: 100 rpm

pH: 6.2 – 7.0 (IN-T4226 test vessels) and 7.5 (control) at test start, 5.6 – 7.1 (IN-T4226 test vessels) and 7.1 (control) at test end

Observations: At 24, 48, 72 and 96 hours cell counts were performed using a haemocytometer and a compound microscope. Additionally morphological changes were recorded.

Analytical measurements: For chemical analysis of the test substance samples were taken at test initiation and test end (day 4).

Method of analysis: HPLC

Statistical evaluation: EC₅₀ values were determined by weighted least-squares non-linear regression (probit analysis). The NOEC was determined from an analysis of variance and a Williams' test.

Recovery test:

The ability of the organisms to recover was assessed for the 30, 40, 50 and 60 mg/L treatment levels. Algae from these test concentrations were exposed to untreated AAP nutrient medium for 216 hours. Cell counts were performed at 72, 144 and 216 hours from the initiation of the recovery test.

Findings:

Analytical results: Initial measured concentrations were 18, 28, 40, 50 and 61 mg/L (90 – 102 % of nominal). After 4 days mean measured concentrations were 1.7, 10, 22, 32 and 43 mg/L (8.5 – 72 % of nominal).

Growth inhibition:

Table B.9.2.6-7: Effects of IN-T4226 on biomass (cell counts and AUC) and growth rate of *Anabaena flos-aquae* after 72 and 96 hours of exposure.

Nominal concentration [mg/L]	Biomass (cell counts) [% inhibition]		Biomass (AUC) [% inhibition]		Growth rate [% inhibition]	
	72 h	96 h	72 h	96 h	72 h	96 h
20	-20	2.5	9.9	1.4	-5.1	0.5
30	49 *	88 *	69 *	78 *	20	44 *
40	64 *	93 *	81 *	86 *	31 *	57 *
50	90 *	97 *	93 *	96 *	69 *	73 *
60	96 *	98 *	94 *	98 *	93 *	88 *

* Statistically significant difference from the control (p<0.05)

Table B.9.2.6-8: Toxicity of IN-T4226 to the freshwater alga *Anabaena flos-aquae*. Toxicity values are based on nominal concentrations.

Endpoint	Time scale	NOEC [mg/L]	EC ₅₀ [µg/L]	95 % CL [µg/L]
Biomass (cell counts)	96 h	20	25.8	24.2 – 26.8
Biomass (AUC)	96 h	20	26.7	24.6 – 27.9
Growth rate	96 h	20 ^a	35.9	33.5 – 38.3

^a In the study report a NOEC of 40 mg/L was stated for growth rate. The RMS did not agree with this NOEC and set the NOEC to 20 mg/L (ANOVA followed by Dunnett's test, p<0.05)

Recovery test:

IN-T4226 was found to be algistatic rather than algicidal for all tested concentrations.

Conclusion:

The following toxicity values are regarded acceptable for risk assessment:

E_bC₅₀ (96 h): 25.8 mg/L

E_rC₅₀ (96 h): 35.9 mg/L

NOEC (96 h) : 20 mg/L

Toxicity values are based on nominal concentrations.

Comment (RMS):

Test concentrations were not arranged in a geometric series as recommended in the test guideline.

However, the RMS is of the opinion that this does not invalidate the study and the results can be used for risk assessment.

EC₅₀ values provided in the study protocol are rather a linear interpolation between the two concentrations encompassing the EC₅₀ than the result from probit analysis (no good model fit with Chi square test). However, viewing at the raw data, the resulting EC₅₀ values seem to be plausible estimates and hence are considered acceptable by the RMS.

Reference: Sloman, T. L. (2001b) IN-W3595: Influence on growth and growth rate of the blue-green alga *Anabaena flos-aquae*. Report/Doc. Number: DuPont-3748

Guideline: US EPA OPPTS 850.5400

GLP: Yes

Deviations: Test concentrations were not arranged in a geometric series.

Validity: The study is considered acceptable.

Material and methods:

Test substance: IN-W3595, batch no.: IN-W3595-3, purity: 94.2 %

Test species: *Anabaena flos-aquae*

Test concentrations: Nutrient medium control, 5, 10, 15, 20 and 25 mg/L

Number of replicates / loading: Per treatment 3 replicates, initial loading: 10000 cells/mL

Test type / duration: Static test system, 96 hours for the definitive test and 216 hours for the recovery test

Dilution water: AAP medium, adjusted to a pH of 7.5 ± 0.1

Test conditions:

Continuous illumination at approximately 1947 – 2127 lux for the definitive test and 2025 for the recovery test

Temperature: 24.3 – 24.6 °C

Shaking rate: 100 rpm

pH: 6.0 – 6.9 (IN-W3595 test vessels) and 7.5 (control) at test start and 7.3 – 6.2 (IN-W3595 test vessels) and 7.3 (control) at test end

Observations: At 24, 48, 72 and 96 hours cell counts were performed using a haemocytometer and a compound microscope. Morphological changes were recorded at the same times.

Analytical measurements: For chemical analysis of the test substance samples were taken at test initiation and test end (day 4).

Method of analysis: HPLC

Statistical evaluation: EC₅₀ values were determined by probit analysis. The NOEC was determined from an analysis of variance and a Williams' test.

Recovery test:

The ability of algae to recover was assessed for each definitive treatment with 50 % or greater growth inhibition (nominal 15, 20 and 25 mg/L). Algae from these test concentrations were exposed to untreated AAP nutrient medium for 216 hours. Cell counts were performed at 72, 144 and 216 hours from the initiation of the recovery test.

Findings:

Analytical results: Initial measured concentrations were 5.3, 11, 16, 21 and 26 mg/L (104 – 110 % of nominal). After 4 days mean measured concentrations were 5.5, 11, 16, 22, and 27 mg/L (107– 110 % of nominal).

Growth inhibition:

Table B.9.2.6-9: Effects of IN-W3595 on biomass (cell counts and AUC) and growth rate of *Anabaena flos-aquae* after 72 and 96 hours of exposure.

Nominal concentrations [mg/L]	Biomass (cell counts) [% inhibition]		Biomass (AUC) [% inhibition]		Growth rate [% inhibition]	
	72 h	96 h	72 h	96 h	72 h	96 h
5	-6	-24	7	-14	-1	-5
10	-1.5	44*	35	37*	0	12
15	56	59*	64*	61*	32*	19
20	56	87*	69*	80*	27	42*
25	94*	99*	94*	98*	93*	92*

Test of significance for 72 hours of exposure was performed by the RMS (ANOVA followed by Dunnett's test, $p < 0.05$)

* Statistically significant difference from the control ($p < 0.05$)

Table B.9.2.6-10: Toxicity of IN-W3595 to the freshwater alga *Anabaena flos-aquae*. Toxicity values are based on nominal concentrations.

Endpoint	Time scale	NOEC [mg/L]	EC ₅₀ [µg/L]	95 % CL [µg/L]
Biomass (cell counts)	96 h	5	12.2	10.6 – 14.0
Biomass (AUC)	96 h	5 ^a	12.7	11.3 – 14.2
Growth rate	96 h	15	19.9	18.4 – 21.0

^a In the study protocol a NOEC of 20 mg/L was determined with Williams test, however, with ANOVA a NOEC of 5 mg/L is derived. Since data fulfil the requirements for ANOVA, this statistical test procedure is preferred.

Recovery test:

At concentrations less than or equal to 20 mg/L the effects of IN-W3595 on growth and growth rate of *Anabaena flos-aquae* were found to be algistatic (logarithmic growth resumed within 216 hours) but algicidal (logarithmic growth did not resume) at 25 mg/L.

Conclusion:

The following toxicity values are regarded acceptable for risk assessment:

E_bC₅₀ (96 h): 12.7 mg/L

E_rC₅₀ (96 h): 19.9 mg/L

NOEC (96 h) : 5 mg/L

Toxicity values are based on nominal concentrations.

Comment (RMS):

Test concentrations were not arranged in a geometric series as recommended in the test guideline.

However, the RMS is of the opinion that this does not invalidate the study and the results can be used for risk assessment.

Reference: Sloman, T. L. (2002a) IN-U3204: Influence on growth and growth rate of the blue-green alga *Anabaena flos-aquae*. Report/Doc. Number: DuPont-9207

Guideline: US EPA OPPTS 850.5400

GLP: Yes

Deviations: Daily growth rates of the control replicate vessels were found to be highly variable over the test duration. Additionally cell density data for replicates within one treatment level were very variable in control cultures. Due to the high variability in growth rates in the control cultures no sound estimate of the toxicity of cymoxanil based on growth and growth rate inhibition relative to the control can be obtained from the study results.

Validity: The study is not considered acceptable.

Reference: Sloman, T. L. (2002b) IN-KQ960: Influence on growth and growth rate of the blue-green alga *Anabaena flos-aquae*. Report/Doc. Number: DuPont-9206

Guideline: US EPA OPPTS 850.5400

GLP: Yes

Deviations: Due to substantial inhibition at the tested limit concentration of 110 mg/L at 72 and 96 hours of exposure no reliable EC₅₀ values can be derived from this study.

Validity: The study is not considered acceptable, because no sound toxicity estimates such as an EC₅₀ or a NOEC could be derived.

Plant protection product Tanos

No toxicity test with the formulation Tanos was submitted for algae. Instead a toxicity test on *Pseudokirchneriella subcapitata* with the formulation DPX-KX007 is available. This formulation contains the same adjuvants in similar amounts as the formulation Tanos. The cymoxanil content of DPX-KX007 is somewhat higher than the content in Tanos.

Reference: Leva, S. E., Sloman, T. L. (1997) DPX-KX007: Influence on growth and growth rate of green alga *Pseudokirchneriella subcapitata* (formerly called *Selenastrum capricornutum*). Report/Doc. Number: AMR 4126-96

Guideline: US EPA 122-2 and 123-2, Directive 92/69/EEC, Method C3

GLP: Yes

Deviations: Initial loading was lower than recommended (3000 cells/mL instead of 10000 cells/mL). Concentrations of the active ingredients were only measured in an abiotic test solution with 10 mg formulation/L.

Validity: The study is considered acceptable.

Material and methods:

Test substance: DPX-KP481-25 50WG, purity: 30.4 % DPX-T3217 (cymoxanil) and 22.7 % DPX-JE874 (famoxadone), batch no.: Not stated in the study report

Test species: *Pseudokirchneriella subcapitata*

Treatments: Nutrient medium control, 0.625, 1.25, 2.5, 5 and 10 mg formulation/L with algae and a 10 mg/L abiotic solution without algae to confirm test substance concentrations

Cymoxanil: 0.19, 0.38, 0.76, 1.52 and 3.04 mg/L

Famoxadone: 0.14, 0.28, 0.57, 1.14 and 2.27 mg/L

Number of replicates / loading: Per treatment 3 replicates, initial loading: 3000 cells/mL

Test type / duration: Static test system, 120 hours

Nutrient medium: AAP medium, adjusted to a pH of 7.5 ± 0.1

Test conditions:

Continuous illumination at approximately 4684 lux

Temperature: 24.7 – 25.1 °C

Shaking rate: 111 rpm

pH: 6.9 – 7.3 at test start and 7.2 – 8.9 at test end

Observations: Morphological observations and cell counts were made by a haemocytometer and a compound microscope after 24, 48, 72, 96 and 120 hours.

Analytical measurements: Chemical analysis of the active ingredients cymoxanil and famoxadone was only performed on samples from the 10 mg/L abiotic control with algae at test start and test end.

Method of analysis: HPLC

Statistical evaluation: EC₅₀ values and respective 95 % confidence limits were estimated using a weighted least squares non linear regression of the log of the test concentration against the measured parameter.

The NOEC was determined by ANOVA and Williams' test.

Recovery test:

A recovery test was performed for the 10 mg/L treatment group (here cell count inhibition relative to the control was greater 50 %). An aliquot from each replicate was diluted with fresh nutrient medium to a test substance concentration below the NOEC and incubated under the same conditions as in the main test for 72 hours.

Findings:

Analytical results: Concentrations of the active ingredients of the tested formulation were only measured in the abiotic control. Measured cymoxanil concentrations were 3.8 mg/L at test start and 0.19 mg/L at test end (125 and 6 % of nominal). Measured famoxadone concentrations were 3.1 mg/L at test start and

2.7 mg/L (137 and 119 % of nominal).

Morphological effects: No information on results of morphological observation is stated in the study report.

Table B.9.2.6-11: Effects of DPX-KX007 on biomass (cell counts and AUC) and growth rate of *Pseudokirchneriella subcapitata* after 72, 96 and 120 hours of exposure.

Nominal concentrations [mg/L]	Biomass (cell counts) [% inhibition]			Biomass (AUC) [% inhibition]			Growth rate [% inhibition]		
	72 h	96 h	120 h	72 h	96 h	120 h	72 h	96 h	120 h
0.625	6	8	-5	-6	4	0	2	1	-1
1.25	11	-4	8	6	0	3	3	0	1
2.5	34	27	19	40	31	26	8	5	3
5	50	53	28	44	50	41	14	11	4
10	90	86	58	90	87	75	47	29	12

Table B.9.2.6-12: Toxicity of DPX-KX007 to the freshwater alga *Pseudokirchneriella subcapitata*. Toxicity values are based on nominal concentrations.

Endpoint	Time scale	NOEC [mg/L]	EC ₅₀ [mg/L]	95 % CL [mg/L]
Biomass (AUC)	72 h	1.25	4.2	3.3 – 5.5
	96 h	1.25	4.4	3.8 – 5.2
	120 h	1.25	5.6	4.9 – 6.5
Growth rate	72 h	1.25	11.0	9.9 – 12.8
	96 h	1.25	18.3	16.0 – 22.2
	120 h	1.25	67	43 – 146

Recovery test:

The effects of DPX-KX007 on *Pseudokirchneriella subcapitata* were found algistatic up to concentrations of 10 mg formulation/L

Conclusion:

The following toxicity values are regarded acceptable for risk assessment:

E_bC₅₀ (72 h): 4.2 mg formulation/L

E_rC₅₀ (72 h): 11.0 mg formulation/L

NOEC (72 h) : 1.25 mg formulation/L

Values are based on nominal concentrations.

Comment (RMS):

The composition of the formulation DPX-KX007 is considered comparable to the lead formulation Tanos and hence the results of this study are regarded acceptable for risk assessment.

Plant protection product Cymoxanil 50 % WP

Reference: Bogers, M. (1999b) Fresh water algal growth inhibition test with Cymoxanil 50 % WP.

Report/Doc. Notox project 262441

Guideline: OECD 201

GLP: Yes

Deviations: The pH of the test solutions was adjusted to a value of 6.5 ± 0.1 to enhance the stability of cymoxanil.

Validity:

In a range finding test algae were exposed to a nutrient medium control (not pH adjusted) and a range of test concentrations between 0.2 and 200 mg formulation/L (increasing factor: 10,). The pH of the test substance treatments was adjusted to 6.5. At 0.2 mg/L no effects on algal growth were observed. At

2.0 mg/L a slight reduction in algal growth was found, at 20 and 200 mg/L algal growth was reduced completely.

Material and methods:

Test substance: Cymoxanil 50 % WP, purity: 505 g cymoxanil/kg batch no.: V345

Test species: *Pseudokirchneriella subcapitata*

Treatments: Nutrient medium controls (not pH adjusted and pH adjusted), 1.0, 2.0, 3.6, 6.4, 10 and 20 mg formulation/L, test solutions were adjusted to a pH of 6.5 ± 0.1

Cymoxanil: 0.505, 1.01, 1.82, 3.28, 5.05 and 10.1 mg/L

Number of replicates / loading: 3 replicates per test substance treatment, 6 replicates for each control, initial loading: 10000 cells/mL

Test type / duration: Static test system, 72 hours

Nutrient medium: M2-medium according to the ISO-Standard "Algal growth inhibition test" (1989)

Test conditions:

Continuous illumination at 3500 - 4000 lux

Temperature: 22 – 22.5 °C

Continuous shaking

pH: 7.0 – 7.1 at test start and 7.3 – 8.2 at test end in pH adjusted control and treatments, 8.1 at test start and test end in the unadjusted control

Observations: At test start cells were counted by microscope using a counting chamber. After 24, 48 and 72 hours cell counts were made by spectrophotometric measurements of samples at 720 nm using a Lambda Spectrophotometer with a cuvette of 5 cm path length.

Analytical measurements: For chemical analysis of the active ingredient cymoxanil samples were taken from all test concentrations at test start, after 24 hours and at test end.

Method of analysis: HPLC

Statistical evaluation: EC₅₀ values and respective 95 % confidence limits were estimated using a linear regression of the log of the test concentration against the measured parameter. The NOEC was determined by ANOVA, Tukey test or Bonferroni t-test as appropriate.

Findings:

Analytical results: Measured cymoxanil concentrations at test start were 0.52, 1.00, 1.45, 3.15, 5.01 and 9.88 mg/L (80 – 103 % of nominal). After 72 hours measured cymoxanil concentrations were < 0.07 mg/L for all test concentrations. From analysis results the following time weighted average concentrations of cymoxanil in the test solutions were calculated for the exposure period of 0 – 72 hours (the concentration of 72 hours was set half the limit of detection = 0.035 mg/L for calculations): 0.110, 0.196, 0.308, 0.555, 0.784 and 1.45 mg ai/L

Morphological effects: No morphological observations were performed.

No difference in cell growth was found between the pH adjusted and the unadjusted control cultures.

Inhibition of the areas under the growth curve and growth rates was calculated relative to the pH adjusted control.

Table B.9.2.6-13: Effects of Cymoxanil 50 % WP on biomass (AUC) and growth rate of *Pseudokirchneriella subcapitata* after 72 hours of exposure.

Treatments Formulation nominal [mg/L]	Treatments (TWA 0 – 72 h) [mg ai/L]	Biomass (AUC) [% inhibition]	Growth rate [% inhibition]
1	0.110	14	3
2	0.196	23 *	4
3.6	0.308	38 *	8 *
6.4	0.555	57 *	14 *

Treatments Formulation nominal [mg/L]	Treatments (TWA 0 – 72 h) [mg ai/L]	Biomass (AUC) [% inhibition]	Growth rate [% inhibition]
10	0.784	70 *	26 *
20	1.45	93 *	49 *

* Statistically significant difference from the control (p<0.05)

Growth rate reduction for the first 48 hours was higher than for the last 24 hours of the test

Table B.9.2.6-14: Toxicity of Cymoxanil 50 % WP to the freshwater alga *Pseudokirchneriella subcapitata*. Toxicity values are based on time weighted average concentrations (0 – 72 h) of cymoxanil and nominal concentrations of the formulation.

Test concentrations	Endpoint	Time scale	NOEC [mg/L]	EC ₅₀ [mg/L]	95 % CL [mg/L]
Cymoxanil TWA (0 – 72 h)	Growth rate	72 h	0.110	0.41	0.24 – 0.71
	Biomass (AUC)	72 h	0.196	0.11	0.065 – 0.20
Cymoxanil 50 % WP based on nominal conc.	Growth rate	72 h	1	4.72	2.47 – 9.0
	Biomass (AUC)	72 h	2	1.08	0.55 – 2.13

Conclusion:

The following toxicity values are regarded acceptable for risk assessment:

E_bC₅₀ (72 h): 0.11 mg ai/L

E_rC₅₀ (72 h): 0.41 mg ai/L

NOEC (72 h): 0.196 mg ai/L (biomass) and 0.11 mg ai/L (growth rate)

Values are based on time weighted average concentrations (0 – 72 h) for the active substance.

B.9.2.7 Effects on aquatic macrophytes (Annex IIA 8.2.8)

Reference: Leva, S. E., Sloman, T. L. (1996) Cymoxanil: Influence on growth and reproduction of *Lemna gibba*. Report/Doc. AMR 3775-96

Guideline: US EPA 122-2

GLP: Yes

Deviations: None of relevance

Validity: The study is considered acceptable.

Material and methods:

Test substance: Cymoxanil technical, purity: 97.27 %, batch no.: DPX-T3217-113

Test species: *Lemna gibba*

Treatments: Nutrient medium controls and 800 µg/L

Number of replicates / loading: 4 replicates for the control and the test substance treatment, initial loading: 5 plants with 3 fronds each per replicate

Test type / duration: Static test system, 14 days

Nutrient medium: 20X AAP nutrient medium, pH adjusted to a value of 7.5 with 0.1 N hydrochloric acid

Test conditions:

Continuous illumination at 5010 ± 810 lux

Temperature: 24.8 – 25.4 °C

pH: 7.7 – 7.8 at test start and 8.7 – 9.5 at test end

Observations: Frond counts were made on days 0, 2, 5, 7, 9, 12 and 14. Dry weight of plants was determined at test termination (day 14)

Analytical measurements: For chemical analysis of cymoxanil samples were taken from all test levels at test start and at test end.

Method of analysis: HPLC

Statistical evaluation: Welch's t-test to compare control and test substance treatment cultures.

Findings:

Analytical results: At test start the measured test substance concentration was 700 µg/L. At test end no cymoxanil could be detected in the test solution.

Morphological effects: No morphological observations were performed.

Table B.9.2.7-1: Effects of cymoxanil on biomass (dry weight) and frond numbers of *Lemna gibba* after 14 days of exposure.

Treatment [µg/L]	Biomass (dry weight) [% inhibition]	Frond numbers [% inhibition]
700	- 4.2	- 2.5

Conclusion:

EC₅₀: > 700 µg/L (for biomass and growth rate)

NOEC (72 h): 700 µg/L

The value is based on an initial measured concentration.

B.9.2.8 Summary of the toxicity of cymoxanil, metabolites of cymoxanil and the formulations Cymoxanil 50 % WP and Tanos to aquatic organisms

Table B.9.2.8-1: Summary of studies on the toxicity of cymoxanil, metabolites of cymoxanil and the formulations Cymoxanil 50 % WP and Tanos to fish.

Test substance	Test organism	Test system / Duration	Endpoints	NOEC [mg/L]	LC ₅₀ [mg/L]	Test conc.	Reference & acceptability
Cymoxanil	<i>Oncorhynchus mykiss</i>	s / 96 h	Mortality Subleth. effects	28	61	mm	Baer (1993a) acceptable
	<i>Lepomis macrochirus</i>	s / 96 h	Mortality Subleth. effects	17	29	mm	Baer (1993b) acceptable
	<i>Cyprinodon variegatus</i>	f / 96 h	Mortality Subleth. effects	11.3	> 47.5	mm	Boeri et al. (1996a) acceptable
	<i>Oncorhynchus mykiss</i>	f / 21 d	Growth (Length)	0.22	1.5	mm	Baer (1992a) acceptable
	<i>Oncorhynchus mykiss</i>	f / 97 d	Growth Fry surviv.	0.12 ^a	-	mm	Boeri et al. (1997) acceptable
	<i>Cyprinodon variegatus</i>	f / 36 d	Growth Fry surviv.	0.0581	-	mm	Boeri et al. (1996) acceptable
IN-T4226	<i>Oncorhynchus mykiss</i>	ss / 96 h	Mortality Subleth. effects	111	> 111	mmL	Boeri et al. 2002a acceptable
IN-KQ960	<i>Oncorhynchus mykiss</i>	s / 96 h	Mortality Subleth. effects	120	> 120	nL	Samel (2002a) acceptable
IN-U3204	<i>Oncorhynchus mykiss</i>	ss / 96 h	Mortality Subleth. effects	97	> 97	mmL	Samel (2002b) acceptable
IN-W3595	<i>Oncorhynchus mykiss</i>	s / 96 h	Mortality Subleth. effects	130	> 130	mmL	Boeri et al. (2002b) acceptable
Cymoxanil	<i>Oncorhynchus</i>	ss / 96 h	Mortality	36 prod	120 prod	n	Bogers (1999a)

Test substance	Test organism	Test system / Duration	Endpoints	NOEC [mg/L]	LC ₅₀ [mg/L]	Test conc.	Reference & acceptability
50 % WP	<i>mykiss</i>			18.2 a.s.	60.6 a.s.		acceptable
Tanos	<i>Oncorhynchus mykiss</i>	f / 96 h	Mortality Subleth. effects	0.014 prod	0.0287 prod	n	Brown (1997a) acceptable
	<i>Oncorhynchus mykiss</i>	s / 96 h	Mortality Subleth. effects	0.008 prod	0.030 prod	n	Hoke (1998a) acceptable

f...flow through, mm...mean measured, mmL...mean measured limit concentration, n...nominal, nL...nominal limit concentration, prod...product, s...static, ss...semi-static

^a NOAEC, for further information see comments of RMS in the respective study summary under point B.9.2.2.1.

Table B.9.2.8-2: Summary of studies on the toxicity of cymoxanil, metabolites of cymoxanil and the formulations Cymoxanil 50 % WP and Tanos on aquatic invertebrates

Test substance	Test organism	Test system / Duration	End points	NOEC [mg/L]	LC ₅₀ /EC ₅₀ [mg/L]	Test conc.	Reference & acceptability
Cymoxanil	<i>Daphnia magna</i>	s / 48 h	Immobility	15	27	mm	Baer (1993c) acceptable
	<i>Mysidopsis bahia</i>	f / 96 h	Mortality Sublethal effects	17.6	> 44.4	mm	Boeri et al. (1996c) acceptable
	<i>Crassostrea virginica</i>	f / 96 h	Shell deposition	28.2	> 46.9	mm	Boeri et al. (1996d) acceptable
	<i>Daphnia magna</i>	ss / 21 d	Mortality Reproduction	0.067	-	mm	Baer (1993d) acceptable
IN-T4226	<i>Daphnia magna</i>	ss / 48 h	Immobility sublethal effects	116	> 116	mm	Boeri et al. (2002c) acceptable
IN-KQ960	<i>Daphnia magna</i>	s / 48 h	Immobility sublethal effects	n.d.	0.8	mm	Samel (2002d) acceptable
	<i>Daphnia magna</i>	ss / 21 d	Mortality Reproduction	0.302	-	mm	Samel (2003) acceptable
IN-U3204	<i>Daphnia magna</i>	ss / 48 h	Immobility sublethal effects	53	100	mm	Samel (2002c) acceptable
IN-W3595	<i>Daphnia magna</i>	s / 48 h	Immobility sublethal effects	126	> 126	mm	Boeri et al. (2002d) acceptable
Cymoxanil 50 % WP	<i>Daphnia magna</i>	ss / 48 h	Immobility	200 prod. 101 a.s.	> 200 prod. > 101 a.s.	nL	Migchielsen (1999) acceptable
Tanos	<i>Daphnia magna</i>	f / 48 h	Immobility sublethal effects	0.014 prod.	0.0555 prod.	n	Brown (1997b) acceptable
	<i>Daphnia magna</i>	s / 48 h	Immobility sublethal effects	0.072 prod.	> 0.2 prod.	n	Hoke (1998b) only additional infor.

f...flow through, mm...mean measured, mmL...mean measured limit concentration, n...nominal, n.d....not derived, nL...nominal limit concentration, prod...product, s...static, ss...semi-static

Table B.9.2.8-3: Summary of studies on the toxicity of cymoxanil, metabolites of cymoxanil and the formulations Cymoxanil 50 % WP and Tanos on aquatic plants.

Test substance	Test organism	Test system / Duration ^a	End points	NOEC [mg/L]	EC ₅₀ [mg/L]	Test conc.	Reference & acceptability
Cymoxanil	<i>Pseudokirchneriella subcapitata</i>	s / 96 h	Growth rate Biomass	n.d.	2.47 < 0.662	im	Boeri et al. (1999) acceptable
	<i>Pseudokirchneriella subcapitata</i>	s / 72 h	Growth rate Biomass	n.d.	0.63 0.35	mm	Bell et al. (1996) acceptable
	<i>Anabaena flos-aquae</i>	s / 96 h	Growth rate Biomass	0.0652 0.034	0.254 0.122	im	Hughes et al. (1996a) acceptable
	<i>Navicula pelliculosa</i>		No valid values available				Hughes et al. (1996b) not acceptable

Test substance	Test organism	Test system / Duration ^a	End points	NOEC [mg/L]	EC ₅₀ [mg/L]	Test conc.	Reference & acceptability
IN-T4226	<i>Anabaena flos-aquae</i>	s / 96 h	Growth rate 72 h Biomass 96 h	20 20	35.9 25.8	n	Sloman (2001a) acceptable
IN-KQ960	<i>Anabaena flos-aquae</i>	No valid values available					Sloman (2002b) not acceptable
IN-U3204	<i>Anabaena flos-aquae</i>	No valid values available					Sloman (2002a) not acceptable
IN-W3595	<i>Anabaena flos-aquae</i>	s / 96 h	Growth rate Biomass	5	19.9 12.7	n	Sloman (2001b) acceptable
Cymoxanil 50 % WP	<i>Pseudokirchneriella subcapitata</i>	s / 72 h	Growth rate Biomass	0.11 ai 0.196 ai	0.41 ai^b 0.11 ai	twa	Bogers (1999b) acceptable
DPX-KX007	<i>Pseudokirchneriella subcapitata</i>	s / 72 h	Growth rate Biomass	1.25 prod	11.0 prod 4.2 prod	n	Leva et al. (1997) acceptable
Cymoxanil	<i>Lemna gibba</i>	s / 14 d	Growth rate Biomass	0.7 0.7	> 0.7 > 0.7	im	Leva et al. (1996)

f...flow through, im...initial measured, mm...mean measured, mmL...mean measured limit concentration, n...nominal, n.d....not derived, nL...nominal limit concentration, s...static, ss...semi-static

^a The duration of some studies was up to 120 hours. Here the time period is stated, for which the acceptable toxicity values were derived. For details see respective study summaries under point B.9.2.6.

^b For classification and labelling of the plant protection product an E_{C50} of 4.72 mg Product/L based on nominal concentrations was used.

B.9.2.9 Risk assessment

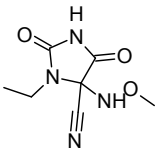
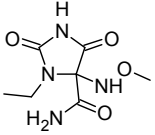
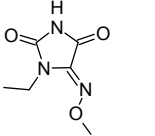
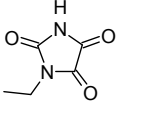
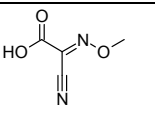
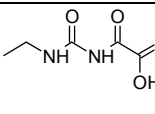
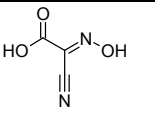
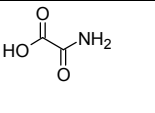
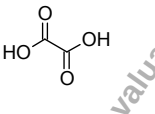
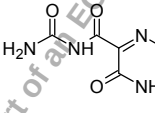
B.9.2.9.1 Lead formulations:

Two notifiers, Du-Pont and Oxon, submitted data. The two notifiers share only Annex II data, Annex III data were considered separately. The lead formulation from Oxon is Cymoxanil 50 % WP and from DuPont Tanos 50 WG.

B.9.2.9.2 Relevant metabolites of cymoxanil according to section fate and behaviour

Table B.9.2.9.2-1: Overview on parent compound, metabolites and identified degradates mentioned in the section B.9 Fate and Behaviour in the Environment.

Code	Chemical structure	Mass [g mol ⁻¹]	Occurrence (maximum amount observed) [% of AR]	Relevance according to section fate and behaviour
Cymoxanil, DPX-T3217		198.2	Parent	
IN-Q8761		198.2	Lab. soil: - Soil photolysis: 6.2 ^a Hydrolysis: - Photolysis: - Water/sediment: -	Not relevant metabolite No risk assessment demanded
IN-U3204		198.2	Lab. soil: 24.7 Soil photolysis: 2.2 Hydrolysis (pH 7): 52.7 Photolysis (pH 5): 0.6 Water/sediment: 24.7	Relevant metabolite Risk assessment demanded

Code	Chemical structure	Mass [g mol ⁻¹]	Occurrence (maximum amount observed) [% of AR]	Relevance according to section fate and behaviour
IN-JX915		198.2	Lab. soil: 8.2 Soil photolysis: 10.9 Hydrolysis (pH 7): 7.2 Photolysis (pH 5): 52.6 Water/sediment: 8.5	Relevant metabolite Risk assessment demanded
IN-KQ960		216.2	Lab. soil: 6.3 Soil photolysis: < 1.0 Hydrolysis (pH 7): 9.0 Photolysis (pH 5): - Water/sediment: 14.1	Relevant metabolite Risk assessment demanded
IN-R3273		171.2	Lab. soil: 2.4 Soil photolysis: < 1.0 Hydrolysis (pH 7): 10.2 Photolysis (pH 5): 35.4 Water/sediment: 5.0	Relevant metabolite Risk assessment demanded
IN-T4226		142.1	Lab. soil: 1.7 Soil photolysis: 1.2 Hydrolysis (pH 7): 5.4 Photolysis (pH 5): 6.7 Water/sediment: 12.0	Relevant metabolite Risk assessment demanded
IN-W3595		128.1	Lab. soil: 10.1 Soil photolysis: 2.3 Hydrolysis (pH 7): 22.6 Photolysis (pH 5): - Water/sediment: 27.5	Relevant metabolite Risk assessment demanded
IN-KP533		160.1	Lab. soil: 2.7 Soil photolysis: < 1.0 Hydrolysis (pH 7): 57.4 Photolysis (pH 5): 7.9 Water/sediment: 26.0	Relevant metabolite Risk assessment demanded
IN-R3274		114.1	Lab. soil: - Soil photolysis: - Hydrolysis (pH 7): - Photolysis (pH 5): - Water/sediment: ?	Relevant metabolite Risk assessment demanded
IN-18474		89.1	Lab. soil: 7.8 Soil photolysis: 4.4 Hydrolysis (pH 7): < 10 Photolysis (pH 5): 2.0 Water/sediment: ?	Not relevant metabolite No risk assessment demanded
Oxalic acid		90.0	Lab. soil: - Soil photolysis: - Hydrolysis (pH 7): < 10 Photolysis (pH 5): - Water/sediment: ?	Not relevant metabolite No risk assessment demanded
Metabolite fraction M5 ^b		188.1 ^c	Lab. soil: - Soil photolysis: - Hydrolysis (pH 7): - Photolysis (pH 5): - Water/sediment: 22.9	Relevant metabolite Risk assessment demanded

^a Also observed in dark control samples with 6.9 % of AR.

^b Metabolite fraction M5 comprises at least 2 compounds, one is considered to be represented by the structure shown in the table, the other compound(s) is/are unknown

^c The stated molar weight refers to the structure shown in the table (one compound of metabolite fraction M5).

B.9.2.9.3 Intended uses and predicted environmental concentrations for surface water bodies (PEC_{sw} values)

In Table B.9.2.9.3-1 notified uses are stated for the two lead formulations for which PEC_{sw} values were

calculated.

Table B.9.2.9.3-1: Intended uses of cymoxanil for the formulations Tanos and Cymoxanil 50% WP.

Crop	South / North EU	Product name	Method	BBCH code	No. of applic.	Application rate [g ai/ha]	Min. Interval [d]	Notifier
Lettuce	South EU	Cymoxanil 50 % WP	Spray	40 - 49	3 – 4	240	7	OXON
Potato	North EU	Cymoxanil 50 % WP	Spray	21 - 95	4 South EU 5 North EU	120	7	OXON
	South EU	Tanos 50 WG ^a	Spray	21 - 95	6 – 8	175 ^b	7 – 10	DuPont

^a Tanos 50 WG = DPX KP481 50 WG (cymoxanil/famoxadone 250/250 g/kg)

^b This equals to 700 g product/ha

In section 'Fate and Behaviour' under point B.8.11 for all intended uses PEC_{sw} values for the active substance and the metabolites IN-U3204, IN-W3595, IN-KQ960, IN-T4226, IN-JX915, IN-R3273, IN-KP533 and the metabolite fraction M5 are presented (derived with FOCUS modelling step 1 and 2).

FOCUS step 1 assumes that the total seasonal amount of active substance is applied at one occasion (worst case assumption).

At FOCUS step 2 the time intervals between individual applications are taken into account for PEC_{sw} modelling. At step 2 PEC_{sw} values were calculated for single applications (just one application at the rates stated in table B.9.2.9.1-1 for the intended uses) and multiple applications (as stated in table B.9.2.9.1-1) to find out which is the worst case scenario (single or multiple application). TER values were calculated with PEC_{sw} values for the application scenario (single or multiple application, North or South EU) which yielded the higher PEC_{sw} value for FOCUS step 2.

For the plant protection product Cymoxanil 50 % WP the use in lettuce is worst case compared to the use in potatoes and hence a risk assessment for the use in lettuce covers a risk assessment for the intended use in potatoes.

In Table B.9.2.9.3-2 PEC_{sw} calculations used for TER calculations are stated.

Table B.9.2.9.3-2: Summary on global maximum PEC_{sw} [µg/L] of cymoxanil and metabolites after application of TANOS 50 WG (DuPont) to potatoes and CYMOXANIL 50 % WP (Oxon) to lettuce (worst case compared to intended use in potatoes).

Formulation		TANOS 50 WG - potatoes (DuPont)			CYMOXANIL 50 % WP – lettuce (Oxon)		
FOCUS STEP	Compound	PEC _{sw} [µg L ⁻¹]	Type of application	Scenario	PEC _{sw} [µg L ⁻¹]	Type of application	Scenario
STEP 1	Cymoxanil	56.7	-	-	77.8	-	-
	IN-U3204	14.3	-	-	19.6	-	-
	IN-W3595	32.4	-	-	22.2	-	-
	IN-KQ960	33.2	-	-	22.7	-	-
	IN-T4226	6.66	-	-	4.57	-	-
	IN-JX915	7.07	-	-	9.70	-	-
	IN-R3273	13.1	-	-	8.98	-	-
	IN-KP533	12.7	-	-	8.72	-	-
	Metabolite fraction M5	0.37	-	-	0.51	-	-
STEP 2	Cymoxanil	1.61	Single	South EU	2.21	Single	South EU
	IN-U3204	0.40	Single	South EU	0.55	Single	South EU
	IN-W3595	0.33	Single	South EU	0.39	Single	South EU

Formulation		TANOS 50 WG - potatoes (DuPont)			CYMOXANIL 50 % WP – lettuce (Oxon)		
FOCUS STEP	Compound	PEC _{sw} [µg L ⁻¹]	Type of application	Scenario	PEC _{sw} [µg L ⁻¹]	Type of application	Scenario
	IN-KQ960	2.12	Multiple	South EU	1.50	Multiple	South EU
	IN-T4226	1.15	Multiple	South EU	0.44	Multiple	South EU
	IN-JX915	0.85	Single	South EU	1.16	Single	South EU
	IN-R3273	2.11	Multiple	South EU	1.06	Multiple	South EU
	IN-KP533	2.04	Multiple	South EU	0.74	Multiple	South EU
	Metabolite fraction M5	0.37	Single	South EU	0.51	Single	South EU

Since the active substance and its metabolites are not likely to partition into sediments (see section fate and behaviour), no PEC_{sed} values are presented here and no risk assessment for sediment dwelling organisms is demanded.

B.9.2.9.4 TER calculations

Cymoxanil: The lowest toxicity value for the respective group of aquatic organisms (fish, daphnids, algae, macrophytes) was compared to the global maximum PEC_{sw} derived with FOCUS step 1 and step 2.

Formulation Cymoxanil 50 % WP: The toxicity of the formulation for fish, daphnids and algae was expressed as active substance and then compared to PEC_{sw} values derived for cymoxanil.

Formulation Tanos: This formulation contains two active ingredients, cymoxanil and famoxadone.

Famoxadone is orders of magnitude more toxic than cymoxanil for fish and daphnids. No procedure for TER calculations has been agreed between member states for such a situation. Therefore the RMS calculated TER values based on the toxicity of the formulation for fish, daphnids and algae and PEC_{sw} values for the formulation assuming only spray drift input into surface waters (based on Ganzelmeier drift values). This procedure was used as no FOCUS PEC_{sw} values can be calculated for a formulation with two active ingredients.

Toxicity exposure ratios (TER) for fish

TER values for intended uses for the product Cymoxanil 50 % WP

Table B.9.2.9.4-1: Toxicity exposure ratios (TER) for fish based on toxicity values for the most sensitive species and maximal PEC_{sw} values derived with FOCUS modelling step 1 and step 2 (South EU).

Test substance	Test organism	Toxicity estimate	Toxicity [mg ai/L]	PEC _{sw} [mg ai/L]		TER		Annex VI trigger
				Step 1	Step 2	Step 1	Step 2	
Cymoxanil	<i>Lepomis macrochirus</i>	LC ₅₀ (96 h)	29	0.0778	0.00221	373	13122	100
	<i>Cyprinodon variegatus</i>	NOEC (36 d)	0.0581	0.0778	0.00221	0.7	26	10
IN-T4226	<i>Oncorhynchus mykiss</i>	LC ₅₀ (96 h)	> 111	0.00457	0.00044	>24289	252273	100
IN-KQ960	<i>Oncorhynchus mykiss</i>	LC ₅₀ (96 h)	> 120	0.0227	0.00150	>5286	80000	100
IN-U3204	<i>Oncorhynchus mykiss</i>	LC ₅₀ (96 h)	> 97	0.0196	0.00055	>4949	176364	100
IN-W3595	<i>Oncorhynchus mykiss</i>	LC ₅₀ (96 h)	> 130	0.0222	0.00039	>5856	333333	100
Cymoxanil 50 % WP	<i>Oncorhynchus mykiss</i>	LC ₅₀ (96 h)	60.6	0.0778	0.00221	779	27421	100

TER values for intended uses for the product TANOS

Table B.9.2.9.4-2: Toxicity exposure ratios (TER) for fish based on toxicity values for the most sensitive species and maximal PEC_{sw} values derived with FOCUS modelling step 1 and step 2 (South EU).

Test substance	Test organism	Toxicity estimate	Toxicity [mg ai/L]	PEC _{sw} [mg ai/L]		TER		Annex VI trigger
				Step 1	Step 2	Step 1	Step 2	
Cymoxanil	<i>Lepomis macrochirus</i>	LC ₅₀ (96 h)	29	0.0567	0.00161	511	18012	100
	<i>Cyprinodon variegatus</i>	NOEC (36 d)	0.0581	0.0567	0.00161	1.0	36	10
IN-T4226	<i>Oncorhynchus mykiss</i>	LC ₅₀ (96 h)	> 111	0.00666	0.00115	>16667	96522	100
IN-KQ960	<i>Oncorhynchus mykiss</i>	LC ₅₀ (96 h)	> 120	0.0332	0.00212	>3614	56604	100
IN-U3204	<i>Oncorhynchus mykiss</i>	LC ₅₀ (96 h)	> 97	0.0143	0.00040	>6783	242500	100
IN-W3595	<i>Oncorhynchus mykiss</i>	LC ₅₀ (96 h)	> 130	0.0324	0.00033	>4012	393939	100

Table B.9.2.9.4-3: TER values for fish based on PEC_{sw} values for the plant protection product Tanos. Only spray drift is assumed to reach surface waters.

Test substance	Test organism	Toxicity estimate	Toxicity [mg prod/L]	Buffer zone	Drift [%]	PEC _{sw} [mg prod./L]	TER	Annex VI trigger
Tanos	<i>Oncorhynchus mykiss</i>	LC ₅₀ (96 h)	0.0287	0 m	2.77	0.006463	4.4	100
				20 m	0.15	0.000350	82	
				30 m	0.10	0.000233	123	

For the metabolites IN-JX915, IN-R3273, IN-KP533 and the metabolite fraction M5 no toxicity values for fish are available. However, these metabolites were found in a 96 hour fish study (Migchielsen, 2003) in the amounts stated in Table B.9.2.9.3-4. In this study no fish died. From both formulations and respective intended uses maximal FOCUS step 2 PEC_{sw} values for the metabolites IN-JX915, IN-R3273, IN-KP533 and the metabolite fraction M5 are 0.00116, 0.00211, 0.00204 and 0.00051 mg/L. When the concentrations of these metabolites in the study of Migchielsen are compared with the maximal PEC_{sw} values derived with FOCUS step 2 modelling it can be seen that predicted environmental concentrations are by far lower than the tested concentrations at which no mortalities occurred. Therefore the risk to fish from exposure to these metabolites can be considered as low.

Table B.9.2.9.4-4: Concentrations of the metabolites IN-JX915, IN-R3273, IN-KP533 and the metabolite fraction M5 in the fish study of Migchielsen (2003)

Hours of exposure	Metabolite fraction M5 [mg/L]	IN-R3273 [mg/L]	IN-JX915 [mg/L]	IN-KP533 [mg/L]
0	0.00	0.00	0.35	0.03
5	0.00	0.00	0.42	0.06
24	0.12	0.09	0.89	0.10
48	2.16	0.16	0.83	0.20
72	2.14	0.22	0.87	0.41
96	2.75	0.25	0.78	0.79

According to SANCO/3268/2001 long-term/chronic tests should only be required if the metabolite is more acutely toxic than the active substance. This trigger is not clearly given for any of the metabolites regarded as environmentally relevant in section fate and behaviour.

Conclusion:

The risk to fish from intended uses of the plant protection product Cymoxanil 50 % WP is low.

The risk to fish from the intended uses of the plant protection product Tanos is low provided that appropriate risk mitigation measures are implemented.

Toxicity exposure ratios (TER) for aquatic invertebrates

TER values for intended uses for the product Cymoxanil 50 % WP

Table B.9.2.9.4-5: Toxicity exposure ratios (TER) for aquatic invertebrates based on toxicity values for the most sensitive species and maximal PEC_{sw} values derived with FOCUS modelling step 1 and step 2 (South EU).

Test substance	Test organism	Toxicity estimate	Toxicity [mg ai/L]	PEC _{sw} [mg ai/L]		TER		Annex VI trigger
				Step 1	Step 2	Step 1	Step 2	
Cymoxanil	<i>Daphnia magna</i>	EC ₅₀ (48 h)	27	0.0778	0.00221	347	12217	100
	<i>Daphnia magna</i>	NOEC (21 d)	0.067	0.0778	0.00221	1	30	10
IN-T4226	<i>Daphnia magna</i>	EC ₅₀ (48 h)	> 116	0.00457	0.00044	25383	263636	100
IN-KQ960	<i>Daphnia magna</i>	EC ₅₀ (48 h)	0.8	0.0227	0.00150	35	533	100
	<i>Daphnia magna</i>	NOEC (21 d)	0.302	0.0227	0.00150	13	201	10
IN-U3204	<i>Daphnia magna</i>	EC ₅₀ (48 h)	100	0.0196	0.00055	5102	181818	100
IN-W3595	<i>Daphnia magna</i>	EC ₅₀ (48 h)	> 126	0.0222	0.00039	5676	323077	100
IN-JX915 ^a	<i>Daphnia magna</i>	EC ₅₀ (48 h)	0.27	0.0097	0.00116	28	233	100
IN-R3273 ^a	<i>Daphnia magna</i>	EC ₅₀ (48 h)	0.27	0.00898	0.00106	30	255	100
IN-KP533 ^a	<i>Daphnia magna</i>	EC ₅₀ (48 h)	0.27	0.00872	0.00074	31	365	100
Metabolite fraction M5 ^a	<i>Daphnia magna</i>	EC ₅₀ (48 h)	0.27	0.00051	0.00051	529	529	100
Cymoxanil 50 % WP	<i>Daphnia magna</i>	EC ₅₀ (48 h)	101	0.0778	0.00221	1298	45701	100

^a Since no toxicity information is available for these metabolites it was assumed that the toxicity is by a factor of 100 higher than the toxicity of the active substance.

TER values for intended uses for the product TANOS

Table B.9.2.9.4-6: Toxicity exposure ratios (TER) for aquatic invertebrates based on toxicity values for the most sensitive species and maximal PEC_{sw} values derived with FOCUS modelling step 1 and step 2 (South EU).

Test substance	Test organism	Toxicity estimate	Toxicity [mg ai/L]	PEC _{sw} [mg ai/L]		TER		Annex VI trigger
				Step 1	Step 2	Step 1	Step 2	
Cymoxanil	<i>Daphnia magna</i>	EC ₅₀ (48 h)	27	0.0567	0.00161	476	16770	100
	<i>Daphnia magna</i>	NOEC (21 d)	0.067	0.0567	0.00161	1	42	10
IN-T4226	<i>Daphnia magna</i>	EC ₅₀ (48 h)	> 116	0.00666	0.00115	17417	100870	100
IN-KQ960	<i>Daphnia magna</i>	EC ₅₀ (48 h)	0.8	0.0332	0.00212	24	377	100
	<i>Daphnia magna</i>	NOEC (21 d)	0.302	0.0332	0.00212	9	142	10
IN-U3204	<i>Daphnia magna</i>	EC ₅₀ (48 h)	100	0.0143	0.00040	6993	250000	100
IN-W3595	<i>Daphnia magna</i>	EC ₅₀ (48 h)	> 126	0.0324	0.00033	3889	381818	100

Test substance	Test organism	Toxicity estimate	Toxicity [mg ai/L]	PEC _{sw} [mg ai/L]		TER		Annex VI trigger
				Step 1	Step 2	Step 1	Step 2	
IN-JX915 ^a	<i>Daphnia magna</i>	EC ₅₀ (48 h)	0.27	0.00707	0.00085	38	318	100
IN-R3273 ^a	<i>Daphnia magna</i>	EC ₅₀ (48 h)	0.27	0.0131	0.00211	21	128	100
IN-KP533 ^a	<i>Daphnia magna</i>	EC ₅₀ (48 h)	0.27	0.0127	0.00204	21	132	100
Metabolite fraction M5 ^a	<i>Daphnia magna</i>	EC ₅₀ (48 h)	0.27	0.00037	0.00037	730	730	100

^a Since no toxicity information is available for these metabolites it was assumed that the toxicity is by a factor of 100 higher than the toxicity of the active substance.

Table B.9.2.9.4-7: TER values for aquatic invertebrates based on PEC_{sw} values for the plant protection product Tanos. Only spray drift is assumed to reach surface waters.

Test substance	Test organism	Toxicity estimate	Toxicity [mg prod/L]	Buffer zone	Drift [%]	PEC _{sw} [mg prod./L]	TER	Annex VI trigger
Tanos	<i>Daphnia magna</i>	EC ₅₀ (48 h)	0.0555	0 m	2.77	0.006463	9	100
				20 m	0.15	0.000350	159	

For the metabolites IN-JX915, IN-R3273, IN-KP533 and the metabolite fraction M5 no toxicity information on daphnids is available. Assuming, that the toxicity of these metabolites to daphnids is by a factor of 100 higher than the toxicity of the active substance results in the TER values above the respective Annex VI trigger (Table B.9.2.8.3-6). This is an indication that the risk to aquatic invertebrates from exposure to these metabolites is low. Looking at the whole toxicity profile of cymoxanil and its metabolites and taking into account that the metabolites IN-JX915, IN-R3273, IN-KP533 and the metabolite fraction M5 are not highly toxic to fish, the RMS is of the opinion, that the risk to invertebrates from these degradation products is low.

According to SANCO/3268/2001 long-term/chronic tests should only be required if the metabolite is more acutely toxic than the active substance. The only metabolite for which this trigger is clearly given is IN-KQ960 (EC₅₀ (48 h) daphnids = 0.8 mg/l for IN-KQ960 and 27 mg/L for cymoxanil). A chronic daphnid study with this metabolite was submitted. The respective TER value is above the trigger of 10 set in Annex VI of Directive 91/414/EEC.

Conclusion:

The risk to aquatic invertebrates from intended uses of the plant protection product Cymoxanil 50 % WP is low.

The risk to aquatic invertebrates from the intended uses of the plant protection product Tanos is low provided that appropriate risk mitigation measures are implemented.

Toxicity exposure ratios (TER) for aquatic plants

TER values for intended uses for the product Cymoxanil 50 % WP

Table B.9.2.9.4-8: Toxicity exposure ratios (TER) for aquatic plants based on toxicity values for the most sensitive species and maximal PEC_{sw} values derived with FOCUS modelling step 1 and step 2 (South EU).

Test substance	Test organism	Toxicity estimate	Toxicity [mg ai/L]	PEC _{sw} [mg ai/L]		TER		Annex VI trigger
				Step 1	Step 2	Step 1	Step 2	
Cymoxanil	<i>Anabaena flos-aquae</i>	E _r C ₅₀ (96 h)	0.254	0.0778	0.00221	3	115	10
		E _b C ₅₀ (96 h)	0.122			2	55	

Test substance	Test organism	Toxicity estimate	Toxicity [mg ai/L]	PEC _{sw} [mg ai/L]		TER		Annex VI trigger
				Step 1	Step 2	Step 1	Step 2	
IN-T4226	<i>Anabaena flos-aquae</i>	E _r C ₅₀ (96 h) E _b C ₅₀ (96 h)	35.9 25.8	0.00457	0.00044	7856 5646	81591 58636	10
IN-W3595	<i>Anabaena flos-aquae</i>	E _r C ₅₀ (96 h) E _b C ₅₀ (96 h)	19.9 12.7	0.0222	0.00039	896 572	51026 32564	10
Cymoxanil 50 % WP	<i>Pseudokirchneriella subcapitata</i>	E _r C ₅₀ (72 h) E _b C ₅₀ (72 h)	0.41 0.11	0.0778	0.00221	5 1	186 50	10
Cymoxanil	<i>Lemna gibba</i>	E _r C ₅₀ (14 d) E _b C ₅₀ (14 d)	> 0.7 > 0.7	0.0778	0.00221	9	317	10

TER values for intended uses for the product TANOS

Table B.9.2.9.4-9: Toxicity exposure ratios (TER) for aquatic plants based on toxicity values for the most sensitive species and maximal PEC_{sw} values derived with FOCUS modelling step 1 and step 2 (South EU).

Test substance	Test organism	Toxicity estimate	Toxicity [mg ai/L]	PEC _{sw} [mg ai/L]		TER		Annex VI trigger
				Step 1	Step 2	Step 1	Step 2	
Cymoxanil	<i>Anabaena flos-aquae</i>	E _r C ₅₀ (96 h) E _b C ₅₀ (96 h)	0.254 0.122	0.0567	0.00161	4 2	158 76	10
IN-T4226	<i>Anabaena flos-aquae</i>	E _r C ₅₀ (96 h) E _b C ₅₀ (96 h)	35.9 25.8	0.00666	0.00115	5390 3874	31217 22435	10
IN-W3595	<i>Anabaena flos-aquae</i>	E _r C ₅₀ (96 h) E _b C ₅₀ (96 h)	19.9 12.7	0.0324	0.00033	614 392	60303 38485	10
Cymoxanil	<i>Lemna gibba</i>	E _r C ₅₀ (14 d) E _b C ₅₀ (14 d)	> 0.7 > 0.7	0.0567	0.00161	12 12	435 435	10

No toxicity value for the plant protection product Tanos is available and hence no respective TER value could be calculated. Therefore the RMS calculated a TER value based on the NOEC derived from a study on the effects of the formulation DPX-KX007 on algae and PEC_{sw} values assuming only spray drift inputs into surface water bodies (see Table B.9.2.9.3-10). The resulting TER is without any buffer zones 193 and hence by an order of magnitude above the trigger of 10. Considering that the active ingredient contents of DPX-KX007 and Tanos are comparable, a NOEC instead of an EC₅₀ was used for TER calculations and that the TER is already high without assuming risk mitigation measures, the RMS considers the risk to algae from the plant protection product Tanos as acceptable.

Table B.9.2.9.4-10: TER values for aquatic plants based on PEC_{sw} values for the plant protection product Tanos. Only spray drift is assumed to reach surface waters.

Test substance	Test organism	Toxicity estimate	Toxicity [mg prod/L]	Buffer zone	Drift [%]	PEC _{sw} [mg prod./L]	TER	Annex VI trigger
DPX-KX007	<i>Pseudokirchneriella subcapitata</i>	NOEC (72 h)	1.25	0 m	2.77	0.006463	193	10

For the metabolites IN-KQ960, IN-U3204, IN-JX915, IN-R3273, IN-KP533 and the metabolite fraction M5 no valid toxicity data are available for algae. However, all studies with algae were performed under static conditions at relatively high pH levels. The fast degradation of cymoxanil is at this pH levels mainly driven by abiotic processes such as hydrolysis and hence from fate and behaviour information on cymoxanil it is reasonable that the metabolites IN-KQ960, IN-U3204, IN-JX915, IN-R3273, IN-KP533 and the metabolite fraction M5 have been present in the test solutions. Therefore the risk to aquatic algae from exposure to these metabolites is considered to be low.

Conclusion:

The risk to aquatic plants from intended uses of the plant protection products Cymoxanil 50 % WP and Tanos is low.

B.9.2.10 Overall conclusion

The risk to aquatic organisms from intended uses of the plant protection product Cymoxanil 50 % WP is low. The risk from intended uses of the plant protection product Tanos is low provided that appropriate risk mitigation measures are implemented.

B.9.3 Effects on other terrestrial vertebrates (ANNEX IIIA 10.3)

B.9.3.1 Summary of effects on mammals

For mammals information on toxicity was obtained from the tests performed under the human health section (Annex B6).

Table B.9.3.1-1: Toxicity data for mammals

Species / scenario	Test substance	Observed Effects	LD ₅₀ (mg/kg bw/d)	NOEC (ppm feed)	NOEL (mg/kg bw/d)	Reference
rat acute oral	cymoxanil	mortality	760 (m) 1200 (f)	/	n.d.	Sarver, 1992
rat acute oral	Cymoxanil 50 % WP (Oxon)	mortality	1200 ¹	/	n.d.	McRea, 1995a
rat acute oral	Tanos (DuPont)	mortality	1732 / 566 ² (m / f)	/	n.d.	Finlay, 1997a
rat 2-generation	cymoxanil	parental and offspring effects (reduced viability and pup weights); no real reproductive effect	/	100	6.5 (m)	Kreckmann, 1993
rat 2-generation	cymoxanil	parental and offspring effects; no real reproductive effect	/	150	10.5 (m)	Ganiger, 2001

¹ equivalent to 603 mg a.s./kg bw

² equivalent to 433 / 142 mg cymoxanil/kg bw

B.9.3.2 Risk assessment for mammals

Toxicity Exposure Ratios (TER) and Estimated Theoretical Exposures (ETE) were calculated based on the methods, i.e. equations and tabulated values described in the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC".

Relevant toxicity figures were taken from laboratory studies with the active substance. These are listed in table 9.3.1-1 above. Acute toxicity data are also given for the two relevant formulations.

The following worst case exposure scenarios were considered:

lettuce	4 x 240 g ai/ha, interval 7 d	CYM 50 (Oxon)	Southern Europe
potatoes	8 x 175 g ai/ha, interval 7 d	Tanos (Du Pont)	Northern + Southern Europe

B.9.3.2.1 First tier risk assessment to mammals

Exposure estimates were performed according to the standard scenarios provided in Sanco/4145/2000. In the category "leafy crops" the guidance document considers a medium herbivore as the relevant mammal of concern. Because potato foliage is not palatable for mammals, insectivorous mammals are considered to be the more relevant category and their risk is also evaluated. In order to calculate the estimated theoretical exposure the respective FIR/bw and RUD figures were chosen to estimate food intake rates and concentrations in the food of concern (see table 9.3.2.1-1). Up to four applications are intended in lettuce, and the respective multiple application factors (MAF) are set at 1.8 for the acute scenario and 2.2 for the short- and long-term scenarios. As up to eight applications are intended in potatoes, the respective multiple application factors (MAF) are set at 2 for the acute scenario and 2.5 for the short- and long-term scenarios. A default half-life of 10 d is assumed for estimating residue decline on herbaceous feed items. In the first tier worst case assessment it is assumed that the mammals feed on a single food type, which is not avoided and that they satisfy their entire food demand in the treated area (PD, PT = 1).

Possible other food items, for which no respective risk assessment is performed, are covered by the calculations presented because residues in these items are expected to be significantly lower.

Choice of long-term/reproductive NOEL:

The lowest NOAEL from multigeneration studies is 6.5 mg/kg bw/d for the male rat. This is based on parental effects (decreased bodyweights, increased testes weights) as well as offspring effects (reduced viability and pup weights). In the second 2-generation study on rats a NOAEL of 10.5 mg/kg bw was established. The NOAEL and LOAEL levels of both studies are linked with the respective effects in the table below to identify a combined NOAEL.

dose level	effect
100 ppm (6.5-6.65 mg/kg bw/d)	none
150 ppm (10.5-14.9 mg/kg bw/d)	none
450 ppm	<u>parental</u> : ↓ bw and bw gain, ↓ food consumption, ↑ testes weight <u>offspring</u> : ↓ 0-4 d viability, ↓ pup weights
500 ppm	<u>parental</u> : ↓ bw, ↓ food consumption <u>offspring</u> : ↓ pup weights

Looking at the combined data the NOAEL of 6.5 mg/kg bw appears to be due to the spacing of dose levels in this study. The overall NOAEL of 10.5 mg/kg bw is considered conservative and appropriate for risk assessment.

In several studies on developmental toxicity a teratogenic potential of cymoxanil was identified. However, the developmental NOAELs derived from these studies are in excess of the above mentioned NOAEL, except one study where no NOAEL could be identified with regard to minor foetal effects, which are considered not ecotoxicologically relevant. According to the notifier these studies of developmental toxicity show no evidence of dose-related effects that might support identification of a teratogenic potential of cymoxanil. The notifier provided a statement on this subject with regard to the toxicology

section.

From test design and relevance of effects, the 2-generation study and the NOAEL of 10.5 mg/kg bw/d is considered most appropriate for ecological risk assessment.

Table B.9.3.2-1: TER values for acute and long-term risk for mammals

Scenario	mammal type	AR (kg/ha)	FIR/bw	RUD	Ftwa	MAF	ETE	Endpoint (mg a.s./kg bw/d)	TER	trigger
lettuce / Oxon										
Acute cymoxanil	medium herbivorous	0.24	0.28	87	-	1.8	10.5	760	72	10
	insectivorous	0.24	0.63	14	-	-	2.1	760	359	10
Acute Cym50WP	medium herbivorous	0.24	0.28	87	-	1.8	10.5	603	57	10
	insectivorous	0.24	0.63	14	-	-	2.1	603	285	10
Long-term	medium herbivorous	0.24	0.28	40	0.53	2.2	3.2	10.5	3.3	5
	insectivorous	0.24	0.63	5.1	-	-	0.8	10.5	14	5
potatoes / DuPont										
Acute cymoxanil	medium herbivorous	0.175	0.28	87	-	2	8.5	760	89	10
	insectivorous	0.175	0.63	14	-	-	1.5	760	492	10
Acute Tanos	medium herbivorous	0.175	0.28	87	-	2	8.5	142	17	10
	insectivorous	0.175	0.63	14	-	-	1.5	142	92	10
Long-term	medium herbivorous	0.175	0.28	40	0.53	2.5	2.6	10.5	4	5
	insectivorous	0.175	0.63	5.1	-	-	0.6	10.5	19	5

FIR = Food intake rate; bw = Bodyweight; RUD = Residue per unit dose; Ftwa = Time weighted average factor; AR = Application rate; ETE = Estimated theoretical exposure; TER = Toxicity exposure ratio.

On the acute time scale, according to the resulting TER figures the risk can be considered low. However, a potential risk was identified in the first tier long-term risk assessment for herbivores, while the risk for insectivorous mammals appears low.

A refined risk assessment for herbivores is considered necessary.

B.9.3.2.2 Higher tier risk assessment

Risk to herbivores in lettuce:

In the six submitted residue decline trials (see B.7.6.1), which were conducted with the 50 WP formulation in Southern Europe, cymoxanil residue concentration in lettuce was found to be near to the limit of quantification within three days. Moreover it was shown that there is no accumulation of residue on lettuce plants due to repeated applications since the residue level before the 4th application was below the limit of detection. Hence an estimated DT₅₀ of 2 days seems a more appropriate and still worst case assumption for the residue calculation in vegetation. (A DT₅₀ of 0.67 d as proposed in the risk assessment of one of the notifiers could not be verified.) In consequence, the MAF is set at 1 and the default time weighted average factor (f_{twa}) is calculated based on an estimated DT₅₀ of 2 d, which results in a refined f_{twa} of 0.38.

Risk to herbivores in potatoes:

This scenario is considered to be of minor relevance, as potato foliage is unpalatable to mammals. Nevertheless, a refinement has been conducted and covers the risk of mammals eating potential ground vegetation beneath potato plants. Results of residue decline studies in potato in Southern and Northern Europe show the same results as for lettuce (residue levels below the limit of detection, no accumulation at all after multiple applications). Therefore, based on residue information as detailed above for lettuce, MAF and f_{twa} are refined to 1 and 0.38.

Table B.9.3.2-2: Refined TER value for the long-term risk for herbivorous mammals

Scenario	mammal type	AR (kg/ha)	FIR/bw	RUD	Ftwa	MAF	ETE	Endpoint (mg a.s./kg bw)	TER	trigger
lettuce / Oxon										
Long-term	medium herbivorous	0.24	0.28	40	0.38	1	1	10.5	10	5
potatoes / DuPont										
Long-term	medium herbivorous	0.175	0.28	40	0.38	1	0.7	10.5	14	5

All resulting TER's exceed the trigger of 5 given in directive 91/414 and indicate a low long-term / reproductive risk to wild mammals after the use of cymoxanil according to GAP.

B.9.3.2.3 Risk to mammals from plant metabolites

In lettuce cymoxanil was fast and extensively metabolised, mainly to glycine. In one of two metabolism studies in lettuce significant amounts of metabolite IN-W3595 were formed (up to 18.1 % of TRR). Other metabolites were detected only in very low amounts (< 6 % TRR). In mammalian metabolism the same metabolite IN-W3595 was the only one detected in significant amounts (up to 45.2 %). Hence it is considered that it's toxicity from an ecotoxicological point of view is covered by toxicity studies with the parent compound.

B.9.3.2.4 Risk to mammals from secondary poisoning

As the LogPow does not exceed 3 the risk to mammals from secondary poisoning can be considered as low. The toxicological evaluation also revealed no potential of bioaccumulation.

B.9.3.2.5 Risk to mammals from the consumption of contaminated drinking water

The water intake of mammals is calculated according to SANCO/4145/2000.

Total water ingestion rate (L/day) = $0.099W^{0.90}$

where W is the body weight in kg.

As cymoxanil is of low acute toxicity, the exposure via drinking from surface water bodies is considered to be negligible. The exposure from puddles of spray liquid is assessed as a worst case. The dilution for spraying is 300 (-600) L water/ha containing 175 g ai for potatoes and 500(-800) L water/ha containing 240 g ai for lettuce. When a dilution factor of 5 is applied for the concentration in the puddle, a $PEC_{\text{drinking water}}$ of 117 / 96 mg ai/L is derived for potatoes and lettuce, respectively.

The total water ingestion rate (daily water intake DWI) of a small mammal (pygmy shrew, 6 g) is calculated as 1 mL/d. Figures for acute toxicity for the a.s. and the formulations Cymoxanil 50 % WP and Tanos are the same as above used for the dietary risk assessment.

The Toxicity Exposure Ratio (TER) for the acute time scale is calculated as LD_{50}/ETE . The ETE_{acute} is calculated as $PEC_{drinking\ water} \times DWI$.

Table B.9.3.2-3: Acute TERs for small mammals drinking contaminated water from puddles

scenario		ETE	TER (ai)	TER (CYM 50WP)	TER (Tanos)
acute	lettuce / Oxon	16	61	38	-
	potatoes / DuPont	19.3	50	-	7.4

The TERs for both the active substance and the 50 WP-formulation are above the trigger and indicate a low risk to wild small mammals consuming drinking water contaminated with cymoxanil. The TER for the formulation TANOS, containing 25 % cymoxanil and 25 % famoxadone, is slightly below the trigger. However, it should be considered that, as this is a formulation with two active substances, the LD_{50} of 142 mg cymoxanil/kg bw is not reflecting the "real" toxicity with regard to cymoxanil. Furthermore the TER calculation includes many worst case assumptions (lowest range of spraying dilution, smallest mammal representative,...). Considering more realistic conditions, the RMS considers the risk via contaminated drinking water to wild mammals as low.

B.9.3.2.6 Overall conclusions

After application of cymoxanil according to GAP the risk to wild mammal populations is considered low.

B.9.4 Effects on bees (Annex IIA 8.3.1, Annex IIIA 10.4)

B.9.4.1 Acute toxicity (ANNEX IIA 8.3.1.1; ANNEX IIIA 10.4.1)

Active Substance Cymoxanil

Reference: Schur, A. (1999). Assessment of side effects of Cymoxanil technical to the honey bee, *Apis mellifera* L. in the laboratory. (Report No. 99063/01-BLEU)

Test guideline: EPPO 170

GLP: Yes

Deviations: The minimum temperature (20°C) and the maximum (90 %) and minimum (35 %) rel. humidities lay outside the relevant ranges ($25 \pm 2^\circ\text{C}$ and $50 \pm 10\%$, respectively) stated in the guideline. As no behavioural abnormalities were evident among the control organisms these deviations are considered not to have influenced the outcome of the study.

Validity: Acceptable

Material and methods:

Test substance: Cymoxanil technical, Batch: 89800028, purity: 98.9 %

Test organism: honeybee (*Apis mellifera carnica*), age 22 - 32 d

Treatments: Contact: 6.25, 12.5, 25, 50, 100 µg a.s./bee (nominal), control: acetone, toxic standard: Perfekthion 0.4 µg a.s./bee

Oral: 6.25 / 5.65, 12.5 / 11.79, 25 / 22.97, 50 / 43.81, 100 / 85.29 µg a.s./bee (nominal / consumed), control: 50 % aqueous sucrose solution with acetone, toxic standard: Perfekthion 0.2 µg a.s./bee

Number of animals: 5 replicates with 10 bees each per treatment

Duration: 48 h of exposure

Test conditions: Feeding: 50 % aqueous sucrose solution; temperature 20 - 28 °C, relative humidity 35 - 90 %

Exposure route: Oral: test substance dissolved in 50 % aqueous sucrose solution

Contact: test substance dissolved in a water/ acetone solution (20:80); topical application on anaesthetised bees

Biological observations: Observations of poisoning symptoms and dead bees at 2 h, 4 h, 24 h and 48 h

Statistical evaluation: mortality in treatment groups corrected according to Schneider-Orelli (1947)

Findings:

Table B.9.4.1-1: Mean mortality of bees (% , not corrected) exposed to cymoxanil in the oral toxicity test

Mortality	Control	5.65 µg a.s./bee	11.79 µg a.s./bee	22.97 µg a.s./bee	43.81 µg a.s./bee	85.29 µg a.s./bee	tox. standard
24h	0	0	0	0	2	0	98
48h	8	0	0	0	2	0	100

Table B.9.4.1-2: Mean mortality of bees (%) exposed to cymoxanil in the contact toxicity test

Mortality	Control	6.25 µg a.s./bee	12.5 µg a.s./bee	25 µg a.s./bee	50 µg a.s./bee	100 µg a.s./bee	tox. standard
24h	0	0	0	0	0	0	72
48h	0	8	0	2	0	2	72

Non-lethal toxicity endpoints: no behavioural abnormalities in treated bees

Conclusions:

LD₅₀ (oral): > 85.29 µg a.s./bee

LD₅₀ (contact): > 100 µg a.s./bee

NOEL (oral): 85.29 µg a.s./bee

NOEL (contact): 100 µg a.s./bee

Plant Protection Product: Cymoxanil/Famoxadon 52.5WG

Reference: Candolfi, M.P. (1996): DPX-KX007-5: Laboratory acute oral and contact toxicity test with the honeybee, *Apis mellifera*, based on the EPPO guideline 170 (1992) and FIFRA guideline 141-1. (Report No.: AMR 3828-96)

Guidelines: EPA 141-1, EPPO 170

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: DPX-KX007 DF, batch: KX007-5, Purity: 30 % cymoxanil and 22.5 % famoxadone

Test organism: honeybee (*Apis mellifera*), age 5 - 12 d

Treatments and exposure route: 12.5, 25, 50, 100, and 200 µg formulation/bee (nominal), oral: test solutions made in 20 % sucrose solutions, contact: test solutions made in 0.1 % Etalfix, an emulsifier; control: 50 % sucrose solutions (oral) and 0.1 % Etalfix (contact), toxic standard: dimethoate 0.16 / 0.18 µg a.s./bee

Number of animals: 3 replicates with 10 bees each per treatment

Duration: 72 h of exposure

Test conditions: Feeding: 20 % aqueous sucrose solution; 25 ± 2°C and 65 ± 15 % humidity

Biological observations: Observations of poisoning symptoms and mortality at 24 h, 48 h and 72 h

Statistical evaluation: effect of treatment tested against control with Fisher exact test

Results:

Table B.9.4.1-3: Acute oral toxicity of DPX-KX007 52.5 WG to honey bees, *Apis mellifera*

Treatment (µg DPX-KX007/bee)	Mean cumulative mortality (%)		
	24 hours	48 hours	72 hours
0	3.3	3.3	6.7
12.5	10.0	16.7	16.7
25	13.3	16.7	20.0
50	6.7	16.7	16.7
100	23.3	30.0	33.3 ^{bc}
186 ^a	13.3	20.0	23.3
Toxic standard (0.18 µg/bee)	60.0	70.0	73.3

^a corrected for actual consumption; all other doses were completely consumed

^b statistically different from control, Fisher exact test, $p \leq 0.05$

^c a single bee showed behavioural effects after 72 h of exposure

Table B.9.4.1-4: Acute contact toxicity of DPX-KX007 52.5 WG to honey bees, *Apis mellifera*

Treatment (µg DPX-KX007/bee)	Mean cumulative mortality (%)		
	24 hours	48 hours	72 hours
0	0	6.7	6.7
12.5	0	0	3.3
25	3.3	3.3	3.3
50	0	3.3	6.7
100	3.3	10.0	13.3
200	0	3.3	6.7
Toxic standard (0.18 µg/bee)	60	70	70

Non-lethal toxicity endpoints: no behavioural abnormalities in treated bees

Conclusions:

72-h acute oral $LD_{50} > 186$ µg product/bee (equivalent to > 55.8 µg cymoxanil/bee), based on actual consumption of the test substance

NOEL (oral) = 50 µg product/bee

72-h acute contact $LC_{50} > 200$ µg product/bee (equivalent to > 60 µg cymoxanil/bee)

NOEL (contact) = 200 µg product/bee

B.9.4.2 Bee brood feeding test (ANNEX IIA 8.3.1.2)

Not required.

B.9.4.3 Residue test (ANNEX IIA 8.3.1.1; ANNEX IIIA 10.4.2)

Not required.

B.9.4.4 Cage test (ANNEX IIIA 10.4.3)

Not required.

B.9.4.5 Field tests (ANNEX IIIA 10.4.4)

Not required.

B.9.4.6 Tunnel test (ANNEX IIIA 10.4.5)

Not required.

B.9.4.7 Summary of effects on bees

Table B.9.4.7-1: Summary of effects of cymoxanil on honeybees

Substance	Media	Species	LC ₅₀	Annex, Reference
cymoxanil	Oral Contact	<i>Apis mellifera</i>	> 85.29 µg a.s./bee * > 100 µg a.s./bee ^x	IIA, 8.3.1 Schur, 1999
Cymoxanil/Famoxadone 52.5WG	Oral Contact	<i>Apis mellifera</i>	> 186 µg formulation/bee * (> 55.5 µg a.s./bee) * > 200 µg formulation/bee ^x (> 60 µg a.s./bee) ^x	IIIA, 10.4.1 Candolfi, 1996

* measured dose ^x nominal dose

B.9.4.8 Risk assessment for bees

Honey-bees may be exposed to formulated cymoxanil by direct spraying of the plant protection product while bees are foraging on flowers and weeds present in or adjacent to the crops treated. They may also be exposed through contact with fresh or dry residues or by oral uptake of contaminated pollen, nectar and honey dew.

As the formulation tested (Cymoxanil/Famoxadone 52.5WG) is not identical with one of the intended formulations, the calculations of hazard quotients were performed using data on the active substance only. There is no indication of the formulation being more toxic than the a.s..

To take into account multiple use scenarios, the RMS usually applies multiple application factors (MAF), as proposed according to Escort 2 for non-target arthropods, in the HQ calculations for bees. However, the EPPO guidance gives no recommendation on this, and in the case of cymoxanil, due to the substance's rapid decline and because it has been shown in residue decline studies that no build-up of residues on crops occurs (see B.7.6.1 and B.9.1.8.2), the use of MAF's is omitted. (furthermore, if MAF's are included, all HQ's still remain < 8).

Table B.9.4.8-1: Risk assessment for bees (*Apis mellifera*) exposed to cymoxanil

Species	Application rate (g as/ha) (MAF)	Media	LD ₅₀ µg a.s./bee	HQ	Annex VI Trigger
lettuce					
Apis mellifera	(4 x) 240	Oral	> 85.29	< 2.8	50
Apis mellifera		Contact	> 100	< 2.4	50
potatoes					
Apis mellifera	(8 x) 175	Oral	> 85.29	< 2.1	50
Apis mellifera		Contact	> 100	< 1.8	50

All hazard quotients for oral and contact toxicity (QH_o and QH_c) are considerably below 50 for the intended uses. Values below the trigger value of 50 indicate a low risk for bees.

B.9.4.8.1 Overall Conclusion:

After application of cymoxanil under the intended representative use conditions in lettuce or potatoes the risk to honeybees is low.

B.9.5 Effects on other arthropod species (Annex IIA 8.3.2, IIIA 10.5)

B.9.5.1 Laboratory, extended laboratory, and semi-field tests (Annex IIA 8.3.2; Annex IIIA 10.5.1)

B.9.5.1.1 Effects on the indicator species *Aphidius rhopalosiphi* and *Typhlodromus pyri* in the laboratory

Plant Production Product: Cymoxanil 50 WP

Reference: Geuijen, W.H.C. (1999a). Effects of Cymoxanil 50 % WP on survival and reproduction of the parasitic wasp *Aphidius rhopalosiphi* in the laboratory (Report No.: 257816)

Test guideline: Polgár (1988), Mead-Briggs (1992)

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: Cymoxanil 50 % WP, Batch V343, Purity: 505 g cymoxanil/kg

Test organism: *Aphidius rhopalosiphi*, < 48 h old adults

Treatments: 960 g formulation/ha, control: water, toxic standard: Asepta Dimethoate 34 g a.s./ha

Number of animals: exposure phase: 4 replicates with 5 males and 5 females each per treatment group; reproduction phase: 1 female/unit, all surviving females

Duration: test duration 2 weeks, exposure duration 48 h, reproduction phase 13 d

Exposure route: contact with freshly dried residues

Test conditions: feeding: solution with honey and water

Environmental parameters: temperature 18 - 22 °C, relative humidity 60 - 85 %, light intensity 500 - 700 lux, 2000 - 3000 lux for reproduction assay

Biological observations: Several assessments for mortality; 24 h after introduction to the reproduction units females were removed and their condition recorded; after 12 d the number of parasitised aphids (mummies) was recorded.

Statistical evaluation: The reproductive capacity was corrected for the control data by the formula of Abbot; total effect calculated according to Overmeer & van Zon (1985).

T-Test for repro data.

Findings:

Table B.9.5.1-1: Mean mortality and fecundity of *Aphidius rhopalosiphi* exposed to cymoxanil.

Treatment (kg product/ha)	Mortality (%)	Corrected mortality (%)	Reproduction (mean no. mummies/ female)	Overall effect E (%)
0 (control)	2.5	-	25.8	-
0.96 kg Cymoxanil 50WP	12.5	10.3	27.4	5 %
toxic standard	100	100	-	100 %

There was no statistically significant difference in number of mummies/females between control and treatment group ($p < 0.05$).

Non-lethal toxicity endpoints: No abnormal behaviour observed in the control or treatment group.

Conclusion:

LR₅₀: > 480 g a.s./ha

Reference: Geuijen, W.H.C. (2000). Effects of Cymoxanil 50 % WP on survival and reproduction of the phytoseiid mite *Typhlodromus pyri* Scheuten (Report No. 257827)

Test guideline: Overmeer (1988), Louis and Ufer (1995), Blümel (1998)

GLP: Yes

Deviations: In the second test, which employed two rates of Cymoxanil 50% WP application, the mean mortality in the dimethoate reference treatment failed to meet the 50 % validity criterion. However, the earlier test performed with a single Cymoxanil 50% WP application rate was valid in this respect and the responses obtained with Cymoxanil 50% WP were broadly similar in both tests.

Validity: Acceptable

Material and methods:

Test substance: Cymoxanil 50 % WP, Batch V343, Purity: 505 g cymoxanil/kg

Test organism: *Typhlodromus pyri*, 4 d old protonymphs

Treatments: study 1: 960 g formulation/ha, study 2: 480 and 960 g formulation/ha, both studies: control: water, toxic standard: Asepta Dimethoate 8 g a.s./ha.

Remark: more experiments were performed, but as these did not meet the reproduction acceptability criterion, they were considered invalid by the study authors and are not reported.

Number of animals: 5 replicates with 20 mites each per treatment group

Duration: test duration 2 / 3 weeks (study 1 / 2)

Exposure route: contact with freshly dried residues

Test conditions: feeding: pollen of pine

Environmental parameters: temperature 25 ± 2 °C, relative humidity 60 - 80 %, light intensity 1200 - 1400 lux

Biological observations: Assessments for mortality, escape and abnormal behaviour on days 1, 2, 5, 7 (relevant assessment for mortality), 8, 12 and 14. Number of eggs and larvae counted on days 7, 8, 12, 14 and 21.

Statistical evaluation: The reproductive capacity was corrected for the control data by the formula of Abbot; total effect calculated according to Overmeer & van Zon (1985).

T-Test for mortality and repro data.

Findings:

Table B.9.5.1-2: Mean mortality and fecundity of *Typhlodromus pyri* exposed to cymoxanil.

Treatment group (kg product/ha)	Mortality (%)*	Corrected mortality (%)*	Reproduction (mean no. eggs/ female)*	Overall effect E (%)
0 (control)	8 / 13	-	3.38 / 3.61	-
0.48 kg Cymoxanil 50WP	- / 21	- / 9.2	- / 0	100 %
0.96 kg Cymoxanil 50WP	4 / 20	0 / 8.1	0 / 0	100 %
toxic standard	100 / 47	100 / 39.1	- / 3.01	nr

* study 1 / 2, nr = not reported

Findings:

There was no statistically significant difference in the mean mortalities between control and treatment groups ($p < 0.05$). An increase in mortality was observed in the second week (after the relevant mortality

Non-lethal toxicity endpoints: It was noted that mites were smaller in the treatment groups compared to the blank control and therefore there were some difficulties to distinguish sexes. Also mites of the treatment groups were described to “look bad” and to show less mobility. In the treatment groups no eggs were produced at all (corrected fertility $R = 0$, statistically significant different from control). To be sure that there is no delayed egg production, the test period of study 2 was prolonged for 1 week.

The LR₅₀ is > 480 g a.s./ha. There was no reproduction at all in cymoxanil treated groups.

Reference: Kuehner, C. (1996b): DPX-KX007, a suspension concentrate (SC) formulation, containing JE874 (90 g/L) and cymoxanil (120 g/L): Acute toxicity to the predatory mite, *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) in the laboratory (Report No. AMR 3530-95)

GLP: Yes

Deviations: none

Validity: Acceptable

Test substance: DPX-KX007 20SC, batch: KX007-4, purity: 120 g cymoxanil/L and 90 g famoxadone/L

Test organism: *Typhlodromus pyri*, protonymphs

Treatments: 1.1 L product/ha (1.21 kg) (132 g cymoxanil/ha); water control; toxic standard: dimethoate 400 g/L formulation at 0.1 L product/ha; calibration was performed

Number of animals: 5 replicates with 20 protonymphs each per treatment

Duration: 1 week (mortality), 1 week (fecundity)

Exposure route: contact with freshly dried residues on glass plate

Test conditions: feeding with pollen; adult mites were transferred to the fecundity assessment (at least one male and 3 females)

Environmental parameters: 24 ± 3 °C, relative humidity 60 ± 15 %, photo period 16 h

Biological observations: the numbers of dead, live, missing, and affected mites were counted at intervals up to eight days after application (21 days in the case of the DPX-KX007 treatment) (until development to adults); the total number of offspring was counted (eggs and larvae)

Statistical / mathematical evaluation: mortality corrected according to Abbott

The average number of offspring per female produced in the control group was 7.5. After eight days exposure, the surviving mites in the DPX-KX007 treatment were still in the larval stage, so their sex could not be determined and the fecundity phase was therefore not initiated. After a further 13 days exposure (Day 21), only one surviving mite was found (sex not determinable). The corrected mortality for DPX-KX007 20SC was 56.8 % after eight days and 98.9 % after 21 days. After eight days, the average escaping rate in the DPX-KX007 treatment was 38 % compared to 10 % in the control, increasing to 48 % after 21 days in the former. Due to this high number of escaped mites, a repellent effect of DPX-KX007 20SC on *T. pyri* is concluded. Because only one mite survived, no mortality assessment was performed for the treatment group.

Table B.9.5.1-3: Effects of DPX-KX007 20SC on survival of *Typhlodromus pyri*

Day	Control			DPX-KX007			Toxic standard		
	A	M	D	A	M	D	A	M	D
4	-	-	-	-	-	-	0	14	86

Day	Control			DPX-KX007			Toxic standard		
	A	M	D	A	M	D	A	M	D
8	88	10	2	38	38	24	-	-	-
15	-	-	-	17	41	42	-	-	-
18	-	-	-	3	48	49	-	-	-
21	-	-	-	1	48	51	-	-	-

Percentage alive (A), missing (M), or dead (D), mean of all replicates

Conclusion:

At a rate of 132 g cymoxanil/ha DPX KX007 20SC led to 98.9 % mortality after 21 days exposure and a 100 % reduction of reproduction.

B.9.5.1.2 Effects on other species than the indicator species *Aphidius rhopalosiphi* and *Typhlodromus pyri* in the laboratory

Plant Production Product: Cymoxanil 50 WP

Reference: Geuijen, W.H.C. (1999b). Effects of Cymoxanil 50% WP on survival of the carabid beetle *Poecilus cupreus* (Report No. 257838)

Test guideline: BBA VI, 23-2.1.8 (1991); IOBC/WPRS (1992)

GLP: Yes

Deviations: none

Validity: Acceptable

Material and methods:

Test substance: Cymoxanil 50 % WP, Batch V343, Purity: 505 g cymoxanil/kg

Test organism: *Poecilus cupreus*, Imagines

Treatments: treatment: 960 g product/ha (480 g a.s./ha nominal); water control; toxic standard: Pyrazofos 294 g a.s./ha; calibration was performed

Number of animals: 5 replicates with 3 male and 3 female beetles each

Duration: 2 weeks

Exposure route: contact with fresh residues

Test conditions: Feeding with pupae of *Musca domestica*

Environmental parameters: 18 - 21 °C, relative humidity 75 - 85 %, photo period 16 h, light intensity 500 - 800 lux

Biological observations: The number of consumed pupae was recorded on days 2, 4, 7, 10 and 14.

Numbers of dead or damaged beetles were recorded regularly.

Statistical evaluation: Mann-Whitney-U-test for effect on food consumption.

Findings:

Table B.9.5.1-4: Mean mortality and fecundity of *Poecilus cupreus* exposed to cymoxanil.

Treatment group (kg product/ha)	Mortality = Corrected mortality (%)	mean pupae consumed/beetle	% reduction in consumption
0 (control)	0	1.3	-
0.96 kg Cymoxanil 50WP	0	1.19	8.3
toxic standard	100	0	100

Non-lethal toxicity endpoints: No abnormal behaviour or mortality was noted in the cymoxanil treated group. In the positive control the majority of beetles were lying on their backs on day 1 and were considered dead on day 2. The reduction in feed consumption of 8.3 % in the cymoxanil treated group was not statistically significant.

Conclusion:

NOAER = 480 g a.s./ha

Reference: Geuijen, W.H.C. (1999c). Effects of Cymoxanil 50% WP on survival and reproduction of the green lacewing *Chrysoperla carnea* (laboratorium test) (Report No. 257849)

Test guideline: Bigler, (1988), Vogt et al., (1998)

GLP: Yes

Deviations: none

Validity: Acceptable for mortality assessment only. The validity criteria for fecundity and fertility (mean no. of eggs/female/d ≥ 15 , mean hatching rate ≥ 70 %) are not met.

Material and methods:

Test substance: Cymoxanil 50 % WP, Batch V343, Purity: 505 g cymoxanil/kg

Test organism: *Chrysoperla carnea*, 2-3 d old larvae

Treatments: treatment: 960 g product/ha (480 g a.s./ha nominal); water control; toxic standard: Dimethoate 18 g a.s./ha; calibration was performed

Number of animals: exposure phase: 30 larvae (= 30 replicates) per treatment/control group, reproduction phase: all emerged adults

Duration: 9 weeks

Exposure route: contact with freshly dried residues

Test conditions: Feeding of larvae 3 x per week with eggs of *Ephestia kuehniella*; Environmental parameters: $23 \pm 2^\circ\text{C}$, relative humidity 70 ± 15 %, photo period 16 h, light intensity > 2000 lux

Biological observations: Mortality recorded daily until pupation. Egg production of adults: Five times per week over 4 weeks no. of eggs counted. Adult mortality and behavioural abnormalities recorded daily. Hatched eggs recorded for 6 - 12 d.

Statistical evaluation: No statistics performed. Egg production data were corrected according to Abbott; the total effect was estimated according to Overmeer & van Zon.

Findings:

Table B.9.5.1-5: Mean mortality (cumulative larval and adult) and fecundity of *Chrysoperla carnea* exposed to cymoxanil.

Treatment group (kg product/ha)	mortality (%)	Corrected mortality (%)	mean no. eggs/female	mean no. viable eggs/female	reduction in benef. capa- city (E%)
0 (control)	6.7	-	10.24	5.53	-
0.96 kg Cymoxanil 50WP	13.3	7.1	9.69	5.04	15.5
toxic standard	83.3	82.1	-	-	-

Mortality in the cymoxanil treated group did not occur during the larval exposure phase, but during pupation or hatching of adults.

Non-lethal toxicity endpoints: Since the number of hatched adults in the toxic standard group was too low, no reproduction phase was started for this group.

One individual of the test substance group pupated late. No other abnormalities were observed. The corrected reproduction factor for Cymoxanil 50WP was 0.91. However, the reproduction assessment is not valid for above mentioned reasons.

Conclusion:

$\text{LR}_{50} > 480$ g a.s./ha

M = 7.1 % at 480 g a.s./ha

Plant Protection Product: Cymoxanil/Famoxadone 20SC

Reference: Kuehner, C. (1995): DPX-KX007, a suspension concentrate (SC) formulation containing JE874 (90 g/L) and cymoxanil (120 g/L): Acute toxicity to the green lacewing, *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae) in the laboratory. (Report No. AMR 3529-95)

Test guideline: Bigler (1988)

GLP: Yes

Deviations: none

Validity: Acceptable

Material and methods:

Test substance: DPX-KX007 20SC, batch: KX007-3, purity: 12 % cymoxanil and 8.3 % famoxadone (analysed)

Test organism: green lacewing *Chrysoperla carnea*, larvae, 2 - 3 d old

Treatments: treatment: 1.1 L product/ha (1.21 kg) (132 g cymoxanil/ha); water control; toxic standard: pyrazophos 294 g/L formulation at 0.8 kg product/ha; calibration was performed

Number of animals: 3 replicates with 15 larvae (kept individually)

Duration: 14 - 21 d (mortality), 4 weeks (fertility)

Exposure route: contact with fresh residues on treated glass plates

Test conditions: feeding with *Sitotroga* eggs

Environmental parameters: 22 ± 2 °C, relative humidity 60 ± 15 %, photo period 16 h

Biological observations: mortality assessment after emergence of adults, fertility test with all emerged adults: egg counting, hatching rate of eggs

Statistical / mathematical evaluation: mortality corrected according to Schneider-Orelli; reduction of reproduction rate calculated according to Abbott; reduction of beneficial capacity calculated according to Overmer & van Zon

Findings:

DPX-KX007 20SC had no effects on pre-imaginal mortality of *C. carnea*. Egg production and fertility per female were slightly reduced (by 17.7 and 17.3 %, respectively, compared to the control) but this was not considered significant by the study author.

Table B.9.5.1-6: Effects of DPX-KX007 20SC on the mortality and reproduction of the green lacewing, *Chrysoperla carnea*

Treatment	Applic. rate	Mean mortality / corrected mortality (%)	Mean number of eggs/female / R (%)	Mean number of fertile eggs/female	Reduction in beneficial capacity (%)
Control	-	8.9 / -	674.4 / -	583.9	-
DPX-KX007 (cymoxanil)	1.1 L/ha (132 g a.s./ha)	2.2 / - 7.3	554.7 / 17.3	483.1	11.2
Pyrazophos	800 g/ha	100 / 100	- / -	-	100

R...reduction in reproduction rate

Conclusion:

DPX-KX007 20SC had no significant effects compared to the control on mortality at an application rate of 1.1 L/ha (132 g cymoxanil/ha). Reproduction and, as a consequence, beneficial capacity of *Chrysoperla carnea* were slightly reduced (< 20 % effect).

Reference: Kuehner, C. (1996a): DPX-KX007, a suspension concentrate (SC) formulation, containing JE874 (90 g/L) and cymoxanil (120 g/L): Acute toxicity to the ground beetle, *Poecilus cupreus* L. (Coleoptera, Carabidae) in the laboratory (Report No. AMR 3528-95)

Test guideline: BBA VI 23-2.1.8 Heimbach (1991)

GLP: Yes

Deviations: none

Validity: Acceptable

Material and methods:

Test substance: DPX-KX007 20SC, batch: KX007-4, purity: 120 g cymoxanil/L and 90 g famoxadone/L

Test organism: *Poecilus cupreus*, Imagines, 8 - 9 weeks old

Treatments: 1.1 L product/ha (1.21 kg) (132 g cymoxanil/ha); water control; toxic standard: pyrazophos 294 g/L formulation at 0.8 kg product/ha; calibration was performed

Number of animals: 5 replicates with 3 males and 3 females each

Duration: 14 days

Exposure route: contact with fresh residues on quartz sand

Test conditions: feeding with *Musca* pupae

Environmental parameters: 20 ± 2 °C; relative humidity 60 ± 10 %; 16 h photo period

Biological observations: mortality, behaviour and food consumption were recorded at intervals

Statistical / mathematical evaluation: mortality corrected according to Schneider-Orelli; reduction of feeding capacity calculated according to Abbott

Results:

Table B.9.5.1-7: Summary of effects of DPX-KX007 on the ground beetle (*Poecilus cupreus*) after 14 days exposure

Treatment	Application rate	Corrected mortality (%)	Average feeding rate (pupae/beetle)	F (%)
Control	-	0	5.10	-
DPX-KX007 (cymoxanil)	1.1 L/ha (132 g a.s./ha)	0	5.23	-2.61
tox.standard	800 g/ha	90	1.78	65.14

F ... feeding capacity

Conclusion:

DPX-KX007 20SC had no effect on either mortality or feeding rate of *P. cupreus* at an application rate of 1.1 L DPX-KX007/ha (132 g cymoxanil/ha).

Plant Protection Product: Cymoxanil/Famoxadone 50 WG

Reference: Kuehner, C. (1997a): DPX-KP481, a water-dispersible granular (WG) formulation, containing JE874 (254 g ai/kg) and cymoxanil (266 g ai/kg): Acute toxicity to the hoverfly, *Episyrphus balteatus* Deg. (Diptera, Syrphidae) in the laboratory (Report No. AMR 4232-96)

Test guideline: Rieckmann (1989) (method for *Syrphus corollae*)

GLP: Yes

Deviations: none

Validity: Acceptable

Material and methods:

Test substance: DPX-KP481 50WG, batch: KP481-25, purity: 25 % cymoxanil and 25 % famoxadone

Test organism: *Episyrphus balteatus*, 2 - 3 d old larvae

Treatments: treatment: 700 g DPX-KP481 50WG/ha (186.2 g cymoxanil/ha based on analysed percentage of 26.6 % cymoxanil); water control; toxic standard: pyrazophos 294 g/L formulation 800 g/ha; calibration was performed

Number of animals: 10 replicates with 6 larvae each per treatment

Duration: 22 d

Exposure route: contact with freshly dried residues on glass

Test conditions: ventilated glass petri dishes, feeding with aphids

Environmental parameters: 20 ± 3°C; relative humidity 60 ± 15 %; 16 h photo period

Biological observations: Total larval mortality was assessed daily until pupation. Pupae were transferred to plastic vessels and adult emergence recorded. The adults were sexed and transferred to a fertility cage (one per treatment), and counts made every 3-4 days of the number of eggs per female for a two-week period (from when the females started to oviposit). A maximum of 100 eggs were transferred to Petri dishes, filled with water on each evaluation date, and hatching monitored.

Statistical /mathematical evaluation: mortality corrected according to Schneider-Orelli, reproduction rate according to Abbott; total effect according to Overmer & van Zon

Findings:

DPX-KP481 50WG had no significant effects on pre-imaginal mortality of *E. balteatus*. Egg production per female was increased by 38.7 % compared to the control, but fertility (fertile eggs per female) showed a reduction of 33.5 % compared to the control. The number of males in the DPX-KP481 50WG treatment was much lower than in the control group (13 and 20, respectively), which may have contributed to the relatively low fertility of the eggs. Reproduction data in syrphid tests generally tend to show a high variability, and the study authors pointed out that the result for DPX-KP481 50WG was within the variability of historical control data from the test facility.

Table B.9.5.1-8: Effects of DPX-KP481 50WG on the mortality and reproduction of *Episyrphus balteatus*

Treatment	Applic. rate	Mean mortality / corrected mortality (%)	Mean number of eggs/female	Mean no. fertile eggs/female	Reduction in reproduction rate (%)	Reduction in beneficial capacity (%)
Control	-	13.3 / -	102.5	68.4	-	-
DPX-KP481 50WG (cymoxanil)	700 (186.2)	15.0 / 1.2	142.2	45.5	33.5	33.8
Pyrazophos form.	800 g/ha	100 / 100	-	-	-	100

Conclusion:

DPX-KP481 50WG had no significant effect on mortality at an application rate of 700 g DPX-KP481 50WG/ha (186.2 g cymoxanil/ha), but reproduction and the overall beneficial capacity of *Episyrphus balteatus* were reduced by 33.5 and 33.8 %, respectively.

Reference: Kuehner, C. (1997b): DPX-KP481, a water-dispersible granular (WG) formulation, containing JE874 (254 g ai/kg) and cymoxanil (266 g ai/kg): Acute toxicity to the ground beetle, *Poecilus cupreus* L. (Coleoptera, Carabidae) in the laboratory (Report No. AMR 4233-96)

Test guideline: BBA VI 23-2.1.8 Heimbach (1991)

GLP: Yes

Deviations: none

Validity: Acceptable

Material and methods:

Test substance: DPX-KP481 50WG, batch: KP481-25, purity: 26.6 % cymoxanil and 25.4 % famoxadone (analysed)

Test organism: *Poecilus cupreus*, Imagines, 3 - 4 weeks old

Treatments: treatment: 700 g test substance/ha (186.2 g cymoxanil/ha); water control; toxic standard: pyrazophos 240 g a.s./ha; calibration was performed

Number of animals: 5 replicates with 3 male and 3 female each

Duration: 14 days

Exposure route: contact with fresh residues on quartz sand

Test conditions: feeding with *Musca* pupae

Environmental parameters: 20 ± 2 °C; relative humidity 60 ± 10 %; 16 h photo period

Biological observations: mortality, behaviour and food consumption were recorded at intervals

Statistical / mathematical evaluation: mortality corrected according to Schneider-Orelli; reduction of feeding capacity calculated according to Abbott

Findings:

Beetles in the control and test substance group showed normal activity.

Table B.9.5.1-9: Summary of effects of DPX-KP481 50WG on the ground beetle (*Poecilus cupreus*) after 14 days exposure

Treatment	Application rate	Corrected mortality (%)	Average feeding rate (pupae/beetle)	R (%)
Control	---	0	5.50	-
DPX-KP481 50WG (cymoxanil)	700 (186.2)	0	5.47	0.61
tox.standard	800 g/ha	100	0.50	90.91

R ... feeding capacity

Conclusion:

DPX-KP481 50WG had no effect on either mortality or feeding rate of *P. cupreus* at an application rate of 700 g/ha (186 g cymoxanil/ha).

Reference: Kuehner, C. (1997c): DPX-KP481, a water-dispersible granular (WG) formulation, containing JE874 (254 g ai/kg) and cymoxanil (266 g ai/kg): Acute toxicity to the green lacewing, *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae) in the laboratory (Report No AMR 4234-96)

Test guideline: Bigler (1988)

GLP: Yes

Deviations: none

Validity: Acceptable

Material and methods:

Test substance: DPX-KP481 50WG, batch: KP481-25, purity: 26.6 % cymoxanil and 25.4 % famoxadone (analysed)

Test organism: green lacewing *Chrysoperla carnea*, larvae, 2 - 3 d old

Treatments: treatment: 700 g test substance/ha (186.2 g cymoxanil/ha); water control; toxic standard: pyrazophos 240 g a.s./ha; calibration was performed

Number of animals: 3 replicates with 15 larvae (kept individually)

Duration: 14 - 21 d (mortality), 4 weeks (fertility)

Exposure route: contact with freshly dried residues on glass plates

Test conditions: feeding with *Sitotroga* eggs until pupation

Environmental parameters: 22 ± 2 °C, relative humidity 60 ± 15 %, photo period 16 h

Biological observations: mortality assessment after emergence of adults, fertility test with all emerged adult: egg counting, hatching rate of eggs

Statistical / mathematical evaluation: mortality corrected according to Schneider-Orelli; reduction of reproduction rate calculated according to Abbott; reduction of beneficial capacity calculated according to Overmer & van Zon

Findings:

DPX-KP481 50WG had no effect on pre-imaginal mortality of *C. carnea* compared to the control. Egg production and fertility per female were slightly reduced (by 2.2 and 4.1 %, respectively, compared to the control) but this was not considered significant.

Table B.9.5.1-10: Effects of DPX-KP481 50WG on the mortality and reproduction of the green lacewing, *Chrysoperla carnea*

Treatment	Applic. rate	Mean mortality / corrected mortality (%)	Mean number of eggs/female / R (%)	Mean number of fertile eggs/female	Reduction in beneficial capacity (%)
Control	-	20.0 / -	139.4 / -	128.5	-
DPX-KP481 50WG (cymoxanil)	700 g/ha (186.2g/ha)	13.3 / - 8.4	136.4 / 4.1	123.2	- 4.1
Pyrazophos	800 g/ha	100 / 100	- / -	-	100

R...reduction in reproduction rate

Conclusion:

DPX-KP481 50WG had no significant effects compared to the control on either mortality, reproduction, or beneficial capacity of *Chrysoperla carnea* at an application rate of 700 g/ha (186.2 g cymoxanil/ha).

Reference: Kuehner, C. (1997d): DPX-KP481, a water-dispersible granular (WG) formulation containing DPX-JE874 (254 g ai/kg) and cymoxanil (266 g ai/kg): Acute toxicity to the staphylinid beetle, *Aleochara bilineata* (Coleoptera, Staphylinidae) in the laboratory (Report No. AMR 4235-96)

Test guideline: Naton (1988) modified according to Heimbach (1991)

GLP: Yes

Deviations: none

Validity: Acceptable

Material and methods:

Test substance: DPX-KP481 50WG, batch: KP481-25, purity: 26.6 % cymoxanil and 25.4 % famoxadone (analysed)

Test organism: rove beetle *Aleochara bilineata*, 2 - 5 d old larvae

Treatments: treatment: 700 g test substance/ha (186.2 g cymoxanil/ha); water control; toxic standard: pyrazophos 240 g a.s./ha; calibration was performed

Number of animals: 3 replicates with 10 pairs each

Duration: 10 weeks

Exposure route: contact with fresh residues on treated sand

Test conditions: plastic vessels filled with quartz sand, feeding with *Chironomus* larvae

Environmental parameters: 20 ± 2 °C (hatching 18 - 25 °C), relative humidity 60 ± 15 %, photo period 16 h

Biological observations: after 7, 14 and 21 d of exposure *Delia* pupae were mixed with the sand, which were collected on day 29 for the parasitisation assessment; adult beetles counted at end; determination of hatching rate

Statistical / mathematical evaluation: mortality corrected according to Schneider Orelli; reduction of reproduction rate according to Abbott

Findings:

DPX-KP481 50WG resulted in a corrected mortality of 7.5 % compared to control. The parasitic capacity, which is expressed as the mean hatching rate of the offspring, was 49.4 % for the DPX-KP481 50WG treatment compared to 50.7 % in the control group and 0.4 % in the toxic standard. The reduction in reproduction rate of the beetles was 2.6 % compared to the control, which can be considered harmless.

Table B.9.5.1-11: Effects of DPX-KP481 50WG on the staphylinid beetle, *Aleochara bilineata* in the laboratory

Treatment	Rate (g product /ha)	Cymoxanil equivalent (g a.s./ha)	Mortality (%)	Corrected mortality (%)	Reduction in parasitic capacity (%)
Control	0	0	11.7	-	-
DPX-KP481 50WG	700	186.2	18.3	7.5	2.6
Toxic Standard	800	-	100	100	99.2

Conclusion:

DPX-KP481 50WG had no detrimental effects on *Aleochara bilineata* at 700 g product/ha (186.2 g cymoxanil/ha) under worst-case laboratory conditions.

B.9.5.1.3 Extended laboratory studies, aged residue studies and semi-field studies

Plant Production Product: Cymoxanil/Famoxadone 20 SC

Reference: Mead-Briggs, M. (1996): DPX-KX007 suspension concentrate (SC) formulation containing DPX-JE874 (90 g/L) and cymoxanil (120 g/L): An extended laboratory test to evaluate the effects on the parasitic wasp *Aphidius rhopalosiphi* when applied in up to six serial applications to tomato plants (Report No. AMR 3593-95)

Test guideline: not stated

GLP: Yes

Deviations: none

Validity: Acceptable

Material and methods:

Test substance: DPX-KX007 20SC, batch: KX007-3, purity: 120 g cymoxanil/L and 90 g famoxadone/L

Test organism: *Aphidius rhopalosiphi*, adult, < 3 d old

Treatments: treatment: DPX-KX007 20SC at a rate of 2.2 L/ha (264 g cymoxanil/ha) up to six times (1, 3, 6 x; 7 d interval); water control; toxic standard: Dimethoate 40 EC at 0.85 L product/ha; calibration was performed

Number of animals: 3 replicates with 5 females each

Duration: 48 h (mortality); 10 d (fecundity)

Exposure route: contact with freshly dried residues on tomato plants

Test conditions: fecundity assessment: ten wasps were recovered from the arenas and individually confined over pots with barley infested with aphids; after 24 h adults were removed and the parasitised aphids were left to develop for 10 d; spraying with a fructose solution

Environmental parameters: 18 - 21°C, photo period 16 h, light intensity ca. 5600 lux

Biological observations: mortality, behavioural effects, number of mummies

Statistical evaluation: t-test

Findings:

There was a small increase in the number of wasps dying in the 3x and 6x regimes (five and four wasps, respectively, compared to two wasps each in the comparable controls). There were almost no records of wasps settled on plants within the first 2.5 h of exposure after any of the treatments. The results of the behavioural observations did not allow any conclusions to be drawn regarding treatment-induced repellency. The mean number of mummies produced in the 1x, 3x, and 6x regimes of DPX-KX007 20SC were 34, 35, and 23 % less than the respective controls; this was only significant for the 1x application ($p < 0.05$). All of the insects were able to reproduce, and it was suggested that the observed differences in mummies may have reflected the large variability in the fecundity of individuals of this species.

Table B.9.5.1-12: Effects of DPX-KX007 20SC on mortality and reproduction of *Aphidius rhopalosiphi*

Treatment Application regime	Mortality (%)			mean no. mummies/female (R %)		
	1x	3x	6x	1x	3x	6x
Control	0	13	13	25.7	13.0	8.4
DPX-KX007 20SC 2.2 L/ha ¹	0	33	25	17.0 (34)	8.5 (35)	6.5 (23)
Dimethoate 40EC	100	100	100	- (100)	- (100)	- (100)

¹ 264 g cymoxanil/ha

R...reduction in reproduction

Conclusion:

At rates up to 6 times 264 g cymoxanil/ha DPX-KX007 20SC led to a maximum of 33 % mortality (compared to 13 % in the controls) and a 35 % reduction in fecundity in *Aphidius rhopalosiphi*. It is concluded that DPX-KX007 20SC presents a relatively low risk to *A. rhopalosiphi* populations.

Reference: Grove, A.J. (1996a): Effects of DPX-KX007-03 on juvenile survival, reproduction and egg hatch success of *Typhlodromus pyri* (Acari: Phytoseiidae), following multiple applications on grapevine leaf: Extended laboratory test (Report No. AMR 3571-95)

Test guideline: Oomen (1988), ESCORT (1994)

GLP: Yes

Deviations: none

Validity: Acceptable

Material and methods:

Test substance: DPX-KX007 20SC, batch: KX007-3, purity: 120 g cymoxanil/L and 90 g famoxadone/L

Test organism: *Typhlodromus pyri*, larvae to protonymphs

Treatments: five spray regimes, with vines being used for bioassays after they had been treated one, three, or six times (experiment 1) and nine or 12 times (experiment 2) at weekly intervals at 1 L product/ha (120 g cymoxanil/ha nominal, ca. 0.01 % product solution sprayed to run-off); water control; toxic standard: Permethrin 250 g/L EC at 0.2 mL product/L to run-off

Number of animals: 5 replicates per treatment with 5 individuals per leaf

Duration: 1 week mortality assessment + 1 week reproductive assessment

Exposure route: contact with fresh residues on vine leaves

Test conditions: Potted grapevines were sprayed and maintained under outdoor conditions (allowing natural weathering of the residues); feeding with pollen of *Vicia faba*; on the last day of treatment five leaves were removed from each vine (six vines/replicate per treatment) for the mortality assessment; surviving females were transferred to a second set of leaves for the assessment of egg production; for each treatment 2 cohorts of max. 30 eggs per plant were isolated for the determination of egg hatch success (only experiment 2)

Environmental parameters: approx. 25 °C, relative humidity 60 %

Biological observations: mortality, egg production and egg hatch success

Statistical evaluation: one-way analysis of variance

Findings:

Control mortality including escaped nymphs was 20 and 24.7 % for experiment 1 and 2. Treatments with DPX-KX007 20SC resulted in increases in juvenile mortality ranging from about 20 to 40 %, and some of these differences were significant (3x + 6x treatment groups compared with the 6x control and 9x + 12x treatment groups compared with the 12x control, $p < 0.05$). However, there was no clear relationship between mortality and the number of applications. No significant differences were observed in egg production or hatching success of eggs between the control and DPX-KX007 20SC treated groups.

The overall reduction in beneficial capacity (E) for the various DPX-KX007 20SC treatments, combining juvenile mortality and reproduction data, ranged from 16.5 % (12 applications) to 28.6 % (three applications). There was no relationship between the number of applications and the magnitude of effect.

Table B.9.5.1-13: Effects of DPX-KX007 20SC on mortality, sublethal toxicity, and reproduction of *Typhlodromus pyri*

Test group	No. applications	Average mortality ^a (%)	Average oviposition ^b (eggs/female/2 d)	Proportion of hatched eggs ^c	Reduction in benefic. capacity (E)
Control	6	-	2.492	-	-
	12	-	2.353	0.907	-
DPX-KX007	1	22.5	2.410	-	24.8
20SC	3	40.0	2.973	-	28.6
	6	37.5	2.895	-	27.5
	9	23.9	2.362	0.879	23.9
	12	21.2	2.498	0.875	16.5
Permethrin	1	100	-	-	100

^a Mortality corrected with Abbott's formula

^b First and second cohorts pooled

^c Cohorts pooled

Conclusion:

When exposed to the accumulated residues of DPX-KX007 20SC after up to 12 applications of 1 L product/ha, the beneficial capacity of the predatory mite *Typhlodromus pyri* was reduced by between 16.5 % (12 applications) and 28.6 % (three applications). There was no relationship between any of the effects measured (mortality, egg production, hatching success, and beneficial capacity) and the number of applications.

Reference: Grove, A.J. (1996b): Extended laboratory test comparing the effects of DPX-KX007 (containing DPX-JE874 and cymoxanil) WG and SC formulations on *Typhlodromus pyri*, following multiple applications on grapevine leaves under greenhouse conditions (Report No. AMR 3812-96).

Test guideline: not stated

GLP: Yes

Deviations: none

Validity: Acceptable

Material and methods:

Test substance: DPX-KX007 20SC, DPX-KX007 52.5WG, batch: KX007-3 (SC), KX007-5 (WG), purity: 120 g cymoxanil/L and 90 g famoxadone/L (KX007-3)
30 % cymoxanil and 22.5 % famoxadone (KX007-5)

Test organism: *Typhlodromus pyri*, larvae to protonymphs

Treatments: five treatments: DPX-KX007 20SC at a nominal concentration of 1.1 mL product/L (159 mg cymoxanil/L, based on analysed content), DPX-KX007 52.5WG at nominal concentrations of 440 and 880 mg product/L (134 and 268 mg cymoxanil/L, based on analysed content); water control; toxic standard: Permethrin 250 g/L EC at 0.2 mL product/L; all sprayed to run-off; the products were sprayed one, three, or six times at weekly intervals

Number of animals: per treatment 3 replicates with 5 leaf discs, 5 *T. pyri* per leaf disc

Exposure route: contact with fresh residues on vine leaves

Test conditions: Potted grapevines were sprayed and maintained under greenhouse conditions; feeding with pollen of *Vicia faba*; on the last day of treatment five leaves were removed from each vine (six vines/replicate per treatment) for the mortality assessment; surviving females were transferred to a

second set of leaves for the assessment of egg production; for each treatment 2 cohorts of max. 30 eggs per plant were isolated for the determination of egg hatch success

Environmental parameters: 25 °C, relative humidity 62 - 76 %, photo period 14 h

Biological observations: mortality (day 3 and 7), counting of females and eggs on days 7, 10, 12 and 14, egg hatch success

Statistical evaluation: 3-way ANOVA, Tukey's multiple comparison test

Findings:

While all mites in the controls had reached adulthood and oviposition had started at the end of the first week, the majority of mites in the DPX-KX007 treated replicates were still juvenile. In the DPX-KX007 WG (134 mg cymoxanil/L), WG (268 mg cymoxanil/L), and SC treatments, 58, 75, and 67 % of the mites identified during the second week, respectively, were still juvenile (most mites did not develop beyond the deutonymphal stage). In addition, the sex ratio in both WG treatments was extremely male biased (65 and 70 % in males for the 134 and 268 mg cymoxanil/L treatments, respectively, compared with 32 % in the controls and 25 % in the SC treatment). Egg production and hatching success of eggs showed marked reductions in all the DPX-KX007 treatments with only the SC replicates producing any significant number of eggs (see table 9.5.1.3-3 below). The overall reduction in beneficial capacity (E) for the various DPX-KX007 treatments, combining juvenile mortality and reproduction data, ranged from 65 (1x, SC) to 100 % (3x and 6x 134 mg cymoxanil/L WG, and all applications of 268 mg cymoxanil/L WG). There was no relationship between any of the effects measured (mortality, egg production, hatching success, and beneficial capacity) and the number of applications.

Table B.9.5.1-14: Effects of DPX-KX007 SC and WG on mortality, sublethal toxicity, and reproduction of *Typhlodromus pyri*

Test group	No. applications	Average mortality (%)	Average oviposition ^b (eggs/female)	Proportion of hatched eggs	Reduction in benefic. capacity (E)
Control	6	15.6	6.52 (n=6)	0.88	-
DPX-KX007	1	25.3 ^a	2.72 (n=4)	0.88	65
SC	3		1.19 (n=3)	0/4 ^c	89
	6		1.49 (n=3)	6/11 ^c	82
DPX-KX007	1	34.7 ^a	1.50 (n=3)	4/5 ^c	85
WG	3		-	-	100
(0.044%)	6		-	-	100
DPX-KX007	1	42.4 ^a	-	-	100
WG (0.088%)	3		-	-	100
	6		-	-	100
Permethrin	1	99.3	-	-	100

^a test product mortality corrected with Abbott's formula. Data pooled for number of applications (1x, 3x, and 6x).

^b Total eggs per female during second week of bioassay

^c Numbers of eggs hatched/total produced (too small for analysis)

Conclusion:

When exposed to the accumulated residues the beneficial capacity of the predatory mite, *Typhlodromus pyri*, was reduced by between 65 % (DPX-KX007 20SC at 159 mg cymoxanil/L) to 100 % (DPX-KX007 WG at 134 mg cymoxanil/L, applied 3- and 6-times, and after 1, 3, and 6 applications at 268 mg cymoxanil/L).

Reference: Nengel, S. (1996a): Assessment of side effects of DPX-KX007 20 SC on the hoverfly, *Episyrphus balteatus* DEG. (Diptera, Syrphidae): Extended lab test (Report No. AMR 3569-95)

Test guideline: BBA VI 23-2.1.7 Rieckmann (1989)

GLP: Yes

Deviations: none relevant

Validity: Acceptable

Material and methods:

Test substance: DPX-KX007 20SC, batch: KX007-4, purity: 120 g cymoxanil/L and 90 g famoxadone/L

Test organism: *Episyrphus balteatus*, larvae, 2 - 3 d old

Treatments: treatment: DPX-KX007 20SC at 2.2 L/ha (289 g cymoxanil/ha) applied one, three, or six times on bean plants (10 - 14 d intervals) outdoors; water control; toxic standard: triazophos - 0.6 L Hostathion/ha; calibration was performed

Number of animals: 4 replicates with 30 larvae per treatment

Duration: mortality until pupation; fertility 3 weeks

Exposure route: contact with freshly dried residues on bean plants

Test conditions: exposure unit: plastic container containing at least three aphid infested broad bean plants; feeding with aphids, imagines with honey solution and pollen

Environmental parameters: fertility test: 18 - 27°C, relative humidity 20 - 80 %, photo period 16 h, light intensity 5000 - 8500 lux

Biological observations: pre-imaginal mortality; number and sex of emerging imagines; fertility: egg number and hatching rate

Statistical / mathematical evaluation: mortality calculated according to Schneider-Orelli, fertility according to Abbott, total effect according to Overmer & van Zon

Findings:

DPX-KX007 20SC had no effects on pre-imaginal mortality of *E. balteatus*, when compared to the control, when applied up to six times at an application rate of 2.2 L/ha. Egg production per female in the DPX-KX007 treatment regimes showed a reduction compared to the control, but this difference was relatively less for the number of fertile eggs. Overall, the reproduction rate in the DPX-KX007 treatment regimes was reduced by up to 33.4 % although this showed no increase with the number of applications.

Table B.9.5.1-15: Effects of DPX-KX007 20SC on the mortality and reproduction of the hoverfly *Episyrphus balteatus*

Treatment	No. applications	Mean pre-imaginal mortality (%)	Mean number of eggs/female	Mean no. of fertile eggs/female
Control	1x	31.1	346	115.1
DPX-KX007 20SC (cymoxanil)	1x	31.8	152	76.6
	3x	28.8	178	84.8
	6x	30.8	165	88.4
Triazophos	1x	68.9	162	0

Table B.9.5.1-16: Summary of effects of DPX-KX007 20SC on *Episyrphus balteatus*

Treatment	No. applications	Corrected mortality (%)	Reduction in reproduction rate (%)	Reduction in benefic. capacity (%)
DPX-KX007 20SC (cymoxanil)	1x	1.1	33.4	34.2
	3x	-3.4	26.3	23.8
	6x	-0.3	23.2	23.0
Triazophos	1x	54.9	100	100

Conclusion:

DPX-KX007 20SC had no significant effect on mortality at an application rate of 2.2 L/ha (264 g cymoxanil/ha) applied up to six times, but reproduction and the overall beneficial capacity of *Episyrphus balteatus* were reduced by up to 33.4 and 34.2 %, respectively.

Reference: Nengel, S. (1996b): Assessment of side effects of DPX-KX007 20SC on the ground beetle, *Poecilus cupreus* L. (Coleoptera, Carabidae): Semi-field test (Report No. AMR 3570-95)

Test guideline: Heimbach (1991)

GLP: Yes

Deviations: none

Validity: Acceptable

Material and methods:

Test substance: DPX-KX007 20SC, batch: KX007-3, purity: 120 g cymoxanil/L and 90 g famoxadone/L

Test organism: *Poecilus cupreus*, Imagines

Treatments: treatment: DPX-KX007 20SC at a rate of 2.2 L/ha (289 g cymoxanil/ha) one, three, or six times 10 - 14 days apart; water control; toxic standard: chlorpyrifos 485.9 g a.s./ha; calibration was performed

Number of animals: 4 replicates with 7 males and 7 females each per treatment

Duration: 14 days mortality + 7 d feeding capacity

Exposure route: contact with fresh residues on soil

Test conditions: beetles were released within square metal frames (0.5 m square and 25 cm high) enclosing a silty sand soil in which two potato plants were grown; feeding with fly pupae

Environmental parameters: average temperature 11.7 - 20.3°C, relative humidity 60 - 92.8 %

Biological observations: mortality, behaviour, food consumption

Statistical / mathematical evaluation: mortality calculated according to Schneider-Orelli, feeding capacity according to Abbott

Findings:

Average mortality in the DPX-KX007 treatment regimes reached a maximum of 23.2 %. There was no consistent increase with the number of applications, as the lowest mortality in the DPX-KX007 treatments occurred after six applications (12.5 %). Similarly, the greatest reduction in food consumption compared to the control occurred after the single application regime (19.5 %) with an increase of 7.8 % being found after three applications. The beetles in the control and DPX-KX007 treatments showed normal activity during the 14-day observation period.

Table B.9.5.1-17: Summary of effects of DPX-KX007 20SC on the ground beetle (*Poecilus cupreus*) after 14 days exposure under semi-field conditions

Treatment	Control	DPX-KX007 20SC (cymoxanil)			Toxic standard
		1x	3x	6x	
Mean mortality (%)	5.4	11.3	23.2	12.5	43.4
Corrected mortality (%)	-	11.3	18.9	7.5	43.4
Food consumption (%)	100	80.5	107.8	92.5	50.3
Reduced feeding capacity (%)	-	19.5	-7.8	7.5	49.7

Conclusion:

DPX-KX007 20SC had little effect on either mortality or feeding rate of *P. cupreus* (< 25 % reduction compared to the control) at an application rate of 2.2 L DPX-KX007 20SC/ha (289 g cymoxanil/ha) applied up to six times.

Plant Production Product: Cymoxanil/Famoxadone 50 WG

Reference: Beech, P., Mead-Briggs, M. (1998): Cymoxanil/Famoxadone (DPX-KP481) 50 WG (25%:25%). An extended laboratory study to evaluate the effects on the ground beetle, *Poecilus cupreus*. (Report No. AMR 4539-97)

Guidance: SETAC-ESCORT (1994)

GLP: Yes

Deviations: none

Validity: Acceptable

Material and methods:

Test substance: Cymoxanil/Famoxadone (DPX-KP481) 50 WG, batch not stated, 26.6 % w/w cymoxanil and 25.4 % w/w famoxadone

Test organism: *Poecilus cupreus*, Imagines, age 4 - 6 weeks

Treatments: treatment: 4 different bioassays with 1, 4, 6 and 9 applications at 700 g product/ha with a 10 d interval (186.2 g cymoxanil/ha); water control; toxic standard: Dimethoate 40 at 0.85 L product/ha

Number of animals: 5 replicates with 3 male and 3 female each (30/treatment)

Duration: 7 d

Exposure route: contact with sprayed soil

Test conditions: Test arenas containing a 4-cm-deep layer of LUFA 2.2 soil were dug into the soil of test plots beneath potato rows, foliage directly above the arenas was removed before applications; appl.s 6 - 9 were made without crop cover; feeding with fly pupae

Environmental parameters: 19 - 23 °C, photo period 16 h, light intensity 5833 lux

Biological observations: Bioassays were carried out after the 1st, 4th, 6th and 9th applications where the condition of the beetles and feeding activity were recorded

Statistical evaluation: t-test

Findings:

Table B.9.5.1-18: Summary of the effects of 0.7 kg/ha DPX-KP481 50WG on *Poecilus cupreus* after exposure under semi-field conditions

Number of appl.s prior to bioassay	Control		DPX-KP481 50WG		Toxic reference	
	% Mortality (7 days)	Mean no. fly pupae eaten	% Mortality (7 days)	Mean no. fly pupae eaten	% Mortality (7 days)	Mean no. fly pupae eaten
1	0	2.87	0	2.96	100	0.07
4	0	5.64	0	4.83	100	0.43
6	0	3.54	0	3.84	90	1.25
9	0	3.86	0	4.03	60	4.27

Non-lethal toxicity endpoints: No abnormal behaviour or mortality was noted in the test product treated group. None of the DPX-KP481 50WG treatments resulted in statistically significant effects as compared to the control on any test date (t-tests, $p > 0.05$).

Conclusion:

DPX-KP481 50WG, when applied up to 9 times at 0.7 kg product/ha (186.2 g cymoxanil /ha) under semi-field conditions, can be considered harmless to *Poecilus cupreus*.

Reference: Thompson, B. (1999a): Cymoxanil/Famoxadone (DPX-KP481) 50 WG (25%:25%) an extended laboratory study to evaluate the effects on the hoverfly, *Episyrphus balteatus* (Report No. AMR 4541-97)

Test guideline: Rieckmann (1989)

GLP: Yes

Deviations: none

Validity: The first test run with 3-fold application is considered not valid because of high control mortality (however, no validity criteria are given in the guideline). Second test run with 6-fold application acceptable.

Material and methods:

Test substance: DPX-KP481 50WG, batch: KP481-25, purity: 25 % cymoxanil and 25 % famoxadone

Test organism: *Episyrphus balteatus*, 3 - 4 d old larvae

Treatments: treatment: DPX-KP481 50WG 0.7 kg product/ha (186.2 g cymoxanil/ha) applied 3 or 6 times with 10 - 14 d intervals; water control; toxic standard: Dimethoate 40 at 0.85 L product/ha; calibration was performed

Number of animals: 30 larvae (3x treatment) and 50 larvae (6x treatment) per treatment kept individually

Duration: mortality until pupation, 3 weeks fecundity

Exposure route: contact with freshly dried residues on potato leaves

Test conditions: test arenas containing potato leaves; pupae transferred to separate containers until emergence of adults; in fecundity boxes aphid infested bean leaves provided for oviposition; feeding with aphids, adults with pollen and honey solution

Environmental parameters: 20 - 25°C, relative humidity 47 - 67 %, photo period 16 h, light intensity 1000 - 4000 lux

Biological observations: pre-imaginal mortality, activity, egg production, egg viability

Statistical / mathematical evaluation: mortality corrected according to Abbott; one-way ANOVA

Findings:

Residues of DPX-KP481 50WG resulted in corrected mortality of 0 % after 3 applications and 7 % after 6 applications. However, the first test run is considered not valid, because of the high control mortality of 64 %. Due to the low number of females evaluated for some treatments, and the variability associated with *E. balteatus* fecundity, the level of effect is difficult to quantify. It is noted that many viable eggs were laid by DPX-KP481 50WG treated insects. Since no trend of increasing effect with greater numbers of applications was observed, it is unlikely that greater effect would be observed following 9 applications as originally planned.

Table B.9.5.1-19: Pre-imaginal mortality and reduction in reproduction of *Episyrphus balteatus* after exposure to field-aged residues of DPX-KP481 50WG on potato leaves following multiple applications

No. of applications	Parameter	% Effect		
		DPX-KP481 50WG 0.7 kg/ha	toxic standard	Untreated control
3	Pre-imaginal mortality	53	100	64
	Corrected mortality	0	100	-
	% reduction in eggs/female/day	49	100	-
	% reduction in viable eggs/female/day	69	100	-
6	Pre-imaginal mortality	22	100	16
	Corrected mortality	7	100	-
	% reduction in eggs/female/day	28	100	-
	% reduction in viable eggs/female/day	50	100	-

Conclusion:

DPX-KP481 50WG at 0.7 kg/ha resulted in corrected mortality of 7 % following 6 applications and in a reduction in eggs/female of 28 %.

Reference: Thompson, B. (1999c): Cymoxanil/Famoxadone (DPX-KP481) 50 WG (25%:25%). An extended laboratory study to evaluate the effects on the green lacewing, *Chrysoperla carnea* (Report No. AMR 4540-97)

Test guideline: Bigler (1988) (adapted)

GLP: Yes

Deviations: none

Validity: Acceptable

Material and methods:

Test substance: DPX-KP481 50WG, batch: KP481-25, purity: 25 % cymoxanil and 25 % famoxadone

Test organism: *Chrysoperla carnea*, larvae, 3 - 4 d old

Treatments: treatment: 0.7 kg test substance/ha 1, 3 and 6 times with a 10 d interval (179 g cymoxanil/ha based on measured content of 25.5 % w/w cymoxanil); water control; toxic standard: Dimethoate 40 applied at 0.85 L product/ha; calibration was performed

Number of animals: 5 replicates with 3 male and 3 female each, singly kept

Duration: mortality until pupation (ca. 10 d)

Exposure route: contact with freshly dried residues on potato leaves (adaxial surface)

Test conditions: glass test arenas; feeding with aphids and *Sitotroga* eggs; pupae removed from test arenas, adults fed with artificial diet

Environmental parameters: 19 - 26°C, relative humidity 34 - 87 %, photo period 16 h

Biological observations: condition of larvae, development to adults

Statistical / mathematical evaluation: mortality corrected according to Abbott; Chi-squared test

Findings:

Corrected mortality of DPX-KP481 50WG treatments was 23, 0, and 13 % for larvae confined on foliage that had been treated with intervals 1, 3, or 6 times, respectively. Mortality was significantly different from controls after the first application of the test substance, but not after 3 or 6 applications.

Table B.9.5.1-20: Summary of effects of DPX-KP481 50WG on the green lacewing, *Chrysoperla carnea*, when exposed to field-applied residues on potato leaves

Treatment	Bioassay	No. larvae tested ^a	No. pupae developing	No. adults developing	% Mortality	% Corrected mortality
Control	1x	29	28	27	7	–
DPX-KP481	1x	29	26	21	28	23 [*]
dimethoate	1x	30	7	7	77	75 [*]
Control	3x	26	24	21	19	–
DPX-KP481	3x	27	25	23	15	0
dimethoate	3x	30	1	1	97	96 [*]
Control	6x	27	20	18	33	–
DPX-KP481	6x	24	15	14	42	13
dimethoate	6x	28	2	2	93	90 [*]

^a Excludes insects that escaped or were accidentally squashed

^{*} Statistically different from controls

Conclusion:

DPX-KP481 50WG applied on potato leaves at 179 g cymoxanil/ha caused < 25 % mortality (statistically significant after single application only) to *Chrysoperla carnea* and mortality did not increase with up to 6 applications.

Reference: Hermann, P. (2001): DPX-KP481-25: A semi-field study to evaluate the effects on the hoverfly, *Episyrphus balteatus* Deg. (Diptera, Syrphidae) (Report No. DuPont-3881)

Test guideline: Tornier & Drescher (1992)

GLP: Yes

Deviations: none

Validity: Acceptable

Material and methods:

Test substance: DPX-KP481 50WG, batch KP481-25, purity: 25 % cymoxanil and 25 % famoxadone

Test organism: *Episyrphus balteatus*, Imagines, 5 - 6 d old

Treatments: treatments: 0.7 and 1.4 kg product/ha (181 and 363 g a.s./ha based on an analysed content of 25.9 % cymoxanil w/w); deionised water control; toxic standard: Tamaron SL 600 (metamidophos) 600 mL product/ha; calibration was performed

Number of animals: 5 replicates with 1 male and 15 females each

Duration: 4 days (mortality) + 10 days reproduction

Exposure route: contact with freshly dried residues on buckwheat plants

Test conditions: gauze covered cages as test units under semi-field conditions; feeding with sugar, honey/water solution and pollen. Reproduction: buckwheat plants were replaced by aphid infested bean plants. Because not enough males were available, one replicate was assessed with females only.

Environmental parameters: 7 - 32 °C, relative humidity 30 - 100 %

Biological observations: mortality and behavioural abnormalities were recorded daily; number of eggs/female and hatching rate were assessed

Statistical evaluation: ANOVA, Dunnett's test for mortality data

Results:

Mean mortality of *E. balteatus* in the control and toxic standard group was 10.2 and 100 %, respectively. No test-substance related behavioural abnormalities were noted. The mean number of fertile eggs produced per female during the 6-h oviposition period per assessment was 9.3 in the control group, 14.7 in the test substance treatment group at 0.7 kg/ha, and 18.2 in the test substance group at 1.4 kg/ha in the cages with males included. Mean values of the other replicates (females only) were 4.1 in the control group, 1.4 in the test substance group at 0.7 kg/ha, and 8.6 in the test substance treatment group at 1.4 kg/ha.

Table B.9.5.1-21: Effects on mortality and reproduction of the hoverfly, *Episyrphus balteatus*, exposed to freshly applied residues of DPX-KP41-25 50WG on buckwheat

Treatment	0.7 kg DPX-KP481-25 50WG/ha	1.4 kg DPX-KP481-25 50WG/ha
% Mortality (Schneider-Orelli correction)	-1.3 %	2.8 %
Reproduction relative to control (males present)	-58.1 %	-94.6 %
Reproduction relative to control (males absent)	65.9 %	-109.8 %

Negative values for the Schneider-Orelli correction indicate that the treatment group performed better than the control

Remark: Reproduction data are generally highly variable in hoverfly tests.

Conclusion:

DPX-KP481 50WG applied to buckwheat plants at rates up to 1.4 kg/ha (363 g cymoxanil/ha) had no significant negative effects on *Episyrphus balteatus*.

Reference: Thompson, B. (1999b): Cymoxanil/famoxadone (DPX-KP481) 50 WG (25%:25%): A semi-field study to evaluate the effects on aphid parasitoids (Hymenoptera, Aphidiinae) in a potato crop (Report No. AMR 4542-97)

Test guideline: /

GLP: Yes

Deviations: none

Validity: Acceptable

Material and methods:

Test substance: DPX-KP481 50WG, batch: KP481-25, purity: 25 % cymoxanil and 25 % famoxadone

Test organism: *Aphidius colemani*, adult females < 48 h old

Treatments: treatments: 0.7 kg test product/ha with 1, 3, 6, and 9 applications at the Chilworth site, and 1, 3, and 6 at the Burrridge site (UK, 10 d intervals) (179 g cymoxanil/ha based on an analysed content of 25.5 % cymoxanil); water control; toxic standard: Dimethoate 40 at 400 g/L g a.s./ha; calibration was performed

Number of animals: 5 replicates with 30 insects

Duration: 2 days + 10 days (fecundity)

Exposure route: contact with fresh residues on potato plants

Test conditions: wasps caged over potato plant; fecundity assessment: an aphid-infested plant was placed in each cage; feeding with honey/water solution

Environmental parameters: semi-field conditions

Biological observations: adult survival (monitored with sticky traps) and parasitisation rate

Statistical evaluation: one-way ANOVA, Tukey's test

Findings:

At the Chilworth site, DPX-KP481 50WG at 0.7 kg product/ha applied 1, 3, 6, and 9 times resulted in a reduction in parasitisation of 5 - 25 % to an increase of 43 %. The highest observed reduction of 25 % occurred after 3 applications. At the Burrridge site, decreases in parasitisation rate occurred after applications 3 and 6 (26 and 52 %, respectively). There were no statistically significant differences between the number of parasitised mummies between the DPX KP481 50WG treatments and the controls for any of the bioassays. Recovery of adults following treatment at the Chilworth site ranged from 27 % greater than the control after 6 applications to 40 % less than the control after 9 applications. At Burrridge a 43 % reduction in recovery of adults occurred after the first treatment and a 31 % increase in recovered adults occurred after the 6th application. None of these differences was statistically significant.

Table B.9.5.1-22: Effect on parasitisation rate and survival of adult *A. colemani* following exposure to residues from multiple applications of DPX-KP481 50WG on potato plants in the semi-field

Site	Parameter	Application number	% Effect relative to the control	
			DPX-KP481 50WG 0.7 kg/ha	toxic standard
Chilworth	Parasitisation rate	1	+7	-77
		3	-25	-63
		6	-5	-44
		9	+43	No data
	Overall mean		+5	-61
	Wasps collected 2-4 DAT	1	+24	-100
		3	+14	-100
		6	+27	-100
		9	-40	No data
	Overall mean		+6	-100
Burrridge	Parasitisation rate	1	No data	No data
		3	-26	+21
		6	-52	No data
	Overall mean		-39	+21
	Wasps collected 2-4 DAT	1	-43	-98
		3	+26	-64
		6	+31	No data
	Overall mean		+5	-81

Conclusion:

At one site, a reduction of parasitisation rate of 52 % was observed after six treatments with DPX-KP481 50WG. However, considering all results, the product applied up to 9 times to a potato crop at

179 g cymoxanil/ha had no statistically significant effect on the activity and parasitism rate of *Aphidius colemani* in the semi-field.

Plant Protection Product: Cymoxanil/famoxadone 52.5WG

Reference: Kuehner, C. (1998): Cymoxanil/famoxadone (DPX-KX007) 52.5% WG (30 % : 22.5 %): An extended laboratory study (field aged residue) to evaluate the effects on the parasitoid *Trichogramma cacoeciae* Marchal (Hym., Trichogrammatidae) (Report No. AMR 4573-97)

Test guideline: Hassan (1989)

GLP: Yes

Deviations: none relevant

Validity: Acceptable

Material and methods:

Test substance: DPX-KX007 52.5WG, Batch: KX007-09, purity: 30 % cymoxanil and 22.5 % famoxadone

Test organism: *Trichogramma cacoeciae*, adults, age: 24 h

Treatments: treatment: DPX-KX007 52.5WG at 400 g product/ha (120 g cymoxanil/ha) applied 1, 3, 6, 9 and 12 times at intervals of 7-9 days from mid June until early September in Southern Germany; control: water or Delan 750SC (non-toxic standard, a.s. dithianon); toxic standard: Lannate 25WP (a.s. methomyl) up to six times; calibration was performed

Number of animals: not exactly determined content of one hatching tube per replicate; 4 replicates

Duration: 8 days

Exposure route: contact with freshly dried residues on grape leaves

Test conditions: eggs of *Sitotroga cerealella* were offered, after 8 d the egg cards were incubated for development of the parasites; feeding with artificial diet

Environmental parameters: 26 ± 3°C, relative humidity 50 - 85 %, photo period 16 h, light intensity ca. 1000 lux

Biological observations: number of parasitised eggs per female counted

Statistical / mathematical evaluation: reduction in reproduction capacity calculated according to Abbott; Dunnett test

Findings:

At all evaluations, immediately after an application of the toxic standard a 100 % reduction in reproduction capacity was observed. No statistically significant reduction in the number of parasitised eggs/female following any of the DPX-KX007 52.5WG applications was achieved.

Table B.9.5.1-23: Reduction in reproduction capacity (R) of *Trichogramma cacoeciae* after exposure to field aged residues of DPX-KX007 52.5WG on grape leaves

Number of applications	Control		DPX-KX007 52.5WG		Toxic reference ^a	
	Mean parasitised eggs/female	Reduction in reproduction capacity	Mean parasitised eggs/female	Reduction in reproduction capacity	Mean parasitised eggs/female	Reduction in reproduction capacity
1	16.2	-	18.1	-11.7	0.0	100
3	21.2	-	31.9	-50.5	0.0	100
6	17.5	-	15.2	13.1	0.0	100
9	27.2	-	25.0	8.1	6.1	77.6
12	23.2	-	24.5	-5.6	24.7	-6.5

negative values signify an increase of reproduction capacity

^a The last application of the toxic standard was made prior to the 9th application. The last two exposure tests were done with leaves having no additional application.

Conclusion:

DPX-KX007 52.5WG applied at 120 g cymoxanil/ha up to 12 times to grape vines resulted in no significant reduction in reproduction capacity of *Trichogramma cacoeciae*.

B.9.5.2 Field tests (Annex IIIA 10.5.2)

Plant Production Product: Cymoxanil/Famoxadone 20 SC

Reference: Oberwalder, C. (1997): A field study to evaluate the effects of DPX-KX007 WG, a formulation containing DPX-JE874 (225 g ai/kg) and cymoxanil (300 g ai/kg), on the predatory mite *Typhlodromus pyri* in vines (two locations in Germany) (Report No.: AMR 3969-96, Revision No.1)

Test guideline: BBA, Part VI, 23-2.3.4 (1991)

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: DPX-KX007 52.5WG, batch: KX007-09, purity: 30 % cymoxanil (225 g/kg nominal) and 22.5 % famoxadone

Biocenosis: *Typhlodromus pyri*; spider mites

Treatments: treatments between middle of June and middle of July: test substance applied 6 times (interval 7 - 10 d) at 0.04 %, equivalent to 400 - 560 g product/ha and 120 - 168 g cymoxanil/ha; reference treatment: propineb applied 6 times up to 1974 g a.s./ha, non-toxic reference: dithianon up to 527 g a.s./ha; 4 replicates for each treatment

Sampling method: Leaf samples were taken immediately before the 1st application, before the 3rd and 6th application and 1 week and 4 weeks (at one site additionally 5 weeks) after the last (6th) application. At all samplings 25 leaves were selected randomly from the middle of each plot and numbers of predatory and spider mites were assessed (washing method). Adults and nymphs were counted.

Test sites: Location: vineyards at two sites in Southern Germany

Environmental parameters: average temperature: 18 - 23 °C, precipitation 0 - 46 mm

Biological observations: population development: the number of predatory mites and their eggs per plot and the number of spider mites and eggs per plot were assessed

Statistical evaluation: Dunnett-test with squareroot-transformed mite numbers; calculation of efficacy according to Henderson and Tilton

Findings:

As the spider mite population was negligible at both sides, no assessment of this group was performed. At the first trial site, predatory mite populations in the control and DPX-KX007-treated plots remained similar until the second evaluation. Between the second and third evaluations, the mite population on the test substance-treated plots decreased to about 50 % of the control population and this difference continued one week after the last application. However, four and five weeks after the last application the populations were nearly the same as the control. There were no statistically significant differences observed between the predatory mite populations in the control and DPX-KX007-treated plots. A similar pattern was shown by the mite eggs, with a lower egg number found on the test substance-treated plots compared to the control at the third evaluation (statistically significantly different, $p < 0.05$) and results similar to controls thereafter.

The results for the second site were similar to the first, with a reduction in predatory mite populations on the test substance-treated plots compared to the control after the third and fourth assessments, although this difference was relatively smaller. Again, there were no significant differences, except at the last

assessment, four weeks after the last application, when there were significantly more predatory mites on the test substance-treated plots than the control.

Table B.9.5.2-1: Mean number of predatory mites, *Typhlodromus pyri*, and their eggs per 25 leaves at site 1

Evaluation	Control		DPX-KX007 52.5WG		Toxic reference	
	mites	eggs	mites	eggs	mites	eggs
1	227.8	12.5	233.0	27.3	179.0	26.0
2	141.8	80.3	150.5	59.5	92.5	20.8
3	141.5	20.3	70.5	5.3	39.5	3.0
4	80.0	5.5	44.3	3.8	16.8	1.8
5	51.0	0.5	47.6	3.5	21.1	0.9
6	78.0	3.0	59.6	2.8	-	-

* Significantly different to the control (p < 0.05)

Table B.9.5.2-2: Mean number of predatory mites, *Typhlodromus pyri*, and their eggs per 25 leaves at site 2

Evaluation	Control		DPX-KX007 52.5WG		Toxic reference	
	mites	eggs	mites	eggs	mites	eggs
1	67.0	21.8	67.5	26.5	56.0	19.0
2	80.0	14.0	97.0	18.8	43.5	4.3
3	80.5	28.8	62.3	19.5	14.8*	1.8
4	70.8	4.0	52.5	1.3	4.3*	0.0*
5	22.1	2.4	29.0*	1.8	2.3	0.0*

* Significantly different to the control (p < 0.05)

Conclusion:

After an application regime of six applications at nominal rates of 120-168 g cymoxanil/ha in vines transient reductions in the predatory mite populations were seen on the test substance-treated plots compared to the control, and none of the differences were significant. The number off eggs was however significantly reduced in one case. Based on the results of this study, it is concluded that DPX-KX007 52.5WG shows no long-term harmful effects on populations of the predatory mite *T. pyri* under field conditions.

Plant Production Product: Cymoxanil 50 WP

Reference: Muther, J. (2002): A field study to evaluate the effects of Cymoxanil 50% WP on predatory mites (Acari: Phytoseiidae) in vines in France (Report No.: 20021069/F1-NFTp)

Test guideline: Blümel et al., IOBC/BART/EPPO (2000), BBA, Part VI, 23-2.3.4 (1991)

GLP: Yes

Deviations: Only three replicate plots were used in the reference toxicant treatment in place of the preferred five to match the number of plots treated with the test material. This is not considered to detract from the outcome of the study.

Validity: Acceptable

Material and methods:

Test substance: Cymoxanil 50% WP, batch: BPL140, purity: 508.8 g cymoxanil/kg

Biocenosis: *Typhlodromus pyri*, spider mites, Tydeid mites

Treatments: treatment T1: 5 x 240 g product/ha, T2: 4 x 480 g product/ha (nominal; intervals 7 ± 1 d), control: tap water (5 x), reference treatment: Ultracid 20 liquide (193 g methidathion/kg) 3 x, first treatment at BBCH 19

Table B.9.5.2-3: Overview on the timings and rates of treatments

Application number	Date	Crop growth stage	Water control (L/ha)	Cymoxanil 50% WP		Reference toxicant (mL/ha)
				T1 (g/ha)	T2 (g/ha)	
1	17-05-02	BBCH 19	600	240	-	1500
2	25-05-02	BBCH 53	600	240	480	-
3	01-06-02	BBCH 57	600	240	480	1500
4	12-06-02	BBCH 68	800	240	480	-
5	20-06-02	BBCH 71	800	240	480	1500

Sampling method: Leaf samples were taken 2 d before the 1st application, 6 - 7 d after each application and 29 d after the last (5th) application. At all samplings 25 leaves were selected randomly from the middle of each plot and numbers of predatory and spider mites were assessed (washing method).

Test sites: Location: Mollans sur Ouveze, S-France, plot size: 15 vines in one row, 1 buffer row between plots

Environmental parameters: average temperature: 17.1 (May) - 22.9 (July), precipitation 50.7 - 60.5 mm

Biological observations: The number of predatory mites and their eggs per plot and the number of spider mites per plot were assessed

Statistical evaluation: Dunnett-test with squareroot-transformed mite numbers

Findings:

The evaluations of predatory mite populations at various time-points during the trial are summarised in the tables below. There were no statistically significant ($p \leq 0.05$) differences in numbers of mites/leaf between treatments at the initial sampling 2 days before the first spray application. There were no significant differences in predatory mite populations between the control group and either the 122 g a.s./ha or the 244 g a.s./ha Cymoxanil 50% WP treatment groups, following a maximum of five and four applications, respectively. Numbers of predatory mites were significantly lower than the control in the reference treatment throughout the trial: during the two weeks that followed each of the three methidathion applications, numbers were reduced by between 65 % and 99 %, relative to the control, with incipient recovery evident 29 days after the last spraying. Counts of predatory mite eggs are shown in table B.9.5.2.-3. There were no significant differences in numbers of eggs/leaf between the control group and either of the two Cymoxanil 50% WP treatments throughout the trial. Egg production in the reference toxicant group was significantly ($p \leq 0.05$) lower than in the control from the second week after the first methidathion application through to day 6 after the last treatment, but had recovered by the time of the final sampling.

Table B.9.5.2-4: Effects of Cymoxanil 50 % WP applications on predatory mite populations in a vineyard

Sampling time	Mean counts of predatory mites/leaf				Effect (%) ¹		
	Control	Cymoxanil 50% WP		Ref. toxicant	Cymoxanil 50% WP		Ref. toxicant
		T1	T2		T1	T2	
App. 1 - 2 d	2.12	1.94	1.98	2.39	-	-	-
App. 1 + 7 d	2.06	1.88	2.15	0.72*	8.74	-4.37	65.05*
App. 2 + 6 d	1.80	1.78	1.71	0.52*	1.11	5.00	71.11*
App. 3 + 6 d	2.14	2.14	2.06	0.33*	0	3.74	84.58*
App. 4 + 7 d	3.61	3.40	3.21	0.09*	5.82	11.08	97.51*
App. 5 + 6 d	4.11	4.30	4.10	0.04*	-4.62	0.24	99.03*
App. 5 + 29 d	4.46	4.67	4.70	2.91*	-4.71	-5.38	34.75*

negative values signify an increase of reproduction capacity

¹ calculated according to Abbott (1925)

* Significant ($p \leq 0.05$) difference from the control (Dunnett's test)

Table B.9.5.2-5: Effects of Cymoxanil 50% WP applications numbers of predatory mite eggs in a vineyard

Sampling time	Mean counts of predatory mite eggs/leaf			
	Control	Cymoxanil 50% WP		Reference toxicant
		T1	T2	
App. 1 - 2 d	0.61	0.74	0.62	0.71
App. 1 + 7 d	0.59	0.61	0.57	0.24
App. 2 + 6 d	0.63	0.52	0.66	0.24*
App. 3 + 6 d	0.74	0.90	0.66	0.08*
App. 4 + 7 d	0.93	1.00	1.02	0.01*
App. 5 + 6 d	1.13	1.30	1.24	0.04*
App. 5 + 29 d	1.20	1.35	1.43	1.11

* Significant ($p \leq 0.05$) difference from the control (Dunnett's test)

The first and last sample collections yielded 378 predatory mites that were all identified as *Typhlodromus pyri*. Spider mites were recorded intermittently in small numbers, but their presence or absence was inconsistent and unrelated to treatment with Cymoxanil 50% WP. Tydeid mites were present among all treatment groups at all sampling occasions and mites of the Eriophyidae family were consistently absent in all treatment groups.

Conclusion:

Multiple exposure to 122 and 244 g cymoxanil/ha resulted in no adverse effect on predatory mite abundance or reproduction under field conditions.

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B.9.5.3 Summary of effects on non-target arthropods

Table B.9.5.3-1: Overview of cymoxanil containing plant protection products used in tests with non-target arthropods

Product	codes, composition
Cymoxanil 50 WP	505 g cymoxanil/kg Lead formulation Oxon
Cymoxanil/Famoxadone 20SC	DPX-KX007 20SC 120 g cymoxanil/L + 90 g famoxadone/L (12 % cymoxanil + 8.3 % famoxadone analysed)
Cymoxanil/Famoxadone 50WG	DPX-KP481 50WG 266 g/kg cymoxanil + 254 g/kg famoxadone/kg (25 % cymoxanil + 25 % famoxadone nominal) Tanos, Lead formulation DuPont
Cymoxanil/Famoxadone 52.5WG	DPX-KX007 52.5 WG 30 % cymoxanil + 22.5 % famoxadone nominal

Remark (RMS): Studies with the 20SC formulation were considered as not essential with regard to the risk assessment for the GAP proposed. They are considered as additional information only and removed from the "references relied on". However, all studies are valid and in the RMS' view should remain in summary tables and the list of endpoints.

Table B.9.5.3-2: Summary of LR₅₀ values of the indicator species *A. rhopalosiphii* and *T. pyri* for Cymoxanil 50WP

Test species	Test substance and duration	LR ₅₀ [g as/ha]	Annex Reference
Aphid parasitoids			
<i>Aphidius rhopalosiphii</i>	Cymoxanil 50 WP exposure 48 h	> 480	IIA, 8.3.2 Geuijen, 1999a
Predatory mites			
<i>Typhlodromus pyri</i>	Cymoxanil 50 WP 2 weeks	> 480	IIA, 8.3.2 Geuijen, 2000

Table B.9.5.3-3: Summary of effects of cymoxanil containing products on non-target terrestrial arthropods

Test species	Study type, test substance Media, duration	Application Rate [g cymoxanil/ha]	Observed effect*	Results ^a (%)	Annex References
parasitoid wasps					
<i>Aphidius rhopalosiphii</i> adults	laboratory, Cymoxanil 50WP glass plate 48 h	480	mortality reproduction	10.3 -6.2	IIA, 8.3.2 Geuijen, 1999a
	extended laboratory, Cymoxanil/F 20SC, leaves 48 h + 10 d	264	mortality	0	IIA, 8.3.2 Mead-Briggs, 1996
		3 x 264	fecundity	34	
		6 x 264	mortality	33	
<i>Aphidius colemani</i>	semi-field, 12 d	179	fecundity	35	IIA, 8.3.2
			fecundity	25	
			fecundity	23	
			parasitization	-7	

Test species	Study type, test substance Media, duration	Application Rate [g cymoxanil/ha]	Observed effect*	Results ^a (%)	Annex References
adults	Cymoxanil/F 50WG, potato leaves	3 x 179 6 x 179 9 x 179	parasitisation parasitisation parasitisation	25 / 26 ¹ 5 / 52 ¹ -43	Thompson 1999b
<i>Trichogramma cacoeciae</i> adults	extended laboratory, Cymoxanil/F 52.5WG, grape leaves 8 d	120 3 x 120 6 x 120 9 x 120 12 x 120	reproduction reproduction reproduction reproduction reproduction	-11.7 -50.5 13.1 8.1 -5.6	IIA, 8.3.2 Kühner 1998
predatory mites					
<i>Typhlodromus pyri</i> protonymphs	laboratory, Cymoxanil 50 WP 14 d	240 480	mortality reproduction mortality reproduction	9.2 100 8.1 100	IIA, 8.3.2 Geuijen, 2000
	laboratory, 21 d Cymoxanil/F 20SC, glass plate	132	mortality reproduction	98.9 100	IIA, 8.3.2 Kühner, 1996b
	extended laboratory, Cymoxanil/F 20SC, leaves 14 d	120 3 x 120 6 x 120 9 x 120 12 x 120	mortality benef.capacity mortality benef.capacity mortality benef.capacity mortality benef.capacity mortality benef.capacity	22.5 24.8 40 28.6 37.5 27.5 23.9 23.9 21.2 16.5	IIA, 8.3.2 Grove, 1996a
	extended laboratory, Cymoxanil/F 20SC, leaves 14 d	1-6 x 159	mortality benef.capacity	25.3 65-89	IIA, 8.3.2 Grove, 1996b
	extended laboratory, Cymoxanil/F 52.5WG, leaves 14 d	1-6 x 134 1-6 x 268	mortality benef.capacity mortality benef.capacity	34.7 85-100 42.4 100	
	field, Cymoxanil/F 52.5WG, vineyards, 2 months	6 x 120 - 168	abundance (mites + eggs)	transient reductions, no long- term effect	IIA, 8.3.2 Oberwalder, 1997
	field, Cymoxanil/F 50WP, vineyards, 2 months	5 x 240 4 x 480	abundance (mites + eggs)	no adverse effects	IIA, 8.3.2 Müther, 2002
Foliage dwelling species					
<i>Chrysoperla carnea</i> larvae	laboratory, 9 w Cymoxanil 50WP glass plate	480	mortality fecundity	7.1 9	IIA, 8.3.2 Geuijen, 1999c
	laboratory, 21+28 d Cymoxanil/F 20SC, glass plate	132	mortality fecundity	-7.3 17.3	IIA, 8.3.2 Kühner, 1995
	laboratory, 21+28 d Cymoxanil/F 50WG, glass plate	186	mortality fecundity	-8.4 4.1	IIA, 8.3.2 Kühner, 1997c
	extended laboratory, Cymoxanil/F 50WG leaves 10 d	179 3 x 179 6 x 179	mortality mortality mortality	23 0 13	IIA, 8.3.2 Thompson, 1999c

Test species	Study type, test substance Media, duration	Application Rate [g cymoxanil/ha]	Observed effect*	Results ^a (%)	Annex References
<i>Episyrphus balteatus</i> larvae to adults	laboratory, 22 d Cymoxanil/F 50WG, glass plate	186	mortality fecundity	1.2 33.5	IIA, 8.3.2 Kühner, 1997a
	extended laboratory, Cymoxanil/F 20SC, leaves 3 w	289 3 x 289 6 x 289	mortality fecundity mortality fecundity mortality fecundity	1.1 33.4 -3.4 26.3 -0.3 23.2	IIA, 8.3.2 Nengel 1996a
	extended laboratory, Cymoxanil/F 50WG leaves 4 w	6 x 186	mortality fecundity	7 50	IIA, 8.3.2 Thompson, 1999a
	semi-field, 14 d Cymoxanil/F 50WG, buckwheat plants	181 363	mortality reproduction mortality reproduction	-1.3 -58.1 2.8 -94.6	IIA, 8.3.2 Hermann, 2001
Ground dwelling species					
<i>Poecilus cupreus</i> adults	laboratory, 14 d Cymoxanil 50WP, glass plate	480	mortality	0	IIA, 8.3.2 Geuijen, 1999b
	laboratory, 14 d Cymoxanil/F 20SC, glass plate	132	mortality	0	IIA, 8.3.2 Kühner, 1996a
	laboratory, 14 d Cymoxanil/F 50WG, glass plate	186	mortality	0	IIA, 8.3.2 Kühner, 1997b
	extended laboratory, Cymoxanil/F 50WG, soil 7 d	186 4 x 186 6 x 186 9 x 186	mortality mortality mortality mortality	0 0 0 0	IIA, 8.3.2 Beech & Mead-Briggs, 1998
	semi-field, 7 d Cymoxanil/F 20SC, silty sand soil	289 3 x 289 6 x 289	mortality mortality mortality	11.3 18.9 7.5	IIA, 8.3.2 Nengel, 1996b
	laboratory, 10 w Cymoxanil/F 50WG, quartz sand	186	mortality parasitic capacity	7.5 2.6	IIA, 8.3.2 Kühner, 1997d
<i>Aleochara bilineata</i> larvae to adults	laboratory, 10 w Cymoxanil/F 50WG, quartz sand	186	mortality parasitic capacity	7.5 2.6	IIA, 8.3.2 Kühner, 1997d

F = Famoxadone

* mortality corrected ^a positive percentages relate to adverse effects compared to control

¹ site 1 / site 2

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B.9.5.3.1 Risk assessment for non-target arthropods

B.9.5.3.2 In-crop risk assessment

Table B.9.5.3.2-1: Hazard Quotient (HQ) calculations for the indicator species in lettuce and potatoes in-field

Test substance	Species	Application rate (AR)	MAF	LR ₅₀ (g/ha)	HQ in-field ¹	Trigger
lettuce						
Cymoxanil 50 WP	<i>Typhlodromus pyri</i>	4 x 240 g/ha	2.7	> 480	< 1.35	2
	<i>Aphidius rhopalosiphi</i>			> 480	< 1.35	2
potato / Oxon						
Cymoxanil 50 WP	<i>Typhlodromus pyri</i>	5 x 120 g/ha	3	> 480	< 0.75	2
	<i>Aphidius rhopalosiphi</i>			> 480	< 0.75	2
potato / DuPont						
Cymoxanil/Famoxadone 50WG	<i>Typhlodromus pyri</i>	8 x 175 g/ha	3.5	> 480	< 1.28	2
	<i>Aphidius rhopalosiphi</i>			> 480	< 1.28	2

¹ HQ = (AR x MAF)/LR₅₀

MAF....multiple application factor

The HQ values for both indicator species are below the trigger of 2 and indicate a low risk for non-target arthropod populations in the in-field area.

However, based on the numerous studies with different species of non-target-arthropods and different cymoxanil containing products, the risk for each of the groups parasitoids, predatory mites, foliage and ground dwelling arthropods is evaluated with regard to the intended multiple application scenarios:

Parasitoids:

In an extended lab study with the 20SC combi-formulation applied up to 6 times 264 g cymoxanil/ha effects on mortality and reproduction of *A. rhopalosiphi* remained below 35 %. With the 50 WG combi-formulation and up to 9 applications of 179 g cymoxanil/ha under semi-field conditions with *Aphidius colemani* only in one instance at one site an effect of > 50 % (52 %) on parasitisation was observed. After the last of the 9 applications, however, no negative effect at all occurred. In a further extended lab study with *Trichogramma cacoeciae* and applications up to 12 times 120 g cymoxanil formulated in the 52.5WG combi-product reproductive effects remained below 20 % in all instances.

It can be concluded that after all intended use situations no unacceptable effects on parasitoid populations are expected.

Predatory mites:

The lab study with Cymoxanil 50WP with *T. pyri* resulted in mortality rates < 10 % and a total reduction in reproduction at a rate of 240 and 480 g a.s./ha. In one glass plate study a rate of 132 g cymoxanil/ha (20SC combi-formulation) led to 98.9 % mortality. Lower effects were observed in three extended lab studies - two with the same formulation and one with the 52.5WG combi-product: At rates of up to 12 times 120 g cymoxanil/ha the highest mortality effect was 40 % after the 3rd application; rates up to 6 times 159 g cymoxanil/ha led to 25 % mortality and a reduction in beneficial capacity of maximal 89 %;

286 g cymoxanil/ha applied up to 6 times (52.5WG) showed a maximal effect of 42 % mortality and 100 % reduction in beneficial capacity. In two field studies using the 50- and 52.5WG combi-formulations with applications of 6 x 120-168 g cymoxanil/ha and 4 x 480 g cymoxanil/ha no or transient population reductions were noted. All results confirmed the observation that multiple applications did not lead to an accumulation of effects. The high reproductive impact that was observed in the laboratory studies did not appear in the field, as the egg abundance was also evaluated.

Overall, cymoxanil containing products under the intended use conditions possibly cause transient effects on mortality and/or reproduction of predatory mites, but based on the available data fast recovery and no unacceptable effects can be expected.

Foliage dwelling arthropods:

In three laboratory and one extended lab studies with *Chrysoperla carnea* and different formulations with rates up to 1 x 480 and 6 x 179 g cymoxanil/ha no significant effects on mortality or fecundity were apparent. In the second species tested, the hoverfly *Episyrphus balteatus*, no increase in mortality was observed in 4 studies (lab to semi-field) with rates up to 6 x 289 g cymoxanil/ha. Effects on fecundity were variable. In conclusion, the risk to this group of arthropods can be considered low under the intended use conditions.

Ground dwelling arthropods:

No significant effects were seen in *Poecilus cupreus* adults in 5 studies from laboratory to the semi-field when cymoxanil was applied in different formulations up to 6 x 289 g/ha. One laboratory study with *A. bilineata* did also not reveal significant effects at a rate of 186 g cymoxanil/ha.

Conclusion:

After application of cymoxanil according to the intended representative uses in lettuce and potatoes the risk to non-target arthropod populations in-field is low.

B.9.5.3.3 Off-crop risk assessment

Table B.9.5.3.3-1: Hazard Quotient (HQ) calculations for the indicator species in lettuce and potatoes off-field

Test substance	Species	Appl. rate (AR)	MAF	vdf	cf	drift (distance)	LR ₅₀ (g/ha)	HQ off-field ¹	Trigger
lettuce									
Cymoxanil 50 WP	<i>T. pyri</i>	4 x 240	2.7	10	10	1.85 (1 m)	> 480	< 0.02	2
	<i>A. rhopalosiphi</i>	g/ha					> 480	< 0.02	2
potato / Oxon									
Cymoxanil 50 WP	<i>T. pyri</i>	5 x 120	3	10	10	1.52 (1 m)	> 480	< 0.01	2
	<i>A. rhopalosiphi</i>	g/ha					> 480	< 0.01	2
potato / DuPont									
Cymoxanil 50 WP	<i>T. pyri</i>	8 x 175	3.5	10	10	1.52 (1 m)	> 480	< 0.02	2
	<i>A. rhopalosiphi</i>	g/ha					> 480	< 0.02	2

vdf.....vegetation distribution factor

cf.....correction factor

The HQ values for both indicator species are far below the trigger of 2 and indicate a low risk for non-target arthropod populations in the off-field area. The results of many first- and higher tier studies with several species and different cymoxanil containing products support the assumption that at drift rates there will be no adverse effects on non-target arthropod populations (see evaluation for the in-field area).

Conclusion:

After application of cymoxanil according to the intended representative uses in lettuce and potatoes the risk to non-target arthropod populations in- and off-field is low.

B.9.6 Effects on earthworms

B.9.6.1 Acute toxicity (ANNEX IIA 8.4.1; ANNEX IIIA 10.6.1.1)

Active substance

Reference: Rodgers, M.H., Cameron, D.M. and Mansell, P. (1995): Cymoxanil technical acute toxicity (LC₅₀) to the earthworm (*Eisenia foetida*) (Report No.: OXN 78A/950675)

Guideline(s): OECD 207

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: Cymoxanil technical; batch: 817, purity: 99.1 %

Test organism: adult clitellate *Eisenia foetida*, 407 - 582 mg bw at test initiation

Treatments: 95, 171, 309, 556 and 1000 mg a.s./kg dry soil; water treated control; the effects of the reference toxicant 2-chloroacetamide were assessed in a separate study.

Number of animals: 4 replicates with 10 worms per treatment and control

Duration: 14 d exposure

Test conditions: test vessels: one-litre glass containers; no feeding; artificial soil (70 % Quartz sand, 20 % kaolin clay, and 10 % sphagnum peat)

Environmental parameters: pH 5.6, temperature 16 - 19°C, water content adjusted for 35 %, light ca. 500 lux; application: test substance mixed in soil

Biological observations: Worms were assessed for mortality and behavioural effects after 7 and 14 days exposure, and body weights (groups of 10 earthworms at start and surviving earthworms at end) were assessed at day 0 and day 14.

Statistical evaluation: none

Findings:

Mortality: No mortality at day 7. At day 14 three and four worms were found dead (7.5 and 10 %) in the 556 and 1000 ppm group, respectively.

Non-lethal toxicity endpoints: Weight loss was observed at 556 and 1000 ppm (- 12 and - 28 %, respectively)

Conclusions:

LC₅₀ > 1000 mg a.s./kg dry soil

NOEC = 309 mg a.s./kg dry soil (based on mortality and weight loss)

Metabolites

Argumentation why no studies are considered necessary is given in chapter B.9.6.5 -> Metabolites.

Formulation: Cymoxanil 50 WP

Reference: M.H. Rodgers (1995): Cymoxanil 50 % WP - Acute toxicity (LC₅₀) to the earthworm (*Eisenia foetida*), (report no. OXN 81A/943245)

Guideline(s): OECD 207; 87/302/EEC, Part C

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: Cymoxanil 50 % WP (batch Q223), purity 505 g a.s./kg

Test organism: adult *Eisenia foetida*, 357 - 486 mg bw at test initiation

Treatments: 0, 95, 171, 309, 556 or 1000 mg product/kg dry soil (nominal); water treated control;
reference toxicant: chloroacetamide

Number of animals: 4 replicates with 10 worms per treatment and control

Duration: 14 d exposure

Test conditions: test vessels: one-litre glass jars; no feeding; artificial soil (70 % Quartz sand, 20 % kaolin clay and 10 % sphagnum peat)

Environmental parameters: pH 5.6, temperature 22 - 23 °C, artificial substrate moisture content 32 – 35 %, light 500 lux; application: product mixed in soil

Biological observations: Worms were assessed for mortality, pathological signs and behavioural effects after 7 and 14 days exposure, and body weights were assessed at day 0 and day 14

Statistical evaluation: none

Findings:

Mortality: 10 out of 40 worms died at 1000 mg/kg soil. The LC₅₀ of the toxic reference was ca. 37 mg/kg soil.

Non-lethal toxicity endpoints: Large treatment-related weight losses were observed at 1000 ppm (29 % vs control). Weight changes in the other groups varied.

Conclusions:

LC₅₀: > 1000 mg product/kg soil dry weight (equivalent to > 505 mg a.s./kg soil dw)

NOEC: 556 mg product/kg soil dry weight (equivalent to 281 mg a.s./kg soil dw)

Formulation: Cymoxanil/Famoxadone 52.5 WP

Reference: Wachter, S. (1997): Acute toxicity of DPX-KX007-5 on earthworms, *Eisenia foetida* using an artificial soil test (Report No.: AMR 4136-96)

Guideline(s): OECD 207

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: DPX-KX007 DF, batch: KX007-5, purity: 30 % cymoxanil and 22.5 % famoxadone (30.4 and 22.7 % analysed)

Test organism: adult clitellate *Eisenia foetida*, 300 - 600 mg bw at test initiation

Treatments: 500, 625, 750, 875, and 1000 mg product/kg dry soil (nominal); water treated control;
reference toxicant: 2-chloroacetamide at 0 - 100 mg/kg soil

Number of animals: 4 replicates with 10 worms per treatment and control

Duration: 14 d exposure

Test conditions: test vessels: one-litre glass jars; no feeding; artificial soil (69 % Quartz sand, 20 % kaolin clay, 1 % Ca carbonate and 10 % sphagnum peat)

Environmental parameters: pH 6.5, temperature 20 ± 2 °C, water content adjusted for 35 %, light 400 - 800 lux; application: product mixed in soil

Biological observations: Worms were assessed for mortality (dead and missing earthworms), pathological signs (colour change) and behavioural effects after 7 and 14 days exposure, and body weights (groups of 10 earthworms at start and surviving earthworms at end) were assessed at day 0 and day 14.

Statistical evaluation: Dunnett test

Findings:

Mortality: Mortalities were noted in all treatment groups. In the range-finding test 80 % mortality occurred at 1000 mg/kg soil. The LC_{50} of the toxic reference was between 10 and 18 mg/kg soil.

Non-lethal toxicity endpoints: At concentrations between 625 and 1000 mg/kg soil abnormalities in behaviour were observed (lethargy, loss of body segments in some cases).

Table B.9.6.1-1: Acute toxicity of DPX-KX007 52.5WG to *Eisenia foetida*

Nominal concentration (mg product/kg soil dw)	Mean cumulative mortality after 14 d (%)	Weight change (%)
		0-14 days
0	0	-11.3
500	7.5	-13.1
625	17.5	-15.5
750	30	-16.5
875	40	-17.3
1000	55	-28.4

Negative values indicate weight losses

* Statistically different from control, Dunnett's test, $\alpha = 0.05$.

Conclusions:

LC_{50} : 989 mg test substance/kg soil dry weight (equivalent to 296.7 mg cymoxanil/kg soil dw).

95% C.I.: 860 - 1138 mg/kg soil

NOEC: could not be determined because of mortalities in the lowest treatment group

B.9.6.2 Sublethal effects (ANNEX IIA 8.4.2; ANNEX IIIA 10.6.1.2)

Active substance

No study provided.

Metabolites

Argumentation why no studies are considered necessary is given in chapter B.9.6.5 -> Metabolites.

Formulation: Cymoxanil/Famoxadone 50 WG = Tanos

Reference: Lühns, U. (2000): Cymoxanil/famoxadone (DPX-KP481): A dose response sublethal study to the earthworm, *Eisenia fetida* (Savigny 1826), in artificial soil (Report No.: DuPont-4611)

Guideline(s): ISO 12268-2 (1998), BBA VI, 2-2 (1994)

GLP: Yes

Deviations: no relevant deviation

Validity: Acceptable

Material and methods:

Test substance: DPX-KP481 50WG, batch: KP481-25, purity: 25.9 % (w/w) cymoxanil and 24.1 % famoxadone

Test organism: adult clitellate *Eisenia fetida andrei*, ca. 11 months old, 300 - 500 mg body weight

Treatments: 1.6, 3.2, 6.4, 12.8, 25.6 mg product/kg dry soil, corresponding to 0.41, 0.83, 1.7, 3.3 and 6.6 mg cymoxanil/kg dry soil (measured); control: deionised water, toxic reference: Carbendazim at 2.3 g a.s./ha; application: test substance mixed in soil

Number of animals: 4 replicates with 10 worms in each control or treatment group

Duration: 56 d (adult exposure 28 d)

Test conditions: Feeding: cattle manure; 2 d acclimatisation before start of study; Soil parameters: 69.6 % sand, 10 % sphagnum peat, 20 % kaolin clay

Environmental parameters: pH 6.3, temperature 19 - 21°C, water content ca. 27 %

Biological observations: Mortality, weight loss and sublethal effects of adults were assessed at day 28; Soil was replaced in the test container and juveniles were allowed to grow for another 28 days, at which time they were removed from the soil, counted, and sublethal effects were assessed.

Statistical evaluation: Mortality data were analysed by Fisher-exact-test (two-sided, $\alpha = 0.05$) and body weight data by Dunett-test

Findings:

Table B.9.6.2-1: Effect of DPX-KP481 50WG on mortality, body weight and reproduction of *Eisenia fetida*.

Treatment (mg a.s./kg soil)	Observation period		
	Day 28	Day 56	
	Cumulative mortality [%]	No of juvenile earthworms per adult	Weight change (%)
Control	2.5	293	51.9
0.41	2.5	308	53.8
0.83	0	298	55.5
1.7	0	257	58.7
3.3	2.5	292	58.8
6.6	0	306	55.3
toxic reference	0	45*	34.0*

* Significantly different ($p < 0.05$) compared to the control

Non-lethal toxicity endpoints: No behavioural abnormalities in any treated group. The amount of food added to the containers of the control and test item treated groups were comparable, whereas less food was added to the toxic reference. It is therefore concluded that exposure to cymoxanil did not affect food consumption. In the toxic standard treatment reproduction was lowered by 84.6 %. Additionally, the body weight increase over the course of the test was significantly lower in the toxic standard treatment, compared to the control.

Conclusions:

There were neither lethal nor sublethal effects on earthworms (*Eisenia fetida*) at DPX-KP481 50WG concentrations corresponding to between 0.41 and 6.6 mg cymoxanil/kg soil. Consequently, the NOEC for sublethal effects of DPX-KP481 50WG on earthworms is equivalent to 6.6 mg cymoxanil/kg dry soil.

B.9.6.3 Field studies (Annex IIIA 10.6.4)

No study required.

B.9.6.4 Summary of effects on earthworms

Table B.9.6.4-1: Summary of effects of cymoxanil (technical and formulated) on earthworms

Substance (purity)	Medium	Species	EC ₅₀	NOEC	Reference
Acute toxicity					
cymoxanil techn. (99.1 %)	artificial soil	<i>Eisenia foetida</i>	> 1000 mg a.s./kg soil dw	309 mg a.s./kg soil dw	Rodgers et al., 1995
Cymoxanil 50 WP	artificial soil	<i>Eisenia foetida</i>	> 505 mg a.s./kg soil dw	281 mg a.s./kg soil dw	Rodgers, 1995
Cymoxanil/Famoxadone 52.5 WP (22.7 %)	artificial soil	<i>Eisenia foetida</i>	989 mg prod./kg soil dw ≈ 296.7 mg cymoxanil/kg soil dw	n.d.	Wachter, 1997
Sublethal toxicity					
Cymoxanil/Famoxadone 50 WG (25.9 %)	artificial soil	<i>Eisenia fetida andrei</i>	/	6.6 mg cymoxanil /kg soil dw	Lühns, 2000

n.d. not determined

B.9.6.5 Risk assessment for earthworms

The acute and long term TER values for earthworms were calculated as the ratio between the acute and the chronic effect concentrations (EC₅₀ and NOEC), respectively, and the initial PECs. Worst case PEC values of the active substance after multiple applications in lettuce and potatoes were calculated to be 0.184 mg/kg and 0.239 mg/kg, respectively; details for calculations see section B.8.3.

Table B.9.6.5-1: TER acute for earthworms

Test substance	14 days LC ₅₀ (mg as/kg soil)	initial PEC (mg/kg)	TER acute	Trigger value
Lettuce / Oxon				
cymoxanil	> 1000	0.184	> 5435	10
Cymoxanil 50 WP	> 505	0.184	> 2745	10
Potatoes / Oxon				
cymoxanil	> 1000	0.159	> 6289	10
Cymoxanil 50 WP	> 505	0.159	> 3176	10
Potatoes / DuPont				
cymoxanil	> 1000	0.239	> 4184	10
Cymoxanil/Famoxadone 52.5 WP	296.7	0.239	1241	10

Table B.9.6.5-2: TER long term for earthworms

Test substance	NOEC (mg as*/kg soil)	initial PEC (mg/kg)	TER It	Trigger value
Lettuce / Oxon				
Cymoxanil/Famoxadone 50 WG	6.6	0.184	36	5
Potatoes / Oxon				
Cymoxanil/Famoxadone 50 WG	6.6	0.159	42	5
Potatoes / DuPont				
Cymoxanil/Famoxadone 50 WG	6.6	0.239	28	5

* cymoxanil alone

Active substance and formulation:

All calculated acute TER values for cymoxanil, Cymoxanil 50 WP and the Cymoxanil/Famoxadone 52.5 WP formulation are well above the trigger value of 10, thus the acute risk to earthworms can be considered as low. The 52.5 WP combi-formulation with famoxadone is not identical with Tanos, the lead formulation of DuPont. However, the compositions of the formulations are very similar. (statement notifier: the components of DPX-KX007-5 are the same as DPX-KP481 but the concentrations are slightly different. DPX-KX007-5 has the same composition as DPX-KX007 WG, with a small difference in cymoxanil content (30.93 % in DPX-KX007-5 compared to 31.25 % in DPX-KX007 WG)). Due to the multiple use scenarios a sublethal / reproduction test was performed with the Cymoxanil/Famoxadone 50 WG formulation (Tanos). As a data requirement according to the Sanco/10329/2002 document a sublethal study is required for the DuPont use in potatoes (> 6 applications) only. Tanos, the DuPont lead formulation, is a wettable granule formulation containing equal amounts (w/w) of cymoxanil and famoxadone. Since no effects occurred up to and including the highest concentration applied in the sublethal study, the issue of whether to attribute impact to either one or both active substances does not arise. The calculated TER value for this long term exposure is significantly above the trigger value of 5. Therefore the long term risk for earthworms after the application of cymoxanil according to GAP is considered acceptable.

Metabolites:

Laboratory studies of the behaviour of cymoxanil under aerobic conditions in soil indicated rapid degradation. On this basis, exposure of earthworms to the metabolites of cymoxanil may be assumed to have occurred during the aerobic conditions that prevailed during the 14-day incubation of cymoxanil in artificial soil. The acute toxicity endpoint therefore takes into account any effects of exposure to degradation products.

Two major metabolites (occurring above 10 % AR) were found in soil metabolism studies: IN-U3204 and IN-W3595 and one in soil photolysis (IN-JX915). Their maximum occurrence takes place on day 0.33, 1 and 7 after application, respectively. Accordingly their toxicity is covered by the 14-d acute toxicity studies. Cymoxanil or its metabolites are not expected to accumulate in soil following multiple applications. Further minor metabolites reach their maximum occurrence in the first three days after application, except metabolite IN-R3273, which maximum occurs on day 15 with an amount of 2.4 % AR and therefore is considered of low relevance. Further testing specifically to determine the acute toxicity of metabolites of cymoxanil to earthworms is therefore unnecessary.

Hence, it can be assumed that cymoxanil soil metabolites pose no unacceptable risk to earthworms.

B.9.6.5.1 Conclusions

The acute and long term risk to earthworms after the application of cymoxanil according to GAP is considered low.

B.9.7 Effects on other soil non-target macro-organisms (Annex IIIA 10.6.2)

No study submitted, not required.

B.9.8 Effects on soil non-target micro-organisms (Annex IIA 8.5, IIIA 10.7)

B.9.8.1 Active Substance: Cymoxanil

Reference: Kölzer, U. (2003): Assessment of the side effects of cymoxanil technical on the activity of the soil microflora (Report No.: 20031314/01-ABMF)

Guidelines: OECD 216 and 217 (2000)

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: cymoxanil techn., batch no.: 198000042, purity: 99.2 %

Test soil: loamy sand, pH 6.28 - 6.74; 60.9 % (w/w) sand, 30.4 % silt and 8.7 % clay

Treatments: 0, 0.32 and 1.6 mg a.s./kg soil dw (corresponding to 0.24 and 1.2 kg a.s./ha, respectively, assuming a soil depth of 5 cm and a bulk density of 1.5 g/cm³), control: deionised water, toxic standard: Dinoterb at 13.3 mg/kg dry soil

Duration: 28 d

Test conditions: 40 % moisture content of soil; incubation temperature 20 ± 2 °C

Biological observations: nitrogen turnover: changes in nitrate-N and ammonium-N were determined 0, 7, 14 and 28 d after application; short-term respiration: oxygene uptake measured for up to 24 h after addition of glucose 0, 7, 14 and 28 d after application and converted to glucose-derived CO₂ production data.

Statistical evaluation: multiple t-test according to Dunnett, student-t-test

Findings:

Table B.9.8.1-1: Effects of cymoxanil on the rate of respiration by soil microflora

Treatment (mg a.s./kg dry soil)	Mean respiration rate (mg CO ₂ /kg dry soil/h) [% divergence from mean control]							
	Day 0		Day 7		Day 14		Day 28	
0	4.85	[-]	4.60	[-]	3.77 ^a	[-]	3.80	[-]
0.32	4.14	[-14.6]	4.36	[-5.22]	3.56	[-5.57]	3.59	[-5.53]
1.60	4.29	[-11.5]	4.31 ^a	[-6.30]	3.46	[-8.22]	3.48	[-8.42*]

^a one replicate omitted due to equipment malfunction

* significantly (p ≤ 0.05) different from the mean control (Student's t-test)

A slight suppression of respiration with respect to the mean control was evident in both treatments throughout the incubation. The maximum divergence from the mean control was a reduction of 14.6 % on day 0 at 0.32 mg/kg. A statistically significant suppression was recorded at 1.60 mg/kg on day 28.

However, effects were below the 25 % trigger for biological significance in both treatments throughout the study.

Table B.9.8.1-2: Effects of cymoxanil on ammonium- and nitrate-nitrogen concentrations in soil amended with lucerne meal

Treatment (mg a.s./kg dry soil)	Mean inorganic nitrogen concentration (mg/kg dry soil)			
	Day 0	Day 7	Day 14	Day 28
NH₄⁺-N				
0	<LOQ	2.40	1.31	<LOQ
0.32	<LOQ	2.30	1.31	<LOQ
1.60	<LOQ	2.58	1.27	<LOQ
NO₃⁻-N				
0	6.29	9.80	19.9	36.1
0.32	8.55	10.6	21.7	38.7
1.60	10.1	9.55	22.3	35.2
Σ inorganic nitrogen^a				
0	6.29	12.2	21.2	36.1
0.32	8.55	12.9	23.0	38.7
1.60	10.1	12.1	23.6	35.2

^a NH₄⁺-N + NO₃⁻-N, excluding nitrite, NO₂⁻-N (not measured)

Table B.9.8.1-3: Effects of cymoxanil on the rate of nitrate formation by soil microflora

Treatment (mg a.s./kg dry soil)	Mean nitrogen transformation rate (mg NO ₃ -N/kg dry soil/day) [% divergence from mean control]					
	Day 0 to 7		Day 0 to 14		Day 0 to 28	
0	0.501	[-]	0.972	[-]	1.06	[-]
0.32	0.293	[-41.5]	0.939	[-3.40]	1.08	[1.89]
1.60	-0.079	[-116]	0.871	[-10.4]	0.896	[-15.5]

The toxic standard led to deviations from the control > 25 % in both nitrogen turnover and respiration test after 28 d.

At concentrations of 0.32 and 1.60 mg a.s./kg soil (equivalent to 0.24 and 1.20 kg a.s./ha, respectively), cymoxanil initially inhibited organic nitrogen mineralisation, but the effects were transient and divergence from the corresponding controls was reduced to < 25 % within 14 days.

Conclusion:

There were no biologically significant long-term effects on nitrogen or carbon transformation by soil microflora at either treatment rate.

B.9.8.1.1 Metabolites

Laboratory studies of the behaviour of cymoxanil under aerobic conditions in soil indicated rapid degradation. On this basis, exposure of microorganisms to the metabolites of cymoxanil may be assumed to have occurred during the 28-day incubation of cymoxanil in soil. The soil microflora studies therefore take into account any effects of exposure to degradation products.

Two major metabolites (occurring above 10 % AR) were found in soil metabolism studies: IN-U3204 and IN-W3595 and one in soil photolysis (IN-JX915). Their maximum occurrence takes place on day 0.33, 1 and 7 after application, respectively. Accordingly their toxicity is covered in the 28-d toxicity studies.

Cymoxanil or its metabolites are not expected to accumulate in soil following multiple applications.

Further minor metabolites reach their maximum occurrence in the first three days after application, except

metabolite IN-R3273, which maximum occurs on day 15 with an amount of 2.4 % AR. Further testing with soil microflora is therefore unnecessary.

Hence, it can be assumed that cymoxanil soil metabolites pose no unacceptable risk to soil microflora.

B.9.8.1.2 Formulation

Plant Production Product: Cymoxanil/Famoxadone 20 SC

Reference: Carter, J.N. (1995): DPX-KX007 (20 SC) effects on soil non-target micro-organisms (Report No.: AMR 3560-95)

Guidelines: SETAC Europe guidelines (1995)

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: DPX-KX007 20SC, batch: KX007-4, purity: 120 g cymoxanil/L and 90 g famoxadone/L

Test soil(s): sandy loam, pH 6.6; 60.9 % (w/w) sand, 30.4 % silt and 8.7 % clay

Treatments: 16 µL test substance/kg soil dw + lucerne meal (corresponding to 12 L product/ha), control: water, water + lucerne meal; toxic standard: Dinoseb acetate at 40 or 100 mg/kg dry soil; 4 replicates/treatment

Duration: 28 d

Test conditions: Lucerne meal was used as a source of nitrogen and carbon. 45 % moisture content of soil; incubation temperature 20 ± 2 °C

Biological observations: Soil samples were taken for analysis of respiration and nitrogen (ammonium, nitrate, and nitrite) content after 3 hours, and 14, 21, and 28 days of incubation

Statistical evaluation: one-way analysis of variance

Findings:

Table B.9.8.1-4: Summary of effects of DPX-KX007 20SC on ammonium and nitrate levels in sandy loam soil amended with lucerne meal

Time	Ammonium (µg N/g soil dw)			Nitrate (µg N/g soil dw)		
	Control ^a	DPX-KX007 20SC	Deviation from control (%)	Control	DPX-KX007 20SC	Deviation from control (%)
3 h	8.6	12.35	43.6	8.8	6.7	-23.8
14 d	0.64	0.64	0	22.2	20.7	-6.8
21 d	0.52	0.29	-44.2	27.4	24.7	-10
28 d	0.08	0	0	36.9	37	-0.3

^a water + lucerne meal

* statistically different from controls, one-way ANOVA, $p < 0.01$

Table B.9.8.1-5: Summary of effects of DPX-KX007 20SC on respiration in a sandy loam soil amended with lucerne meal

Time	Mean cumulative respiration (ppm C as CO ₂ /g soil dw)		
	Control	DPX-KX007 20SC	Deviation from control (%)
3 h	20	11	-44.6
2 d	282	187	-33.6
7 d	578	484	-16.2
14 d	759	651	-14.3
21 d	856	762	-11.1
28 d	924	831	-10.0

The toxic standard led to deviations from the control > 25 % in both nitrogen turnover and respiration test after 28 d.

Conclusion:

DPX-KX007 20SC at 0.016 mL/kg soil dry weight (equivalent to 1.44 kg cymoxanil/ha) had no significant effect on soil nitrogen transformation or carbon mineralisation at the end of a 28-day incubation period.

B.9.8.2 Summary of effects on soil non-target microorganisms

Table B.9.8.2-1: Summary of effects on non-target microorganisms.

Substance	Soil type	Application rate	Test duration	Effect (%)	Annex
cymoxanil	loamy sand	1.6 mg a.s./kg soil (equivalent to 1.2 kg a.s./ha)	28 d	< 25 %	IIIA, 10.7 Kölzer, 2003
Cymoxanil/Famoxadone 20SC	sandy loam	0.016 mL/kg soil dw (equivalent to 1.44 kg a.s./ha)	28 d	< 25 %	IIIA, 10.7 Carter, 1995

B.9.8.3 Risk assessment for soil non-target micro-organisms

Neither respiration nor nitrogen mineralisation of soils treated with cymoxanil up to rates that correspond with all intended multiple use scenarios differed from untreated soils by greater than 25 % after 28 days. The maximum PEC_{soil} for cymoxanil is significantly below the highest test concentration with no lasting effects greater than 25 %.

As regards metabolites, they are assumed to pose low risk to soil-microorganisms, as detailed under B.9.8.2.

In conclusion, cymoxanil is not expected to cause any significant effects on soil microbial populations when applied according to GAP.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be a risk (Annex IIA 8.6)

B.9.9.1 Toxicity to non target flora (ANNEX IIA 8.6; ANNEX IIIA 10.8)

Reference: Balluff, M. (2003): Vegetative vigour limit test for non target plants following single rate application of Cymoxanil 50 WP (Oxon Report No.: 20033057/S1-FGW)

Guideline(s): Draft OECD 208 B (2000), BBA consensus paper (2001), EPPO 1/135(2)

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: Cymoxanil 50 WP, 500 g a.s./kg

Test species: Monocotyledonous: *Allium cepa*, *Avena sativa*, Dicotyledonous: *Beta vulgaris*, *Cucumis sativus*, *Glycine max*, *Helianthus annuus*

Treatments: 240 g a.s./ha solved in 500 L/ha water, control: deionised water

Number of plants: 8 (monocot) or 20 (dicot) replicates with each pot containing 2 (dicots) or 5 (monocot) young plants (BBCH 12-14)

Duration: 21 d

Test conditions: Test pots irrigated with a water and nutrient source. Application method: spraying

Environmental parameters: temperature 16.5 - 23.5 °C, photoperiod: 16 h light / 8 h dark, 5000 - 34000 lux, relative humidity 53 - 95 %; Soil pH 7.5

Biological observations: Observations on plant condition and growth were made regularly. Endpoints: phytotoxicity, fresh weight of shoot at study termination

Statistical evaluation: Student's t-test

Findings:

Table B.9.9.1-1: Effects of Cymoxanil 50 WP (0.24 kg a.s./ha) on vegetative vigour of six non-target plant species

Plant species	Fresh shoot biomass (g)		
	Mean control	Mean treated	% reduction ^a
Monocot species			
<i>Allium cepa</i>	8.74	4.83*	44.77
<i>Avena sativa</i>	25.16	21.69*	13.78
Dicot species			
<i>Beta vulgaris</i>	9.60	7.42*	22.75
<i>Cucumis sativus</i>	36.84	26.21*	28.85
<i>Glycine max</i>	57.49	52.72*	8.30
<i>Helianthus annuus</i>	22.85	19.19*	16.04

* significantly ($p < 0.05$) different from the control (Student's t-test)

^a relative to the control

Chlorosis, absent in *B. vulgaris* and *H. annuus*, was evident among some plants of the other four species at termination, for two species from day 14 onwards. There were no effects of treatment on plant development. Weights of shoots treated with Cymoxanil 50 WP were significantly reduced by comparison with the appropriate water-treated controls for all species, with reductions ranging from 8.30 % (*G. max*) to 44.77 % (*A. cepa*).

Conclusion:

The ER₅₀ of Cymoxanil 50 WP for vegetative vigour, based on shoot biomass measurements made 21 days post-treatment, exceeded the limit rate of 0.24 kg a.s./ha for plants of six non-target species. The most sensitive species, *Allium cepa*, showed a reduction of shoot biomass of 44.77 %.

Table 9.9.1-2: Effect of Cymoxanil 50WP on plant growth: Most Sensitive Monocot and Dicot Plant Species

Endpoint	Vegetative vigour
Monocot	
Most sensitive species	<i>Allium cepa</i>
NOEC for the most sensitive parameter (e.g., dry weight)	n.d.
EC50 for the most sensitive monocot	> 240 g a.s./ha
Dicot	
Most sensitive species	<i>Cucumis sativus</i>
NOEC for the most sensitive parameter (e.g., dry weight)	n.d.
EC50 for the most sensitive dicot	> 240 g a.s./ha

B.9.9.2 Effects on other non-target organisms (ANNEX IIA 8.6; ANNEX IIIA 10.8)

No data submitted.

B.9.9.2.1 Summaries from biological screening data on fungicidal/insecticidal/herbicidal activity (ANNEX IIA 8.6; ANNEX IIIA 10.8)

Not submitted.

B.9.9.3 Summary of effects on other non-target organisms

B.9.9.3.1 Summary of toxicity to terrestrial non-target plants

Table B.9.9.3.1-1: Summary of toxicity to terrestrial non-target plants.

Test-substance	Species	Endpoint	Phytoxicity	Plant weight	Reference
Cymoxanil 50WP	<i>Allium cepa</i>	NOEC EC ₅₀	chlorosis n.d. n.d.	Shoot biomass n.d. > 240 g a.s./ha	IIIA 10.8 Balluff, 2003

n.d. not determined

B.9.9.3.2 Risk assessment

Terrestrial non-target plants may be exposed to cymoxanil by spray drift in the vicinity of the treated area. A quantitative risk assessment approach was taken according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002). TERs were determined based on drift deposition at 1 m from the edge of the field considering a multiple application factor (MAF) and the worst case application scenario for the crops lettuce and potatoes.

Table B.9.9.3.2-1: Risk assessment for non-target plants

Test substance	Application rate (MAF)	ER ₅₀ (g/ha)	Bufferzone (m)	% spray drift	Exposure ¹ (g/ha)	TER	Trigger
lettuce							
Cymoxanil 50WP	4 x 240 g a.s./ha (2.7)	> 240 g a.s./ha	1	1.85 %	12	> 20	5
potatoes							
Cymoxanil 50WP	8 x 175 g a.s./ha (3.5)	> 240 g a.s./ha	1	1.52 %	9.3	> 26	5

¹ based on Ganzelmeier drift data

The resulting TERs exceed the trigger for all intended GAPs and indicate that at a distance of 1 m to the edge of the field no significant damage will occur to non-target terrestrial plants.

This is based on the effects of the "single formulation" Cymoxanil 50WP and also covers the cymoxanil component of the formulation TANOS. For national authorisations of TANOS the RMS recommends a risk assessment based on effects data for the combination product.

B.9.9.3.3 Conclusion

The submitted studies on terrestrial plants and the calculated TER-values indicate that the risk for non-target plants after the use of cymoxanil according to GAP is low at a distance of 1 m to the treated area.

B.9.9.4 Effects on biological methods of sewage treatment (Annex IIA 8.7)

B.9.9.4.1 Laboratory studies

Reference: Desmares-Koopmans, M.J.E. (2001): Activated sludge respiration inhibition test with cymoxanil technical (contact time: 3 hours) (Oxon Report No.: 308767)

Guideline(s): OECD 209

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: cymoxanil technical, batch no.: 89800028, purity 98.9 %

Test system: Microorganisms in activated sludge from a sewage treatment plant

Treatments: nominal concentrations of 0 (control), 0.1, 0.32, 1.0, 3.2, 10 and 32 mg/L, toxic reference: 3,5-dichlorophenol

Test conditions: Contact time: 3 h; test substance mixed with synthetic sewage feed and aerated; glass test vessels of 1 L, synthetic sewage feed; environmental parameters: pH 7.7, temperature of test medium 20.9 °C

Biological observations: respiration rate and percentage reductions in each of the cymoxanil and reference treatments were assessed in relation to the mean control rate

Statistical evaluation: EC determined using linear regression

Findings:

Table B.9.9.4.1-1: Effects of cymoxanil technical on the respiration rate of activated sludge

Cymoxanil concentration (mg/L)	Mean respiration rate (mg O ₂ /L/h)	Inhibition of respiration rate (%)
Control	36	-
0.1	42	-18 ^a
0.32	36	-1
1.0	33	7
3.2	27	24
10	20	44
32	16	55

^a negative values indicate stimulation of respiration

The inhibition data obtained for cymoxanil show a dose-response relationship whereas respiration was stimulated at 0.1 mg/L, but the effect was reversed at 1.0 mg/L and statistically significant (>10 %) inhibition occurred at concentrations of 3.2 mg/L and above. The maximum inhibition (55 %) occurred at the highest nominal concentration (32 mg/L). The two individual control rates met the 15 % conformity requirement.

EC₅₀ of positive control (3,5-DCP) = 7 mg/L (range given in guideline: 5 - 30 mg/L)

Conclusions:

EC₅₀ of cymoxanil techn. = 19.4 mg/L, 95 % C.I.: 6.6 - 56.7 mg/L

B.9.9.4.2 Summary and risk assessment of impact of water treatment procedures

Table B.9.9.4.2-1: Summary of cymoxanil toxicity to biological methods for sewage sludge

Substance	Media	Process	EC ₅₀ (mg/L)	Reference
cymoxanil techn.	activated sludge	respiration rate	19.4	Desmares-Koopmans, 2001

B.9.9.4.3 Overall conclusion

For the effects of cymoxanil on the inhibition of the respiration rate of aerobic wastewater micro-organisms a value EC_{50} of 19.4 mg/L was determined. Therefore it can be assumed that adverse effects on methods of sewage treatment are unlikely, when cymoxanil is applied according to GAP.

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B.9.10 References relied on

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company. Report No GLP or GEP status (where relevant). Published or not	Data Protection Claimed Y/N-R/NR	Owner
Annex II Data and Information					
IIA, 8.1.1	Rodgers, M. H.	1996	Cymoxanil Technical acute oral toxicity (LD50) to the bobwhite quail. [REDACTED] OXN 69A/950758 GLP: Yes Published: No	N	Oxon
IIA, 8.1.1	Heijink, E.	1999	Acute oral toxicity study in the mallard duck with cymoxanil technical [REDACTED] 257377 GLP: Yes Published: No	Y	Oxon
IIA, 8.1.1	Campbell, S.M., Lynn, S.P.	1992	H-19,062-02: An acute oral toxicity study with the mallard [REDACTED] HLO 139-92 GLP: Yes Published: No	N	DuPont
IIA, 8.1.2	Johnson, A.J.	1997	Cymoxanil Technical dietary LC50 to the bobwhite quail [REDACTED] OXN 215/970985 GLP: Yes Published: No	N	Oxon
IIA, 8.1.2	Freiling W.J.A.M., van Dreumel, I.F.	1999	5-Day dietary toxicity study in mallard duck [REDACTED] 257401 GLP: Yes Published: No	Y	Oxon
IIA, 8.1.3	Gallagher, S.P., Foster, J.W., Beavers, J.B., Jaber, M.	1996a	DPX-T3217-113 (cymoxanil): A reproduction study with the northern bobwhite (<i>Colinus virginianus</i>) [REDACTED] AMR 3507-95 GLP: Yes Published: No	N	DuPont
IIA, 8.1.3	Gallagher, S.P., Foster, J.W., Beavers, J.B., Jaber, M.	1996b	DPX-T3217-113 (cymoxanil): A reproduction study with the mallard (<i>Anas platyrhynchos</i>) [REDACTED] AMR 3508-95 GLP: Yes Published: No	N	DuPont
IIA, 8.2.1	Baer, K.N.	1993a	Static, acute, 96-hour LC ₅₀ of DPX-T3217-113 (cymoxanil) to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] HLR 735-92 GLP: Yes Published: No	N	DuPont

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IIA, 8.2.1	Baer, K.N.	1993b	Static, acute, 96-hour LC ₅₀ of DPX-T3217-113 (cymoxanil) to bluegill sunfish, <i>Lepomis macrochirus</i> [REDACTED] HLR 834-92 GLP: Yes Published: No	N	DuPont
IIA, 8.2.1	Boeri, R.L., Kowalski, P.L., Ward, T.J.	1996a	Acute toxicity of DPX-T3217-113 (Cymoxanil) to the sheepshead minnow, <i>Cyprinodon variegatus</i> [REDACTED] HLO 634-96 GLP: Yes Published: No	N	DuPont
IIA, 8.2.1	Boeri, R.L., Wyskiel, D.C., Ward, T.J.	2002a	IN-T4226: Static-renewal, acute, 96-hour limit test to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] DuPont-9386 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.1	Samel, A.	2002a	IN-KQ960: Static, acute, 96-hour limit test to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] DuPont-9560 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.1	Samel, A.	2002b	IN-U3204: Static-renewal, acute, 96-hour limit test to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] DuPont-9558 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.1	Boeri, R.L., Wyskiel, D.C., Ward, T.J.	2002b	IN-W3595: Static, acute, 96-hour limit test to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] DuPont-9384 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.1	Migchielsen, M.H.J., Brekelmans, M.J.C. Willems, H.	2003	Demonstrating exposure of rainbow trout to cymoxanil degradation products during a 96-hour acute toxicity study in rainbow trout (limit test under static conditions) [REDACTED] 363904 GLP: Yes Published: No	Y	Oxon
IIA, 8.2.2.1	Baer, K.N.	1992a	Flow-through, 21-day toxicity of DPX-T3217-113 (cymoxanil) to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] HLR 545-92 GLP: Yes Published: No	N	DuPont

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IIA, 8.2.2.2	Boeri, R.L., Magazu, J.P., Ward, T.J.	1997	DPX-T3217-113 (cymoxanil): Early life-stage toxicity to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] HLO 1013-96, Vol 1-3 GLP: Yes Published: No	N	DuPont
IIA, 8.2.2.2	Boeri, R.L., Kowalski, P.L., Ward, T.J.	1996b	Early life-stage toxicity of DPX-T3217-113 (cymoxanil) to the sheepshead minnow, <i>Cyprinodon variegatus</i> [REDACTED] HLO 913-96 GLP: Yes Published: No	N	DuPont
IIA, 8.2.4	Baer, K.N.	1993c	Static, acute, 48-hour EC ₅₀ of DPX-T3217-113 (cymoxanil) to <i>Daphnia magna</i> DuPont Haskell Laboratory HLR 736-92 GLP: Yes Published: No	N	DuPont
IIA, 8.2.4	Boeri, R.L., Kowalski, P.L., Ward, T.J.	1996c	Acute toxicity of DPX-T3217-113 (cymoxanil) to the mysid, <i>Mysidopsis bahia</i> T. R. Wilbury Laboratories, Inc. HLO 632-96 GLP: Yes Published: No	N	DuPont
IIA, 8.2.4	Boeri, R.L., Kowalski, P.L., Ward, T.J.	1996d	Acute flow-through mollusc shell deposition test with DPX-T3217-113 (cymoxanil) T. R. Wilbury Laboratories, Inc. HLO 633-96 GLP: Yes Published: No	N	DuPont
IIA, 8.2.4	Boeri, R.L., Wyskiel, D.C., Ward, T.J.	2002c	IN-T4226: Acute, 48-hour EC ₅₀ to <i>Daphnia</i> <i>magna</i> T. R. Wilbury Laboratories, Inc. DuPont-9385 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.4	Boeri, R.L., Wyskiel, D.C., Ward, T.J.	2002d	IN-W3595: Acute, 48-hour EC ₅₀ to <i>Daphnia</i> <i>magna</i> T. R. Wilbury Laboratories, Inc. DuPont-9383 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.4	Samel, A.	2002c	IN-U3204: Static-renewal, acute, 48-hour EC ₅₀ to <i>Daphnia magna</i> DuPont Haskell Laboratory DuPont-9557 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.4	Samel, A.	2002d	IN-KQ960: Static, acute, 48-hour EC ₅₀ to <i>Daphnia</i> <i>magna</i> DuPont Haskell Laboratory DuPont-9559 GLP: Yes Published: No	Y	DuPont

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IIA, 8.2.5	Baer, K.N.	1993d	Chronic toxicity of DPX-T3217-113 (cymoxanil) to <i>Daphnia magna</i> : 24-Hour renewal DuPont Haskell Laboratory HLR 354-93, Revision No. 1 GLP: Yes Published: No	N	DuPont
IIA, 8.2.5	Samel, A.	2003	IN-KQ960: 21-Day chronic toxicity to <i>Daphnia magna</i> DuPont Haskell Laboratory DuPont-11971 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.6	Boeri, R.L., Magazu, J.P., Ward, T.J.	1999	Cymoxanil technical: Growth and reproduction test with the freshwater alga, <i>Selenastrum capricornutum</i> T.R. Wilbury Laboratories, Inc. DuPont-2498 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.6	Bell, G.	1996	Cymoxanil technical: algal growth inhibition Huntingdon Life Sciences, Ltd OXN 107A(a)/950955 GLP: Yes Published: No	N	Oxon
IIA, 8.2.6	Hughes, J.S., Williams, T.L., Conder, L.A.	1996a	DPX-T3217-113 (cymoxanil): Influence on growth and reproduction of <i>Anabaena flos-aquae</i> Carolina Ecotox, Inc. AMR 4109-96 GLP: Yes Published: No	N	DuPont
IIA, 8.2.6	Sloman, T.L.	2001a	IN-T4226: Influence on growth and growth rate of the blue-green alga <i>Anabaena flos-aquae</i> DuPont Haskell Laboratory DuPont-3747 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.6	Sloman, T.L.	2001b	IN-W3595: Influence on growth and growth rate of the blue-green alga <i>Anabaena flos-aquae</i> DuPont Haskell Laboratory DuPont-3748 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.8	Leva, S.E., Sloman, T.L.	1996	Cymoxanil: Influence on growth and reproduction of <i>Lemna gibba</i> G3 DuPont Stine-Haskell Research Center AMR 3775-96 GLP: Yes Published: No	N	DuPont

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IIA, 8.3.1.1	Schur, A.	1999	Assessment of side effects of cymoxanil technical to the honey bee, <i>Apis mellifera</i> L. in the laboratory GAB Biotechnologie GmbH and IFU Umweltanalytik GmbH 99063/01-BLEU GLP: Yes Published: No	Y	Oxon
IIA, 8.3.2	Geuijen, W.H.C.	1999a	Effects of Cymoxanil 50% WP on survival and reproduction of the parasitic wasp <i>Aphidius rhopalosiphii</i> in the laboratory NOTOX BV. 257816 GLP: Yes Published: No	Y	Oxon
IIA, 8.3.2	Geuijen, W.H.C.	2000	Effects of Cymoxanil 50% WP on survival and reproduction of the phytoseiid mite <i>Typhlodromus pyri</i> Scheuten NOTOX BV. 257827 GLP: Yes Published: No	Y	Oxon
IIA, 8.3.2	Geuijen, W.H.C.	1999b	Effects of Cymoxanil 50% WP on survival of the carabid beetle <i>Poecilus cupreus</i> NOTOX BV. 257838 GLP: Yes Published: No	Y	Oxon
IIA, 8.3.2	Geuijen, W.H.C.	1999c	Effects of Cymoxanil 50% WP on survival and reproduction of the green lacewing <i>Chrysoperla carnea</i> (laboratorium test) NOTOX BV. 257849 GLP: Yes Published: No	Y	Oxon
IIA, 8.3.2	Müther, J.	2002	A field study to evaluate the effects of Cymoxanil 50% WP on predatory mites (Acari: phytoseiidae) in vines in France Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Germany, 20021069/F1-NFTp GLP: Yes Published: No	Y	Oxon
IIA, 8.4.1	Rodgers, M.H., Cameron, D.M., Mansell, P.	1995	Cymoxanil technical acute toxicity (LC50) to the earthworm (<i>Eisenia foetida</i>) Huntingdon Life Sciences Ltd. OXN 78A/950675 GLP: Yes Published: No	N	Oxon

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IIA, 8.4.2	Lührs, U.	2000	Cymoxanil/famoxadone (DPX-KP481): A dose response sublethal study to the earthworm, <i>Eisenia fetida</i> (Savigny 1826), in artificial soil IBACON DuPont-4611 GLP: Yes Published: No	Y	DuPont
IIA, 8.5	Kölzer, U.	2003	Assessment of the side effects of cymoxanil technical on the activity of the soil microflora Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH. 20031314/01-ABMF GLP: Yes Published: No	Y	Oxon
IIA, 8.6	Balluff, M.	2003	Vegetative vigour limit test for non target plants following single rate application of Cymoxanil 50 WP Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH. 20033057/S1-FGW GLP: Yes Published: No	Y	Oxon
IIA, 8.7	Desmares-Koopmans, M.J.E.	2001	Activated sludge respiration inhibition test with cymoxanil technical (contact time: 3 hours) NOTOX BV. 308767 GLP: Yes Published: No	Y	Oxon
Annex III Data and Information					
IIIA, 10.1.1.	Lueschner, J.	2003	Avian toxicity study of Cymoxanil 50 WP oral toxicity in the Japanese quail – limit test according to SETAC guideline document. [REDACTED] Report 17232/03 GLP: Yes Published: No	N	Oxon
IIIA 10.2.1	Bogers M.	1999a	96-hour acute toxicity study in rainbow trout with Cymoxanil 50 % WP (semi-static) [REDACTED] Report 262428 GLP: Yes Published: No	Y	Oxon
IIIA, 10.2.1	Brown, M.R.	1997a	DPX-KP481-25 50WG: Flow-through, acute, 96-hour LC ₅₀ to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] HL-1997-00440 GLP: Yes Published: No	N	DuPont
IIIA 10.2.1	Hoke, R.A.	1998a	DPX-KP481-25 50WG: Static, acute, 96-hour LC ₅₀ to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] DuPont-1148 GLP: Yes Published: No	N	DuPont

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IIIA 10.2.1	Migchielsen M.H.J.	1999	Acute toxicity study in <i>Daphnia magna</i> with Cymoxanil 50 % WP (semi-static) NOTOX B.V., The Netherlands, Report 262439 GLP: Yes Published: No	Y	Oxon
IIIA, 10.2.1	Brown, M.R.	1997b	DPX-KP481-25 50WG: Flow-through, acute, 48- hour EC ₅₀ to <i>Daphnia magna</i> DuPont Haskell Laboratory HL-1997-00437 GLP: Yes Published: No	N	DuPont
IIIA, 10.2.1	Hoke, R.A.	1998b	DPX-KP481-25 50WG: Static, acute, 48-hour EC ₅₀ to <i>Daphnia magna</i> DuPont Haskell Laboratory DuPont-1076 GLP: Yes Published: No	N	DuPont
IIIA 10.2.1	Bogers M.	1999b	Fresh water algal growth inhibition test with Cymoxanil 50 % WP NOTOX B.V., The Netherlands, Report 262441 GLP: Yes Published: No	Y	Oxon
IIIA, 10.2.1	Leva, S.E., Sloman, T.L.	1997	DPX-KX007: Influence on growth and growth rate of the green alga <i>Pseudokirchneriella subcapitata</i> (formerly called <i>Selenastrum capricornutum</i>) DuPont Stine Research Center AMR 4116-96 GLP: Yes Published: No	N	DuPont
IIIA, 10.4.1	Candolfi, M.P.	1996	DPX-KX007-5: Laboratory acute oral and contact toxicity test with the honeybee, <i>Apis mellifera</i> , based on the EPPO guideline 170 (1992) and FIFRA guideline 141-1 Springborn Laboratories (Europe) Ag AMR 3828-96 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1.	Beech, P. Mead-Briggs, M.	1998	Cymoxanil/Famoxadone (DPX-KP481) 50 WG (25%:25%). An extended laboratory study to evaluate the effects on the ground beetle, <i>Poecilus cupreus</i> University of Southampton, Agrochemical Evaluation Unit (AEU) AMR 4539-97 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1.	Grove, A.J.	1996b	Extended laboratory test comparing the effects of DPX-KX007 (containing DPX-JE874 and cymoxanil) WG and SC formulations on <i>Typhlodromus pyri</i> , following multiple applications on grapevine leaves under greenhouse conditions MITOX Stichting Bevordering Duurzame Plaagbestijding AMR 3812-96 GLP: Yes	N	DuPont

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			Published: No		
IIIA, 10.5.1.	Hermann, P.	2001	DPX-KP481-25: A semi-field study to evaluate the effects on the hoverfly, <i>Episyrphus balteatus</i> Deg. (Diptera, Syrphidae) GAB Biotechnologie, GmbH DuPont-3881 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1.	Kuehner, C.	1997a	DPX-KP481, a water-dispersible granular (WG) formulation, containing JE874 (254 g ai/kg) and cymoxanil (266 g ai/kg): Acute toxicity to the hoverfly, <i>Episyrphus balteatus</i> Deg. (Diptera, Syrphidae) in the laboratory GAB Biotechnologie GmbH AMR 4232-96 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1.	Kuehner, C.	1997b	DPX-KP481, a water-dispersible granular (WG) formulation, containing JE874 (254 g ai/kg) and cymoxanil (266 g ai/kg): Acute toxicity to the ground beetle, <i>Poecilus cupreus</i> L. (Coleoptera, Carabidae) in the laboratory GAB Biotechnologie GmbH AMR 4233-96 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1.	Kuehner, C.	1997c	DPX-KP481, a water-dispersible granular (WG) formulation, containing JE874 (254 g ai/kg) and cymoxanil (266 g ai/kg): Acute toxicity to the green lacewing, <i>Chrysoperla carnea</i> Steph. (Neuroptera, Chrysopidae) in the laboratory GAB Biotechnologie GmbH AMR 4234-96 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1.	Kuehner, C.	1997d	DPX-KP481, a water-dispersible granular (WG) formulation containing DPX-JE874 (254 g ai/kg) and cymoxanil (266 g ai/kg): Acute toxicity to the staphylinid beetle, <i>Aleochara bilineata</i> (Coleoptera, Staphylinidae) in the laboratory GAB Biotechnologie, GmbH AMR 4235-96 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1.	Thompson, B.	1999a	Cymoxanil/Famoxadone (DPX-KP481) 50 WG (25%:25%) an extended laboratory study to evaluate the effects on the hoverfly, <i>Episyrphus balteatus</i> University of Southampton, Agrochemical Evaluation Unit (AEU) AMR 4541-97 GLP: Yes Published: No	N	DuPont

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company. Report No GLP or GEP status (where relevant). Published or not	Data Protection Claimed Y/N-R/NR	Owner
IIIA, 10.5.1.	Thompson, B.	1999b	Cymoxanil/Famoxadone (DPX-KP481) 50 WG (25%:25%): A semi-field study to evaluate the effects on aphid parasitoids, (Hymenoptera, Aphidiinae) in a potato crop University of Southampton, Agrochemical Evaluation Unit (AEU) AMR 4542-97 GLP: Yes Published: No	N	DuPont
IIIA, 10.5.1.	Thompson, B.	1999c	Cymoxanil/Famoxadone (DPX-KP481) 50 WG (25%:25%). An extended laboratory study to evaluate the effects on the green lacewing, <i>Chrysoperla carnea</i> University of Southampton, Agrochemical Evaluation Unit (AEU) AMR 4540-97 GLP: Yes Published: No	N	DuPont
IIIA, 10.6.1.1.	Rodgers, M.H.	1995	Cymoxanil 50 % WP - Acute toxicity (LC50) to the earthworm (<i>Eisenia foetida</i>) Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England OXN 81A/943245 GLP: Yes Published: No	N	Oxon
IIIA, 10.6.1.1	Wachter, S.	1997	Acute toxicity of DPX-KX007-5 on earthworms, <i>Eisenia foetida</i> using an artificial soil test GAB Biotechnologie, GmbH AMR 4136-96 GLP: Yes Published: No	N	DuPont