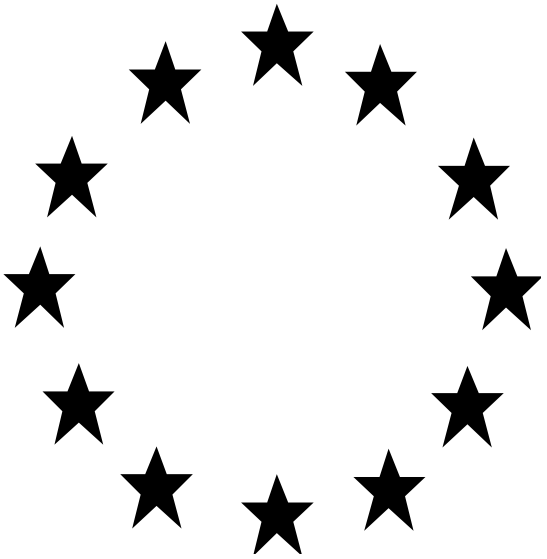

Final addendum to the

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State the United Kingdom for the new active substance

PROQUINAZID

as referred to in Article 8(1) of Council Directive 91/414/EEC

July 2009

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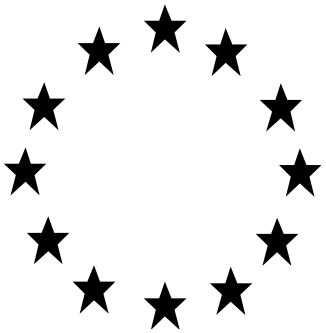
Council Directive 91/414/EEC



Proquinazid

Addendum 1
to
Annex B
to the Report and Proposed Decision of the United Kingdom made to the European Commission under Article 8 of 91/414/EEC

B.5 Methods of Analysis

Date December 2007

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B.5.2 Analytical methods (residue) for treated plants, plant products, foodstuffs of plant and animal origin and feedingstuffs (IIA 4.2.1, IIIA 5.2)

B.5.2.1 Plant Matrices - DFG S19 Modified

Watery and dry crops were extracted with acetone:water (2:1 v/v) followed by the addition of NaCl and ethyl acetate/cyclohexane which caused the partition of proquinazid into the organic phase. Oily crops were extracted with acetone/acetonitrile (25:225 v/v) after the addition of Calflo E and Celite. Following concentration, gel permeation chromatography (GPC), and further fractionation on a silica gel mini-column residues of proquinazid were determined as follows:

1) By GC-ECD with a DB-5MS column (30m x 0.25 mm i.d., film thickness 0.25µm) or equivalent.

2) Residues were also determined by GC/MSD in selected crops for selected fortification levels using the same column as for GC-ECD (or equivalent) through the acquisition of three of the ions, m/z 216, 245, 272, 288 and 330.

Validation data have been submitted for wheat grain, oilseed rape, grapes, apple and wheat straw and are presented in Table B.5.1.

(Class, 1999)

Independent laboratory validation was performed on wheat grain, oilseed rape and grapes (dry, watery and oily crops). For the ILV samples were analysed by GC/MSD only with quantification on the m/z 288 ion and further confirmation by m/z 245 and 272. Validation data are presented in Table B.5.1.

(Reichert, 2003a)

In response to questions regarding the validation data for the GC-ECD method and the use of GC-MSD only for the ILV the Notifier provided further information (e-mail from Brown, 2004a and reference to the study of Class and Hornshuh 2002 for further validation of wheat straw). From this the Notifier proposed using GC-MSD as the primary means of detection for determining residues.

(Class and Hornshuh, 2002; Brown, 2004a)

Evaluation of Analytical method (residue) for treated plants, plant products, foodstuffs of plant and animal origin and feedingstuffs

GC-ECD was the primary determination technique used in the method validation study with GC-MS used as the confirmatory method. However, analysis by GC-ECD was found to be unsuitable for some matrices due to interfering peaks. Some, but not all of the validation data in the original report were determined by GC-ECD and GC-MS. The ILV data were determined by GC-MS only. Consequently whilst there is fully acceptable validation data using GC-MS available for the ILV, the initial validation data is lacking in some fortification levels determined by GC-MS and some of the data obtained using GC-ECD is unacceptable according to current guidelines (e.g. recovery and repeatability data for wheat and apples at a fortification level of 0.01 mg/kg). The validation data available for oilseed rape measured by GC-ECD in the original study were corrected for interferences present in the control samples (See note 2 for Table B.5.1).however as the levels found in controls were less than 20% of the LOQ the data are considered acceptable.

The Notifier has proposed using GC-MS as the primary means of detection and confirmation of residues through the use of ions with m/z ratios 245 and 272.

Although the data generated by GC-MS for the original validation study do not fully meet the current guidance, the validation data provided at the LOQ and determined by GC-MS are acceptable for watery and dry crops. The ILV validation data are acceptable for watery, dry and oily crops. It is considered that the weight of evidence indicates that method is acceptable.

Table B.5.1 Summary of method description and validation for the determination of proquinazid residues (treated plants, plant products, foodstuffs, feedingstuffs)

Substrate	Quantification (ion)	Specificity and Interference	Limit of quantif-ication (mg/kg)	Recovery fortifica-tion level (mg/kg)	Recoveries % range (mean)	Repeatabil-ity RSD (%) (n)	Linearity demon-strated	Ref.
Apple	GC-ECD	Interferences noted at levels < 10% of LOQ	0.01	0.01 0.02 0.20	83 - 113 (98) 78 – 88 (82)	13 (n=5) 7 (n=5)	See note (1)	Class, 1999;
	GC/MS (m/z 288)	No interference.	0.01	0.01	80-105 (89)	13 (n=5)	0.002 – 0.50 µg/ml	Class, 1999;
Grape	GC-ECD	Interferences noted at levels < 10% of LOQ	0.01	0.01 0.02 0.20	78 - 107 (92) 71- 85 (76) 72 - 80 (74)	11 (n=5) 7 (n=5) 5 (n=5)	See note (1)	Class, 1999;
	GC/MS (m/z 288)	No interference.	0.01	0.01	79-95 (85)	8 (n=5)	0.002 – 0.50 µg/ml	Class, 1999;
Wheat Grain	GC-ECD	Interferences noted at levels < 10% of LOQ	0.01	0.01 0.02 0.20	71 - 149 (94) 74 – 94 (80) 71 – 82 (78)	10 (n=5) 6 (n=5)	See note (1)	Class, 1999;
	GC/MS (m/z 288)	No interference.	0.01	0.01	75-101 (93)	14 (n=5)	0.002 – 0.50 µg/ml	Class, 1999
Wheat Straw	GC-ECD	Interferences noted at levels < 10% of LOQ	0.1	0.1 1.0	79 – 94 (87) 69 – 94 (81)	8 (n=5) 14 (n=5)	See note (1)	Class, 1999;
	GC/MS (m/z 288)	No interference.	0.1	0.1 1.0	71–93 (84) 68-102 (82)	10 (n=6) 18 (n=4)	0.002 – 0.50 µg/ml	Class and Hornshuh, 2002
Oilseed Rape Seed	GC-ECD	Interferences noted at levels < 20% of LOQ	0.02	0.02 0.2	86-111 (100) 64 – 85 (74) See note (2)	11 (n=5) 11 (n=5)	See note (1)	Class, 1999;
	GC/MS – m/z 288	No data	No data	No data	No data	No data	No data	Class, 1999;

I.L.V.								
Wheat Grain	GC/MS – m/z 288	No interference	0.01	0.01 0.10	69-79 (75) 73-80 (77)	5 (5) 3 (5)	0.002 – 0.50 µg/ml	Reichert, 2003a
Oilseed Rape	GC/MS – m/z 288	No interference	0.02	0.02 0.20	77-101 (91) 78-101 (93)	10 (5) 10 (5)	0.002 – 0.50 µg/ml	Reichert, 2003a
Grapes	GC/MS – m/z 288	Small interference in blank matrix, applicant states that this is well under 30 % of LOQ.	0.01	0.01 0.10	70-89 (78) 81-85 (83)	9 (5) 2 (5)	0.002 – 0.50 µg/ml	Reichert, 2003a

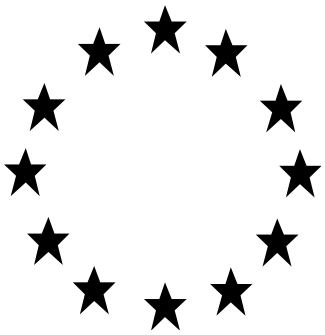
Notes: (1) The ECD method gave a non-linear response between 0.002 – 0.05 µg/ml. The study reported that two separate quadratic calibration functions ranging from 0.002 – 0.05 µg/ml (for extracts containing < 0.05 µg/ml) and 0.002 to 0.5 µg/ml (for extracts containing > 0.02 µg/ml) were therefore used. Typical calibration plots were submitted, with $R^2 = 0.998$ for both ranges.

(2) The results were presented corrected for the level of interference found in control samples. The uncorrected recovery data are for the 0.02 mg/kg level: 99-125% (mean = 113%), RSD 9% (n=5); for 0.2 mg/kg level: 66 -85% (mean = 76%), RSD 10 % (n=5).

References

Annex point	Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
IIA, 4.2.1./01	Class, T.	1999	Method assessment and validation of an analytical multi-residue enforcement method (DFG S19 Modified) for the determination of residues of DPX-KQ926 in cereal, oilseed and fruit PTRL Europe AMR 4943-98 GLP: Yes Published: No	Y	DuPont
IIA, 4.2.1./05	Reichert, N.	2003a	Independent laboratory validation of the analytical multi-residue enforcement method (DFG S19 modified) for the determination of residues of DPX-KQ926 in cereal, oilseed and fruit Institut Fresenius Chemische und Biologische/GmbH DuPont-11254 GLP: Yes Published: No	Y	DuPont
IIA 4.2.1	Brown, J	2004a	DuPont response to questions from PSD dated 13 September 2004 regarding proquinazid crop residue method validation. (email of 28/9/04) GLP: No Published: No	Y	DuPont

Council Directive 91/414/EEC



Proquinazid

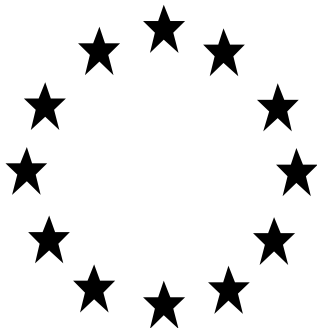
Addendum
to
Annex C
to the Report and Proposed Decision of the United Kingdom made to the European Commission under Article 8 of 91/414/EEC

Confidential

Date December 2007

Confidential information available at the RMS

Council Directive 91/414/EEC



Proquinazid

Addendum 2
to
Annex B
to the Report and Proposed Decision of the United Kingdom made to the European Commission under Article 8 of 91/414/EEC

B.2 Physical and chemical properties

March 2009

CONTENTS

B.2 PHYSICAL CHEMICAL PROPERTIES

B.2.1 Physical and chemical properties of the active substance

Table B.2.1 Summary of the physical and chemical properties of the active substance (studies were completed to an acceptable standard and results were considered to be valid unless specified otherwise) [Pure = 99.2 %; Technical. = 97 %, unless stated otherwise]

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.2 (IIA 2.1)	Boiling point	99.6%	EEC A1	Not observed at temperatures below 360°C. Thermal decomposition occurred at 367°C	To GLP	Burwood, C.E; 2007
B.2.1.3 (IIA 2.1)	Temperature of decomposition or sublimation	99.6%	EEC A1	Thermal decomposition occurred at 367°C	To GLP	Burwood, C.E; 2007

B.2.2 Physical, chemical and technical properties of the plant protection product

Product name: „Proquinazid 200 g/L EC'

section (Annex point)	study	method	results	comment	reference
B.2.2.15 (IIIA 2.7)	Shelf life		1L HDPE/EVOH, 24 months ambient: Appearance Initial: Brown/amber liquid, pungent sweet ester-like odour. 24 months: Brown liquid, pungent sweet ester-like odour. A.s. content. Initial: 200.9 g/L 24 months: 198.7g/L pH (CIPAC MT 75) Initial: 6.8 (1% dilution) 24 months: 5.2 (1% dilution) Emulsion characteristics (CIPAC MT 36.3) 24 months: uniform emulsion after 30 seconds, 30 mins and 2 hours Re-emulsification after 24 hrs acceptable. Approx. 1 mm of creamy foam noted after standing for 30 mins after re-emulsification. Results for both CIPAC waters A & D were the same. Pack weight changed from 1083.79g to 1081.77g (= loss of 0.19% w/w). Slight paneling noted at one corner of pack, however no evidence of leakage.		Wong, DKH; 2006a

section (Annex point)	study	method	results	comment	reference
B.2.2.15 (IIIA 2.7) Cont'd	Shelf life		250 ml PET, 27 months ambient: Appearance Initial: Brown/amber liquid, pungent sweet ester-like odour. 27 months: Brown liquid, pungent sweet ester-like odour. A.s. content. Initial: 200.9 g/L 27 months: 199.9 g/L pH (CIPAC MT 75) Initial: 6.8 (1% dilution) 27 months: 5.2 (1% dilution) Emulsion characteristics (CIPAC MT 36.3) 27 months: uniform emulsion after 30 seconds, 30 mins and 2 hours Re-emulsification after 24 hrs acceptable. Approx. 1 mm of creamy foam noted after standing for 30 mins after re-emulsification. Results for both CIPAC waters A & D were the same. Pack weight changed from 285.3g to 285.6g (= increase of 0.12% w/w). Slight paneling noted on one side, however no evidence of leakage.		Wong, DKH; 2006b

B.2.3 Summary of physical and chemical properties

B.2.3.1 Active substance

Pure proquinazid is a white crystalline solid with a melting point of 62°C and is very slightly volatile (9×10^{-5} Pa at 25°C). The technical material (purity 97 %) is a brown wax-like crystalline solid. Proquinazid is slightly soluble in water (0.97 mg/l at 25°C in HPLC grade water) and is soluble (>250 g/l at 25°C) in acetone, dichloromethane, dimethylformamide, ethyl acetate, n-hexane, 1-octanol and *o*-xylene.

The octanol/water partition coefficient (Log k_{OW} = 5.5 at 25°C) indicates a potential to bio-accumulate. The pure active substance is hydrolytically stable at pH 4, 7 and 9 after 20 days at 30°C, has a photolytic half life of 0.03 days at pH 7 in sterile aqueous buffered solution (pH 7), producing two products initially, IN-MM671 and IN-MM986 which also degraded further to IN-MT884 and IN-MM991 respectively.

The pure active substance does not require classification with regards to flammability, explosive and oxidising properties.

B.2.3.2 Plant protection product

Proquinazid is formulated as an emulsifiable concentrate, „Proquinazid 200g/L EC', containing 200 g/l of active substance. Data submitted indicated that the formulation was a brown liquid that was chemically and physically stable for 2 weeks at 54°C and at ambient temperatures for 2 years in the sales pack. The formulation is not oxidising, explosive or flammable and the data does not indicate that it is an aspiration hazard. The formulation readily forms a stable emulsion that can be easily re-emulsified.

B.2.4 Conclusion

The information submitted on physical and chemical properties of the active substance and plant protection product is sufficient to support inclusion in Annex I.

References

Active substance

Annex point	Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
B.2.2.15 (IIIA 2.7)	Burwood C E	2007a	DPX-KQ926 (PROQUINAZID): Laboratory study of boiling and decomposition temperatures Covance Laboratories Ltd DuPont-23153, Revision No. 1 Covance Report No. 0550/108-D2149 GLP: Yes Published: No	Y	

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Plant protection product

Annex point	Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
B.2.2.15 (IIIA 2.7)	Wong, D.K.H; Craig, W.B.	2006a	Proquinazid (DPX-KQ926) 200 g/L Emulsifiable Concentrate (EC) Fungicide Formulation: Laboratory Study of Shelf Life Stability in a High Density Polyethylene/ Ethyl Vinyl Alcohol (HDPE/EVOH) Container Inveresk Research DuPont-12184 GLP: Yes Published: No	Y	
B.2.2.15 (IIIA 2.7)	Wong, D.K.H; Craig, W.B.	2006b	Proquinazid (DPX-KQ926) 200 g/L Emulsifiable Concentrate (EC) Fungicide Formulation: Laboratory Study of Shelf Life Stability in a Polyethylene Terephthalate (PET) Container Inveresk Research DuPont-12186 GLP: Yes Published: No	Y	

B.6 TOXICOLOGY AND METABOLISM

Introduction.

The information in this section is provided to aid discussion at the PRAPeR toxicology meeting. Information is included relating to:

1. Reporting Table Open Point 0.1

Technical specification of batches of proquinazid used for toxicology testing compared with proposed technical specification for which Annex I listing sought (see reporting table)

2. Evaluation Table Open Points 2.1 (and 2.2)

Ocular discharge in dogs (see open points 2.1 and 2.2 in evaluation table)

3. Evaluation Table Open Point 2.2

ARfD (open point 2.2 in evaluation table)

4. Evaluation Table Open Point 2.3

Dermal absorption (open point 2.3 in evaluation table)

B.6 Reporting Table Open Point 0.1

Technical specification of batches of proquinazid used for toxicology testing compared with proposed technical specification for which Annex I listing sought

This point is not mentioned in the Evaluation Table however it is identified in the Reporting Table where the conclusion is that the point is addressed with the requirement for the RMS to consider the new information in an Addendum to the DAR. Therefore the point is included here to draw attention to the new information provided and included in the confidential Addendum.

a) Reporting table

At General point 0.3 in the reporting table the following comments appear:

EFSA commented (column 2) that the level of many impurities will be increased compared to the batches tested in tox.

RMS replied (column 3): The notifier has presented further studies to support commercial production. This includes a **revised technical specification for which Annex I listing is sought.** The evaluation is presented in the revised Annex C to the DAR dated March 2009. This Addendum to the confidential volume replaces in its entirety the original Annex C and the earlier Addendum to Volume C, dated December 2007. The revised Annex C is made available in the confidential area of CIRCA.
The Confidential Addendum considers the toxicological significance of the impurities listed in the new proposed technical specification in section C.1.2.d. A comparison table of the batches used for toxicological testing compared with the original and new proposed technical specifications is given at Table C.1.8 of the Confidential Addendum.

All impurities in the proposed technical specification are present at <1% apart an organic solvent (max 2%) and another impurity (max 1.5%).

The toxicological commentary at C.1.2.d of the Confidential Addendum takes into account the view that at concentrations of <1% the main concern from impurities comes from potential genotoxicity (see guidance document on the assessment of the equivalence of technical materials SANCO/10597/2003 –rev7 final 2, 14 December 2005).

It is notable that none of these impurities contain obvious structural alerts for potential DNA reactivity according to the model of Tennant and Ashby (1991). Other information, including the magnitude of the difference in impurity levels, has also been considered.

RMS conclusions are:

- None of the listed impurities, at the levels in the proposed technical specification are considered to be of clear toxicological concern.
- The proposed technical specification is adequately supported by the submitted toxicology studies with proquinazid synthesised by the original and current production process.
- RMS considers that the point raised by EFSA is addressed.

Column 4 states **: Addressed: tox to consider the new information in the addendum.**

b) Evaluation table

There is no mention of this issue in the mammalian toxicology section of the evaluation table. The only mention of this issue being at 1.1 of the identity section(but with no mention of a need for further toxicological consideration).

c) Relevant information from the DAR

In case further consideration is needed at the PRAPeR toxicology meeting the following information from the DAR (which is referred to in the Confidential Addendum) is included below for ease of reference.

Table B.6.1 Batches of proquinazid used for the toxicology studies

Test substance code	**Purity**	**Production process**	**Studies submitted**
DPX-KQ926-21	99.2%	Not stated	ADME studies
DPX-KQ926-45	96.4 - 98%*	Original process	Full package (except for ADME, acute, irritancy and sensitisation), including 90-day feeding/subchronic neurotoxicity study in rats Ames In vivo micronucleus Multigeneration
DPX-KQ926-75	97.9-98.3%	Current process (pilot plant)	Acute oral Acute dermal Skin irritation Eye irritation Skin sensitisation 90-day feeding study in rats (bridging study) Ames (bridging study) In vivo micronucleus (bridging study) Mouse lymphoma (additional genotoxicity study requested by UK ACP)
DPX-KQ926-85	97.7%	Current process (pilot plant)	Acute inhalation Multigeneration (further study, to modern protocol)

* The test substance purity varied slightly on re-characterisation.

Table B.6.52 Summary of genotoxicity studies with proquinazid

Type of study	Concentration range evaluated	Result	Reference
Batch DPX-KQ926-45			
In vitro bacterial mutagenicity (Ames)	10-5000 μg/plate (with and without S9)	Negative	Mathison, 1997
In vitro chromosome aberration (clastogenicity) human lymphocytes	Without S9 Up to 5000 μg/mL With S9 up to 1250 μg/mL	Negative for structural and numerical aberrations However there are limitations to this study: use of only a short exposure period in absence of S9, high levels of cytotoxicity, low positive control responses.	Gudi and Schadly, 1999
In vitro mammalian cell mutagenicity (CHO/HPRT)	Without S9 Up to 35 μg/mL With S9 up to 70 μg/mL	**Findings were not interpretable.** There is concern as to the sensitivity of CHO/HPRT studies. In this study there was considerable variance in mutation frequency between assays and a very low mutation frequency in some negative controls.	San and Clarke, 1997
In vitro unscheduled DNA synthesis rat primary hepatocytes	7.8-125 μg/mL	Negative	San, 1999
In vivo micronucleus mouse bone marrow single dose by gavage	male and female: 360-1440 mg/kg bw	Negative	Gudi, 1999

Type of study	Concentration range evaluated	Result	Reference
Batch DPX-KQ926-75			
In vitro bacterial mutagenicity (Ames)	10-5000 μg/plate (with and without S9)	Negative	Cox, 1998
In vitro mammalian cell mutagenicity (mouse lymphoma assay)	Without S9 Up to 105 μg/mL for 3h, up to 90 μg/mL for 24h With S9 up to 135 or 180 μg/mL for 3h	Negative	Ballantyne, 2005
In vivo micronucleus mouse bone marrow single dose by gavage	male: 720-2000 mg/kg bw female: 360-1440 mg/kg bw	Negative	Wun-Kim, 1999a

B.6.2.6 Skin sensitisation (IIA 5.2.6)

Study	DPX-KQ926 technical: Evaluation of the potential dermal sensitization in the guinea pig (Magnusson-Kligman Maximization Test)
Reference	Hershman, R.J. (1999)
Date performed	in-life phase: 10 November 1998-18 December 1998
Test facility	[redacted]
Report reference	DuPont Report No.: DuPont-1603
Guideline(s)	OECD 406 (1992)
Deviations from the guideline	None
GLP	Mostly conducted to GLP. Dosing preparations were not checked for stability, homogeneity or accuracy of concentration, but this is an acceptable deviation.
Test material	DPX-KQ926 technical **Batch:** KQ926-75 **Purity:** 97.9%
Study acceptable	Yes

Materials and methods:

The dermal sensitisation potential of proquinazid (after being ground to a fine powder) was evaluated by the Magnusson-Kligman Maximisation method in male Hartley albino guinea pigs.

Based on a range-finding study, twenty animals were intradermally induced on Day 1 with a 3% concentration of proquinazid in propylene glycol emulsified with Freund's complete adjuvant. On Test Day 8, animals were topically induced with 0.5 g of the test substance moistened with 0.5 mL of propylene glycol, under an occlusive dressing,

following pre-treatment with SLS. Animals were challenged on Test Day 21, using Hill Top Chambers on separate sites, with 0.5 g of the test substance moistened in 0.5 mL propylene glycol (considered 100%), 0.5 mL of a 1:3 w/v concentration of the test substance in propylene glycol, and 0.5 mL propylene glycol. Due to the similarity of irritation scores at all challenge sites treated with the test substance, animals were rechallenged on day 28 with 0.5 mL of a 1:3 w/v concentration of the test substance in propylene glycol.

Approximately 24 and 48 hours after the challenge and rechallenge phases, the test sites were evaluated for signs of elicited sensitisation. Very faint redness (usually nonconfluent, score of 0.5) was not considered a positive dermal reaction and these scores were not included in the study report. Scores of 1 (faint redness, usually confluent) or greater were required to be indicative of sensitisation.

The same procedures were carried out on a contemporaneous control group of 10 animals except that for the induction phases the test article was replaced by propylene glycol (vehicle control).

Satisfactory positive control results with α-hexylcinnamaldehyde were provided for a study conducted within a month of the present study.

Findings:

Results of range-finding study: Intradermal irritation potential: 1% concentration in propylene glycol produced no redness at 24 hours and no redness to scattered mild redness at 48 hours; the 3% concentration produced no redness to scattered mild redness at 24 and 48 hours; and the 5% concentration produced moderate redness at 24 hours and scattered mild redness to moderate redness at 48 hours. All these concentration in FCA produced moderate redness at 24 and 48h.
In the topical and challenge range-finding studies, no irritation was seen with concentrations of up to 100% a.s.

Results of main study: Two animals died while on study. Gross necropsy findings were not believed to be test substance related.

Skin responses to induction were not reported.

Positive skin responses (all were grade 1) following challenge and re-challenge are shown in Table B.6.20. There was essentially no difference in skin responses between the test substance and control groups.

No test substance-related clinical signs of toxicity were observed. There were no test substance-related body weight effects noted.

Table B.6.20 Maximisation test: challenge and re-challenge results

	Number of animals with positive dermal response/number tested			
	Test group		Control Group	
Hours after exposure	24	48	24	48
After challenge				
0.5 g of proquinazid moistened in 0.5 mL propylene glycol	3/18 (17%)	2/18 (11%)	1/10 (10%)	0/10
0.5 mL of a 1:3 w/v concentration of proquinazid in propylene glycol	3/18 (17%)	2/18 (11%)	1/10 (10%)	0/10
0.5 mL propylene glycol	0/18	0/18	0/10	0/10
After re-challenge				
0.5 mL of a 1:3 w/v concentration of proquinazid in propylene glycol	1/18 (6%)	0/18	1/10 (10%)	0/10

Conclusion: Proquinazid does not possess skin sensitising potential under the conditions of the Maximisation test. No classification is required according to the criteria specified in Directive 67/548/EEC.

(Hershman, 1999)

B.6 Evaluation Table Open Points 2.1 (and 2.2)

Ocular discharge in dogs

90-Day dietary study in dogs

An increased incidence of clear discharge from the eye was seen in all dose groups compared to controls (DAR Table B.6.33). This was most notable at the time of feeding. Purulent discharge from the eye was also noted at the top dose, and sporadically from other doses but not in the controls. An increased incidence of green or brown material around the eye was noted at the top dose. The study author considered that the incidence of the ocular findings generally increased throughout the study.

An increased incidence of ear reddening was noted at all dose levels compared to controls 1-2 h after feeding (DAR Table B.6.33).

These findings indicate that proquinazid was having an effect on eyes and ears at all dose levels, possibly due to irritation.

Overall study conclusion by RMS (copied from DAR)

Substance-related toxicity (ocular discharge, red ears and increased liver weight) was seen at all dose levels, i.e. ≥ at 500 ppm (≥ 17-18 mg/kg bw/day). The increased liver weight in this study is not considered to be an adverse effect. Although proquinazid is not classified as an eye irritant based on acute exposure of rabbits, the extent to which ocular discharge in this dog study was due to a local irritation rather than to a systemic effect is unclear. The presence of red ears at all doses and conjunctivitis in one top dose animal is consistent with an irritant response. However increased ocular discharge was also seen in the 1-year dog study with dosing by capsule. **Hence it is not possible to propose, with confidence, a NOAEL for systemic effects in this 90-day feeding study in dogs**

DAR Table B.6.33 Some notable clinical findings in 90-day dog study: total occurrence/number of animals (4/sex/concentration)

Sex	Effect	0 ppm	500 ppm	2000 ppm	4000/3000 ppm*
Examination before feeding (detailed weekly physical examination)					
Male	Clear discharge left eye	0/0	4/1	4/1	5/2
	Clear discharge right eye	0/0	2/1	3/1	6/2
Female	Clear discharge left eye	2/1	4/1	0/0	15/3
	Clear discharge right eye	0/0	6/1	12/2	13/3
Examination at time of feeding (daily examination)					
Male	Clear discharge left eye	0/0	36/1	17/1	32/2
	Clear discharge right eye	0/0	12/1	8/2	19/2
Female	Clear discharge left eye	15/1	42/2	14/3	82/4
	Clear discharge right eye	0/0	40/2	66/3	78/4
Examination 1-2h after feeding (daily examination)					
Male	Clear discharge left eye	0/0	10/1	9/1	18/2
	Clear discharge right eye	0/0	1/1	1/1	9/2
	Reddened left ear	0/0	15/3	28/2	13/1
	Reddened right ear	0/0	23/4	33/2	17/2
Female	Clear discharge left eye	14/2	21/2	4/3	56/3
	Clear discharge right eye	0/0	15/1	33/2	43/2
	Reddened left ear	0/0	6/2	1/1	6/2
	Reddened right ear	2/2	11/2	4/3	9/2

* Owing to decreased food consumption and body weight loss, the 4000 ppm groups received basal diet during Study Week 5 and resumed test substance administration during Study Week 6 at 3000 ppm.

Mean daily intakes were:
Males: 0, 17, 62, and 87 mg/kg bw/day
Females: 0, 18, 56, and 95 mg/kg bw/day

Ocular discharge in 90-day study: relevance for setting ARfD
(copied from DAR)

Individual data for ocular discharge have been examined. Ocular discharge was seen at the time of feeding on first day of exposure in one dog from each test group but in none of the controls (DAR Table B.6.33a). However, apart from the low dose animal this response was not seen 1-2 h after feeding and hence this response would seem to be of limited concern (especially in the absence of any dose response) for acute risk assessment.

At the first examination before feeding (at the end of week 1), ocular discharge was recorded in 2 dogs per dose at 2000 (1 male, 1 female) and 4000 ppm (2 females) but in none at 500 ppm or in the controls. One control showed ocular discharge at the end of week 2 (but this seems to be related to an enlarged nictitiating membrane).

Overall, following a precautionary approach, it is considered that ocular discharge seen in one female dog at the time of first exposure to 500 ppm (= 19 mg/kgbw/day for the first week of exposure) is of relevance for setting an ARfD*.
[*Note: this is stated in the DAR, RMS now willing to accept that this may be too precautionary, see section 2 below]

DAR Table B.6.33a First occurrence of ocular discharge for each dog in the 90-day dietary study

Female dogs

0 ppm		500 ppm		2000 ppm		4000/3000 ppm	
At time of feeding	**1-2h after feeding**	**At time of feeding**	**1-2h after feeding**	**At time of feeding**	**1-2h after feeding**	**At time of feeding**	**1-2h after feeding**
D8 Dog 4267	**D15**	**Not seen** Dog 4264	**Not seen**	**D10** Dog 4265	**D36**	**D15** Dog 4268	**2 months**
Not seen Dog 4275	**2 months**	**Not seen** Dog 4272	**D26**	**Not seen** Dog 4270	**D25**	**D4** Dog 4271	**1 month**
Not seen Dog 4276	**Not seen**	**D1** Dog 4274	**D1**	**D3** Dog 4277	**D4**	**D1** Dog 4278	**D4**
Not seen Dog 4282	**Not seen**	**D7** Dog 4279	**Not seen**	**2 months** Dog 4281	**2.5 months**	**D20** Dog 4280	**D26**

Male dogs

0 ppm		500 ppm		2000 ppm		4000/3000 ppm	
At time of feeding	**1-2h after feeding**	**At time of feeding**	**1-2h after feeding**	**At time of feeding**	**1-2h after feeding**	**At time of feeding**	**1-2h after feeding**
Not seen Dog 4245	**Not seen**	**D32** Dog 4246	**D34**	**Not seen** Dog 4247	**Not seen**	**Not seen** Dog 4249	**2.5 months**
Not seen Dog 4253	**Not seen**	**Not seen** Dog 4251	**Not seen**	**Not seen** Dog 4250	**Not seen**	**D9** Dog 4252	**D9**
Not seen Dog 4259	**Not seen**	**D23** Dog 4254	**D28**	**D1** Dog 4256	**D8**	**D9** Dog 4255	**D18**
Not seen Dog 4262	**Not seen**	**Not seen** Dog 4261	**Not seen**	**2.5 months** Dog 4260	**Not seen**	**Not seen** Dog 4263	**Not seen**

D=day of exposure

Oral 1-year study in dogs (administration by capsule)

At the top two doses, an increased incidence of clear ocular discharge was seen in females at 60 mg/kg bw/day and in both sexes at 180 mg/kg bw/day (DAR Table B.6.36). This was seen particularly at the time of dosing.

At 15 mg/kg bw/day there were slight increases in the incidence of some clinical findings. The most consistent effect was the increased incidence of clear ocular discharge in females (Table B.6.36) at/soon after dosing; no importance is attached to an apparent increase in males at 15 mg/kg bw/day in the absence of an increase at 60 mg/kg bw/day. The study author considered that there were no substance related clinical signs at 15 mg/kg bw/day.

Overall study conclusion by RMS (as in DAR)

A NOAEL of 15 mg/kg bw/day is proposed for males based on effects seen at 60 mg/kg bw/day (reduced body weight gain).

A NOAEL of < 15 mg/kg bw/day is proposed for females based on an increased incidence of ocular discharge, at the time of dosing, at 15 mg/kg bw/day. This is however a minor effect at 15 mg/kg bw/day, with no significant response persisting through to the following day.

Ocular discharge in 1-year study: relevance for setting ARfD
(copied from DAR)

Individual data for ocular discharge, emesis and salivation have been examined. There is no strong evidence for a substance-related increased incidence after a single dose. The only suggestion of a possible acute effect was ocular discharge in 2 high-dose females when first examined before dosing at the end of week 1. In these 2 dogs (4998 and 4995), ocular discharge was first noted at the time of dosing on days 3 or 5, and 1-2 h after dosing on days 5 or 14.]

DAR Table B.6.36 Some notable clinical findings in 1-year dog study: total occurrence/number of animals (5/sex/dose)

Sex	Effect	0 ppm	15 mg/kg bw/day	60 mg/kg bw/day	180 mg/kg bw/day*
Examination before dosing (detailed weekly physical examination)					
Male	Clear discharge left eye	0/0	0/0	0/0	40/1
	Clear discharge right eye	1/1	0/0	0/0	15/1
	Clear nasal discharge	0/0	0/0	0/0	0/0
Female	Clear discharge left eye	1/1	4/1	22/2	98/3
	Clear discharge right eye	0/0	3/1	8/2	96/3
	Clear nasal discharge	0/0	0/0	0/0	9/2
Examination at time of dosing (daily examination)					
Male	Clear discharge left eye	4/3	37/3	6/3	265/4
	Clear discharge right eye	2/1	27/2	4/3	125/4
	Clear nasal discharge	1/1	0/0	16/3	4/2
Female	Clear discharge left eye	26/3	41/3	154/2	451/3
	Clear discharge right eye	10/2	36/2	111/2	452/3
	Clear nasal discharge	0/0	0/0	0/0	57/2
Examination 1-2h after dosing (daily examination)					
Male	Clear discharge left eye	7/2	24/2	1/1	102/2
	Clear discharge right eye	2/1	16/2	2/1	47/2
	Clear nasal discharge	0/0	1/1	8/4	4/3
Female	Clear discharge left eye	13/2	37/3	88/2	251/4
	Clear discharge right eye	7/3	39/4	64/2	242/4
	Clear nasal discharge	0/0	1/1	1/1	35/4

* The dose level for the high dose group was 60 mg/kg/day for Study Week 0, 120 mg/kg/day for Study Week 1, and 180 mg/kg/day for the remainder of the study.

Commentary on the likely cause of ocular discharge in dogs following dietary and capsule administration of proquinazid (copied from DAR)

The **UK ACP** (January 2005) thought it unlikely that the increased ocular discharge seen following dietary and capsule administration was due to infection.

The **applicant** (Frost 2005) considers that the ocular discharge is most likely the result of local ocular irritation due to the presence of test substance crystals either mixed with the diet or from capsules (minimal exposure to residues) because:

a) In both studies, ocular discharge was most frequently seen at the time of dosing (as compared with 1-2h after dosing and before dosing).

b) Following ocular examination in study week 12 of the 90-day study, a veterinary ophthalmologist concluded that the presence of conjunctivitis (in one dog) in association with ocular discharge makes ocular irritation more likely than impairment in tear outflow.

c) In the 90-day dietary study both clear (serous) and purulent discharge were seen. In the 1-year capsule study, only clear (serous) discharge was seen. Purulent discharge is more severe clinically than clear discharge. The more severe response in the dietary study is consistent with the greater potential for direct ocular exposure (and hence an irritant response). RMS also notes that in the one-year study no test substance-related ocular findings were seen on ophthalmologic examination during week 53.

d) Although it is not classified as an ocular irritant based on a study in rabbits (see B.6.2.5) it did cause mild irritation in rabbits, including conjunctival discharge.

RMS notes that technical grade proquinazid (purity 97 %) is a brown wax-like crystalline solid. [Additional note: proquinazid has a low vapour pressure: 9×10^{-5} Pa at 25°C.]

The applicant does not explain how there could have been direct ocular exposure to proquinazid crystals when the test substance is dosed in a capsule. The RMS notes it is theoretically possible that ocular exposure could have resulted from:

- crystals on the outside of the capsule (but there is no evidence for this), and/or
- emesis at the time of dosing (particularly common in high dose males dosed by capsule).

RMS notes that substance-related ocular discharge was not a finding in most studies with rodents however the following observations are notable:

- Black ocular discharge in one rat given a single gavage dose of 5000 mg proquinazid/kg bw (B.6.2.1).
- Red, black or clear ocular discharge in several rats given 2000 or 3000 mg/kg bw of the environmental metabolite IN-MM671 (not a rat metabolite) by gavage (B.6.8.4a). This metabolite is structurally very similar to proquinazid; IN-MM671 just lacks the iodine atom present in proquinazid.
- Crusty eyes in mice given a single gavage dose of 1440 mg proquinazid/kg bw in a micronucleus study (B.6.4.2a).
- Dark red eyes were noted in rats given 600 ppm and above in the 2-year dietary study.

These rodent data suggest that at high gavage doses there may be some excretion of proquinazid/metabolites in tears. As proquinazid is a mild irritant its presence in the eye/tears following systemic exposure may aggravate tear production.

Overall, RMS considers that as ocular discharge in dogs was most frequent at the time of test substance administration (dietary or capsule) suggests that ocular discharge was principally due to direct (non systemic) ocular contact with the test substance at the time of dosing. However systemic exposure of the eye to the test substance/metabolites may have contributed to the ocular irritation seen at other times

Relevance of ocular discharge in dogs for human risk assessment
(new commentary, not in DAR)

Since the cause of this consistent and frequent finding in dogs exposed to proquinazid is unclear, and there was some evidence for ocular discharge in rodents at high doses, a precautionary approach is justified when considering the relevance of ocular discharge in dogs for human risk assessment.

B.6 Evaluation Table Open Point 2.2

ARfD

ARfD proposal by RMS in DAR

The following ARfD proposal for proquinazid is also applicable for the metabolite IN-MW977 (a residue in grain) because both substances are expected to be of similar toxicity (see B.6.8.6b).

An ARfD of 0.2 mg/kg bw is proposed for proquinazid based on applying a 100-fold assessment factor to a dose level of 500 ppm (= 19 mg/kg bw/day for the first week of exposure) at which an increased incidence of ocular discharge was seen in one dog at the time of first exposure in a 90-day dietary study with batch KQ926-45 (Mertens 1997).

A margin of c.100 on a dose level associated with an increased incidence of a minor and largely transient effect is considered to be sufficient. This margin is preferable to a margin of 65 for ocular effects in dogs which would have resulted if an ARfD of 0.3 mg/kg bw had been proposed based on a NOAEL of 30 mg/kg bw/day for acute effects in the rat developmental toxicity study. In this rat study, loss of body weight and reduced food consumption in dams were seen over the first 2 days of dosing (gestation days 7-9) at 60 mg/kg bw/day, with a NOAEL of 30 mg/kg bw/day.

The applicant proposed an ARfD of 0.025 mg/kg bw based on the developmental study in rabbits

This proposal by the applicant is not supported because there were no clear adverse effects in dams immediately after dosing and effects on fetuses at the end of the study were limited to body weight effects. On gestation days 7-9, there was no clear substance-related reduction in body weight gain and no reduction in food consumption. Although mean weight loss over days 7-9 was greater at 5 and 10 mg/kg bw/day than in controls, the difference was not statistically significant (individual data were very variable and contained notable outliers); also the mean weight loss at 5 and 10 mg/kg bw/day was similar to the mean weight loss seen in controls from days 9-11.

Other data considered when proposing the ARfD included notably the following:

- Acute oral (gavage) toxicity data for rats (and mice micronucleus studies): no deaths up to 1000 mg/kg bw, see and B.6.2.1 and B.6.4.2.

- Acute oral (gavage) neurotoxicity study in rats: NOAEL for reduced motor activity (seen on day of dosing) of 50 mg/kg bw, based on an effect at 100 mg/kg bw, see B.6.7a

- 28-day oral (dietary) mechanistic study in rats: although changes in thyroid hormones were seen at all dose levels after exposure for one week, i.e. at 10 ppm (0.6 mg/kg bw/day) and above, these are not considered to be adverse findings because a substance related increase in thyroid follicular cell hypertrophy was not seen after exposure for 1 week nor after exposure for 2 weeks; it was only seen at 300 ppm (19 mg/kg bw/day) after exposure for 4 weeks, see B.6.8.1. It is also reassuring that humans are less sensitive than rats to chemically-induced disruption of thyroid hormone homeostasis.

- 90-day oral (dietary) study in rats: over the first week of dosing, statistically significant reductions in body weight gain were seen in males at ≥300 ppm (> 28 mg/kg bw/day for first week), body weight gain was similar to controls over the second week of the study. In females body weight gain over the first week was reduced at 600 ppm (60 mg/kg bw/day over first week) with an NOAEL of 300 ppm (31 mg/kg bw/day over first week).

DE comment on RMS proposal (see 2.8 in reporting table)

An ARfD of 0.3 mg/kg bw is proposed instead of 0.2 mg/kg bw.

The developmental toxicity study in rats should be used to derive the ARfD. In the rat study, loss of bodyweight and reduced feed consumption in dams were seen over the first 2 days of dosing at 60 mg/kg bw/d (NOAEL: 30 mg/kg bw/d).

The proposal by the RMS is not supported because there was only one low dose female dog affected (ocular discharge). Safety factor of 100 should be applied deriving the ARfD of 0.3 mg/kg bw

RMS response to DE comment

RMS acknowledges the concerns expressed by DE, and can accept the DE proposal for an ARfD of 0.3 mg/kg bw (because 0.2 mg/kg bw may be too conservative/precautionary). However the views of other members of the PRAPeR toxicology meeting are welcomed.

Please note that PSD initially proposed an ARfD of 0.3 mg/kg bw derived in the same way as proposed by DE in a UK draft of the DAR.

However the UK ACP was concerned about the evidence for ocular discharge at the time of feeding on the first day in one out of 4 female dogs at 500 ppm (=19 mg/kg bw/day at this time) in the 90-day study, see Table B.6.33a and accompanying text reproduced above. The UK ACP considered there was a need to have a safety margin of 100 between this dose of 19 mg/kg bw and the ARfD; the ACP therefore asked PSD to lower the proposed ARfD to 0.2 mg/kg bw. The document was therefore amended to reflect this more conservative position

RMS can agree to the DE proposal because the occurrence of ocular discharge in one dog at the time of feeding on the first day of exposure at each test substance concentration as compared with one female control for the first time on day 8 is not very convincing evidence for substance-related ocular discharge after a single dose. Of these dogs showing an ocular response on the first day of exposure, only the female at the lowest test concentration still showed ocular discharge 1-2 h after the time of feeding

B.6 Evaluation Table Open Point 2.3

Dermal absorption

To aid discussion at PRAPeR some information/comments additional to those in the DAR are presented below.

a) Tape strip data

This additional information is provided because tape strip data has been an issue for discussion at recent PRAPeR toxicology meetings.

In vitro dermal absorption study

In the DAR, data are provided on the % dose present in tape strips as a whole, but there is no information on the number of tape strips taken or on the % dose present in individual strips (this information was not provided in the study report).

However this lack of tape strip information is not considered critical because:

- For the concentrate, the dermal absorption value proposed by the RMS includes the total amount radioactivity found in all tape strips.

- For the in-use dilution, inclusion of the total amount of radioactivity in tape strips was considered to be too conservative an approach. This is because it would have meant dermal absorption through rat skin was the same as through human skin (which is not consistent with flux data from the study, nor with what is typically expected for rat and human skin). Hence pragmatically absorption through rata skin was considered to be only slightly greater (1.5x) than through human skin.

In vivo dermal absorption study

Some information on % dose in tape strips is included in the DAR. Notably at the last sample time (concentrate: 330 h post dose; in-use dilution 498h post dose) the vast majority of radioactivity in tape strips was present in the first strip, with the mean amount of radioactivity decreasing to minimal levels (0.01% dose) at the lowest strip sampled.

In case further clarification is required for discussion at the PRAPeR meeting tape strip data from the *in vivo* dermal absorption study is tabulated below.

Table 1. Distribution of radioactivity in tape strips in *in vivo* rat dermal absorption study with Proquinazid 20 EC formulation

Concentrate

Tape strip	Mean % applied dose		
	At end of 6h exposure	18h after 6h exposure	330h after 6h exposure
1	4.03	1.99	1.16
2	2.17	2.48	0.09
3	0.87	1.71	0.06
4	0.41	0.50	0.07
5	0.36	0.27	0.05
6	0.73	0.24	0.02
7	0.66	0.17	0.05
8	0.51	0.28	0.05
9	0.60	N.S	0.01
10	0.31	N.S	0.01
11	N.S	N.S	N.S
12	N.S	N.S	N.S
13	N.S	N.S	N.S
14	N.S	N.S	N.S
15	N.S	N.S	N.S
Total	8.54	7.21	1.52

Dilution

Tape strip	Mean % applied dose		
	At end of 6h exposure	18h after 6h exposure	498h after 6h exposure
1	9.15	9.29	3.17
2	7.39	6.93	0.10
3	5.14	3.85	0.06
4	2.23	1.18	0.03
5	1.26	0.90	0.03
6	0.93	1.22	0.02
7	3.28	0.76	0.02
8	2.28	0.38	0.01
9	1.86	0.19	0.01
10	1.30	N.S	0.01
11	0.76	N.S	N.S
12	0.68	N.S	N.S
13	0.21	N.S	N.S
14	0.29	N.S	N.S
15	0.12	N.S	N.S
Total	28.79	22.33	3.42

NS = no sample

b) **Rat: human correction factor to apply to *in vivo* rat dermal absorption data**

The applicant and the RMS (in the DAR) based this rat: human correction factor on the percentage of the applied dose absorbed (taking account of the amount of radiolabel in skin) in the *in vitro* study.

The SANCO guidance document (SANCO/222/2000 rev 7 March 2004)
states that the preferred approach is to base the correction factor on the maximum flux.

The *in vitro* study report includes the value for mean flux (not maximum flux) over 2 different time periods (see Table Y).

Table 2 **Mean flux (penetration rate) calculated in the *in vitro* dermal absorption study**

Concentrate

Time period	**Penetration rate µg equiv/cm^2/h**		**Penetration rate greater in rat skin by a factor of**
	Rat skin	Human skin	
0-6h	9.23	1.29	7.2x
6-24h	10.4	1.07	9.7x

Dilution

Time period	**Penetration rate µg equiv/cm^2/h**		**Penetration rate greater in rat skin by a factor of**
	Rat skin	Human skin	
0-6h	0.28	0.03	9.3x
6-24h	0.17	0.05	3.4x

There are limitations for using these data to calculate the rat: human correction factor:

- Although the 0-6 h data was based on five measurements, the
- 6-24 h data was just based on two measurements (calculation of rate from just 2 measurements is not very reliable).
- For the concentrate: the 0-6h data for human skin included a 2h lag phase and for rats the rate clearly increased with time. Hence the flux calculated

over the whole 0-6 h cannot be considered the maximum flux. It is however reassuring that for both rat and human skin the flux over 0-6 h was similar to that over 6-24h

- For the dilution: the 0-6h data for rat skin included a 1h lag phase. Hence for rats flux calculated over the whole 0-6 h cannot be considered the maximum flux. It is also of concern that the flux for human skin was significantly higher from 6-24h than from 0-6h. However as 6-24h value was based on just two values there is uncertainly if this value reflects maximum flux.

Another factor to consider is that the *in vivo* data indicate that following exposure for 6h significant dermal penetration occurs after 24h (particularly from the tested dilution) apparently due to delayed penetration of material from the stratum corneum

B.8 ENVIRONMENTAL FATE AND BEHAVIOUR

B.8.1.4 Field studies

B.8.1.4.1 Field dissipation

Study a) The following is intended to provide some clarification to the fate and behaviour assessment in volume 3 of the DAR for proquinazid in response to point 4(12) in the reporting table for proquinazid and Open point 4.2 of the proquinazid evaluation table. In these points it was noted that „Comparing the results of table B.8.24 and B.8.25 either there is a significant procedural loss during the identification of the residues components, significant unextracted radioactivity and / or unidentified components.'

The results reported in Table B.8.24 are total radioactivity (TRR) in the soil horizons for each replicate plot reported as the concentration equivalent to proquinazid. TRR was determined by combustion of the homogenized soil sample. Table B.8.25 reports the mean concentration of proquinazid and three metabolites in the two replicate plots following extraction of the soil and analysis of the extract by HPLC. Minor unidentified metabolites and unextractable residues were not reported in Table B.8.25 and therefore a full mass balance cannot be anticipated in Table B.8.25.

However for the 0 DAT data point presented as an example in the reporting table, the appropriate comparison is between the mean TRR from Table B.8.24 (0.22+0.18/2=0.2) and the sum of residues for 0 DAT in Table B.8.25 (0.125+0.01+<0.01+0.02), plus unextractable residues (0.03 mg/kg), an unidentified metabolite (<0.01 mg/kg), and unresolved radioactivity reported as "Other" (0.01 mg/kg). Treating < values as actual values results in a total mass balance of 0.215 mg/kg or 107.5 %. Using the convention that results < LOD may be represented by one-half the detection limit in the calculation, the sum of the components, (0.125+0.01+ 0.005+0.02+0.005+0.01+0.03 = 0.205 mg/kg) a recovery of 102.5 % is obtained.

The RMS has updated Table B.8.25 below by adding in results for the unextracted radioactivity. This table is now titled Table B.8.25b and is reported below. The original Table B.8.24 is also reproduced below for ease of reference.

Table B.8.24 Total radioactivity in the „Evesham 3' soil horizons from Alconbury after three applications of [phenyl(U)-^{14}C] proquinazid at a total rate of 400 g a.s. / ha. Values in brackets are comparative % AR rates for each of the two replicate plots

Days after last application	mg equivalents proquinazid / kg soil dry weight (% applied radioactivity)			
	Nominal soil depth (cm)			
	0-15 cm	15-30 cm	30-60 cm	60-90 cm
0	0.22 (100.0)	nd	na	ns
0	0.18 (100.0)	nd	na	na
1	0.25 (113.6)	nd	na	na
1	0.20 (111.1)	0.02 (11.1)	nd	na
2	0.21 (95.5)	nd	na	ns
2	0.20 (111.1)	nd	na	na
4	0.33 (150.0)	nd	na	ns
4	0.30 (166.7)	nd	na	ns
7	0.31 (140.9)	<0.01 (<4.5)	nd	ns
7	0.18 (100.0)	0.01 (5.6)	na	ns
14	0.18 (81.8)	<0.01 (<4.5)	nd	na
14	0.24 (133.3)	0.01 (5.6)	0.01 (5.6)	ns
21	0.19 (86.4)	nd	na	na
21	0.29 (161.1)	0.01 (5.6)	nd	ns
30	0.23 (104.5)	nd	na	na
30	0.24 (133.3)	<0.01 (<5.6)	<0.01	ns
60	0.14 (63.6)	nd	na	na
60	0.22 (122.2)	0.04 (22.2)	nd	ns
90	0.18 (81.8)	nd	na	ns
90	0.19 (105.6)	<0.01 (<5.6)	<0.01	ns
120	0.10 (45.5)	nd	na	na
120	0.24 (133.3)	nd	na	na
151	0.15 (68.2)	nd	na	na
151	0.17 (94.4)	0.04 (22.2)	nd	ns
180	0.17 (77.3)	nd	na	na
180	0.13 (72.2)	nd	na	ns
215	0.12 (54.5)	nd	ns	na
215	0.11 (61.1)	0.02 (11.1)	nd	ns
239	0.17 (77.3)	nd	na	na
239	0.21 (117)	nd	na	ns
300	0.09 (40.9)	nd	na	ns
300	0.16 (88.9)	nd	na	ns
361	0.10 (45.5)	nd	na	na
361	0.14 (77.8)	nd	na	ns
420	0.11 (50.0)	nd	na	na
420	0.10 (55.6)	nd	na	ns
480	0.13 (59.1)	nd	na	na
480	0.12 (66.7)	nd	na	ns

(Note: nd = not detected, below LOQ; na = not analysed; ns = no sample).

Table B.8.25b Concentration of proquinazid and degradates in the 0-15 cm Evesham 3 soil horizon from Alconbury (mg / kg)

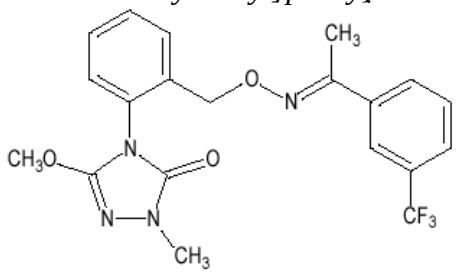
Days after last application	Component as mean mg / kg soil dry weight				
	Proquinazid	**IN-MM671**	**IN-MM991**	**IN-MM986**	**Unextracted Residue**
0	0.125	0.01	<0.01	0.02	0.03
1	0.145	0.01	<0.01	0.02	0.03
2	0.13	0.01	<0.01	0.03	0.02
4	0.18	0.03	0.01	0.04	0.04
7	0.14	0.01	0.01	0.04	0.03
14	0.115	0.01	0.01	0.04	0.03
21	0.12	0.02	0.01	0.04	0.04
30	0.11	0.01	0.01	0.04	0.05
60	0.07	0.02	0.01	0.02	0.04
90	0.055	0.03	0.01	0.02	0.05
120	0.035	0.02	0.02	0.02	0.05
151	0.03	0.03	0.01	0.01	0.06
180	0.025	0.03	0.01	0.01	0.05
215	0.015	0.02	0.01	<0.01	0.04
239	0.03	0.03	0.01	0.01	0.09
300	0.015	0.03	0.01	0.01	0.05
361	0.01	0.02	0.01	<0.01	0.08
420	0.01	0.02	0.01	<0.01	0.06
480	0.01	0.03	0.01	<0.01	0.07

NB. Metabolite M1 was also observed, but never at concentrations > 0.01 mg/kg. Other unidentified radioactive peaks were observed, however total unidentified radioactivity in any sample was ≤ 0.02 mg/ kg.

b) and c) The following is intended to provide some clarification to the fate and behaviour assessment in volume 3 of the DAR for proquinazid in response to point 4(13) in the reporting table for proquinazid and a resulting data requirement in the proquinazid evaluation table. The Applicant was requested to provide some information on the identity of DPX-KZ165 co-formulated with proquinazid in the field dissipation studies (Zietz et al., 2003a; Zietz et al., 2003b) and soil residue studies. This has been provided in the evaluation table, however the Applicants response is reproduced below for completeness.

'The test substance was a commercial formulation containing proquinazid (4.6%) and DPX-KZ165 (4.7%). Development of DPX-KZ165 was halted in 1999.

IUPAC name and structure of DPX-KZ165:
(E)-3-Methoxy-1-methyl-4-{2-[1-(3-trifluoro-methylphenyl) ethylideneaminooxymethyl]pheny} -1H-1,2,4-triazol-5(4H)-one '



B.8.1.4.2 Soil residue studies

a), b), c) See B.8.1.4.1 b) and c) above.

B.8.5.2 Surface waters and sediment

B.8.5.2.1 FOCUS Steps 1 and 2

The following is intended to provide some clarification to the fate and behaviour assessment in volume 3 of the DAR for proquinazid in response to point 4(30) in the reporting table for proquinazid and Open point 4.5 of the proquinazid evaluation table. It was requested that the RMS provide an explanation on the selection of the DT50whole system for metabolite IN-MM671 used in FOCUS SW calculation.

The RMS had a concern over use of the 497 day value calculated for the metabolite IN-MM671in the water/ sediment study due to the decline phase being unclear. Hence a 300 day default value was selected. The RMS has checked the impact of using DT50s of both 497 d and a 1000 d default on the Steps 1 and 2 PEC values. There is no change to the initial PEC values, but a change to PEC values over time (actuals and TWAs). Therefore, in the context of the risk assessment, there is no actual impact of using a longer whole system DT50.

B.9 ECOXICOLOGY

B.9.1 Effects on birds (IIA 8.1, IIIA 10.1)

B.9.1.4.2 Risk of exposure to active substance/plant protection product

Open point: 5.2
MS to discuss in a PRAPeR expert meeting the relevant species proposed by the applicant to refined the long-term risk identified for the insectivorous birds in vines.

We have received the paper published in J. Ornithology (Vol 149, pages 301-312). The paragraph concerning the diet of the Stonechat (Saxicola torquata) is reproduced below:

> Using the neck-collar technique, we collected 301 prey items from chicks (n = 141 in TEC[1], 160 in RIC[2], from six and five broods, respectively). These were assigned to 12 categories (mostly taxonomic orders; Fig. 2). The frequency of prey categories did not differ between TEC and RIC (contingency table, v2 = 9.514, df = 8, P = 0.301) after the three less numerous categories with a mean item dry biomass \10 mg had been grouped together. Three prey categories dominated in the diet at the two study plots, both in numbers and biomass: Orthoptera made up 32 and 30% of the diet biomass in TEC and RIC, respectively; Lepidoptera (mostly caterpillars) made up 27 and 36%, respectively, and Coleoptera made up 23 and 12%, respectively. Combined, these three categories represented 82% of the total biomass supplied to chicks in TEC and 78% in RIC. Shannon–Weaver indices of diet diversity did not differ between TEC (0.88) and RIC (0.86; Mann–Whitney U-test U = 14, n = 6 and 5, P = 0.86).
>
> [1]Revitalised intensively cultivated farmland
> [2]Traditional extensively cultivated farmland

RMS: The paper potentially shows that the diets of Yellowhammer and Stonechat are broadly similar. However we consider the information is not conclusive and we re-iterate our previous opinion that if the indicator species used in the risk assessment are not considered to be representative for certain Member States, then this issue should be considered at product re-registration as a Member State issue.

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

B.9.2.1 Acute aquatic toxicity of active substance and its metabolites (IIA 8.2)

c. Algae (IIA 8.2.6)

The following summary was provided by the Notifier

The acute toxicity of proquinazid to the freshwater green alga, *Pseudokirchneriella subcapitata* (*P. subcapitata*), under static conditions was determined in a 72-hour exposure test. The results of this study are based on geometric mean measured proquinazid active substance (a.s.) concentrations. The test was conducted in accordance with the OECD 201 guideline (2006).

The solubility limit of proquinazid in algal assay procedure (AAP) nutrient medium was determined to be 0.139 mg/L. The study was conducted with five concentrations of proquinazid (0.0072, 0.015, 0.031, 0.058, and 0.12 mg a.s./L), a solvent control and a dilution water (AAP nutrient medium) control at a temperature range of 23 to 24°C. Three replicates with an initial cell density of approximately 1.0 x 104 cells/mL per replicate were initiated for each test substance concentration, three replicates for the control, and six replicates for the solvent control. A single additional test flask at a nominal concentration of 0.038 mg a.s./L was initiated for the abiotic (stability) control.

The blank control and solvent control responses for the 72-hour cell density, area under the growth curve, and growth rate based on cell count were not statistically significantly different. Based on guidance in the OECD 201 guideline, the treatment data were compared to the solvent control data to determine treatment effects.

Cell growth increased in the solvent control by at least a factor of 16 in 72 hours. The coefficient of variation (CV) for section-by-section specific growth rates (day 0 to 1, 1 to 2, and 2 to 3) in the solvent control replicates did not exceed 35%, and the CV for the average specific growth rate of the solvent control for the entire test period (0-72 hour growth rate) did not exceed 7%. Therefore, the study met all the OECD 201 guideline (2006) test validity criteria.

Geometric mean measured concentrations of proquinazid in the test concentrations ranged from 77 to 81% of the nominal proquinazid concentrations. Geometric mean measured concentrations of proquinazid were used in the determination of EC50 values. Endpoints are presented in the table below.

ENDPOINT	NOEC MG A.S./L	EC50 MG A.S./L
0-72 HOUR GROWTH RATE	0.12	>0.12
0-72 HOUR BIOMASS	0.12	>0.12
72 HOUR CELL DENSITY	0.12	>0.12

(Hoberg, J R 2007b)

RMS comment

The study was conducted to OECD 201 and in accordance with the principles of GLP. The study met its validity criteria and is suitable for the risk assessment. The relevant endpoints are EbC50, ErC50 of >0.12 mg a.s./L.

B.9.2.2 Plant protection products (IIIA 10.2.1)

c. Algae (IIA 10.2.1)

The following summary was provided by the Notifier

The acute toxicity of Proquinazid 200 g/L EC to the freshwater green alga, *Pseudokirchneriella subcapitata* (*P. subcapitata*), under static conditions was determined in a 72-hour exposure test. The results of this study are based on nominal Proquinazid 200 g/L EC formulation concentrations and nominal proquinazid active substance concentrations. The test was conducted in accordance with the OECD 201 guideline (2006). Additionally, a recovery phase was initiated after 72 hours of exposure to determine if the test substance was algistatic or algicidal.

The study was conducted with six concentrations of Proquinazid 200 g/L EC (0.10, 0.20, 0.40, 0.80, 1.6, and 3.2 mg Proquinazid 200 g/L EC/L) and one dilution water (algal assay procedure (AAP) nutrient medium) control at a temperature range of 23 to 24°C. Three replicates with an initial cell density of approximately 1.0 x 104 cells/mL per replicate were initiated for each test substance concentration and six replicates for the control. A single additional test flask at a nominal concentration of 0.40 mg formulation/L was initiated for the abiotic (stability) control.

Cell growth increased in the blank control by at least a factor of 16 in 72 hours. The mean coefficient of variation (CV) for section-by-section specific growth rates (day 0 to 1, 1 to 2, and 2 to 3) in the control replicates did not exceed 35%, and the CV for the average specific growth rate of the control for the entire test period (0-72 hour growth rate) did not exceed 7%.

Therefore, the study met all the OECD 201 guideline (2006) test validity criteria.
Geometric mean measured concentrations of the active substance proquinazid in the test concentrations ranged from 75 to 91% of the nominal proquinazid concentrations. At the request of the Study Sponsor, nominal concentrations of the formulation Proquinazid 200 g/L EC and the active substance proquinazid were used in the determination of EC50 values. Endpoints are presented in the table below.

ENDPOINT BASED ON NOMINAL FORMULATION CONCENTRATIONS	**NOEC MG FORMULATION/L**	**EC50 (95% CI) MG FORMULATION/L**
0-72 HOUR GROWTH RATE	**0.80**	**2.5 (2.2-2.7)**
0-72 HOUR BIOMASS	**0.80**	**1.4 (1.3-1.4)**
72 HOUR CELL DENSITY	**0.80**	**1.3 (1.3-1.4)**
ENDPOINT BASED ON NOMINAL PROQUINAZID CONCENTRATIONS	**NOEC MG A.S./L**	**EC50 (95% CI) MG A.S./L**
0-72 HOUR GROWTH RATE	**0.16**	**0.50 (0.46-0.55)**
0-72 HOUR BIOMASS	**0.16**	**0.28 (0.25-0.30)**
72 HOUR CELL DENSITY	**0.16**	**0.28 (0.26-0.30)**

(Hoberg, J R 2007b)

RMS comment

The study was conducted to OECD 201 and in accordance with the principles of GLP. The study met its validity criteria and is suitable for the risk assessment. The relevant endpoint is the EbC50 of 1.4 mg formulation/L.

Open Point 5.6 in the Evaluation Table. Correction.

The following is replacement text for Section B. 9.2.3.2 in the original DAR.

This is provided to address Open point 5.6 in the Evaluation Table. In the original DAR the text stated that Daphnia magna was the most sensitive aquatic species. This is now corrected and the correction highlighted. The classification is unchanged by this correction.

B.9.2.3.2 Hazard classification/labelling of the plant protection product

The most sensitive aquatic test species was *Pseudokirchneriella subcapitata* with a 72 hour EbC50 of 1.3 mg product/l. Given that this is <10 mg product/l and >1 mg product /l, „Proquinazid 200 g/l EC' should carry the „N' symbol and „Dangerous for the environment' classification with the following risk phrases:

R51 *'Toxic to aquatic organisms'*

In the absence of evidence that the product is „readily degradable', it must also carry the phrase:

R53 *'May cause long-term effects in aquatic environment.*'

On the basis of the R51/53 classification, the product should also carry the following safety phrases:

S35 *'This material and its container must be disposed of in a safe way'*
S57 *'Use appropriate containment to avoid environmental contamination'*

Open Point 5.8 in the Evaluation Table. Correction.

The following is replacement text for Section B. 9.2.5.3 in the original DAR.

This is provided to address Open point 5.8 in the Evaluation Table. In the original DAR the cross reference was unclear. This is now corrected and the correction highlighted.

B.9.2.5.3 Risk to aquatic life from metabolites

Three metabolites of proquinazid are formed in soil or water/sediment, at or above 10%, these are: IN-MM671 (41-65% of applied radioactivity (AR) in soil and 6-68% of AR in water/sediment), IN-MM986 (2-74% of AR in soil and up to 1% of AR in water/sediment) and IN-MM991 (2-13% of AR in soil

and 1-2% of AR in water/sediment). A fourth metabolite; IN-MT884, was only formed under aqueous photolysis in the laboratory (30.5% of parent dose), but not detected in field soil dissipation and residue studies reported in Section B.8.1.4. Maximum PECsw values used in this risk assessment are based on the results of FOCUS surface water modelling (Step 1 and Step 2) which are detailed in Table B.8.89.

Open Point 5.7 in the Evaluation Table. Correction.

The following is replacement text for Section B 9.2.5.5 in the original DAR.

This is provided to address Open point 5.7 in the Evaluation Table. In the original DAR the cross references to the tabulated information in the fate section was incorrect. This is now corrected and the correction highlighted.

B.9.2.5.5 Risk to sediment dwelling invertebrates

The use of liquid scintillation to measure concentrations in the *Chironomus riparius* study provides a measure of the radioactivity within the system. However, if the radiolabelling occurred on one of the two carbon rings within the structure of proquinazid, it will not have been able to differentiate between parent and metabolites, as the rings remain intact when degraded to the main metabolites (IN-MM671, IN-MM986, IN-MM991 and IN-MT884). Therefore, where levels of proquinazid are referred to in terms of applied radioactivity (AR) this is likely to be a measure of both proquinazid and its metabolites.

As proquinazid was found at >10% of the applied radioactivity (AR) in the sediment phase of the aerobic water/sediment study and the reported 21-day *Daphnia* NOEC was <0.1 mg a.s./l, a study was conducted to determine the effects of proquinazid on the sediment dwelling organism *Chironomus riparius*.

As it is unclear as to where the proquinazid readily partitions into sediment, up to 85% of applied radioactivity (AR) as proquinazid (section B.8.4.4). Therefore, for risk assessment purposes, it is most relevant to compare worst-case exposure of *Chironomus riparius* to both the maximum initial concentrations in the overlying water immediately after application („total loading' maximum PECsw, using FOCUS Step 1, but assuming that the total load was present in the water phase) see Section B.8.6.2.1 and the maximum actual concentration in the sediment (the maximum PECsed using FOCUS Step 1) for proquinazid. For the cereal use (2 applications of 50 g a.s./ha) the „total loading' maximum PECsw value for proquinazid was 34.12 µg a.s./l (0.03412 mg.a.s./l) and the maximum PECsed value was 240.8 µg a.s./kg (0.2408 mg a.s./kg). For the vine use (4 applications of 75 g a.s./ha) the „total loading' maximum PECsw value for proquinazid was 107.6 µg a.s./l (0.1076 mg.a.s./l)

and the maximum PECsed value was 759.45 µg a.s./kg (0.75945 mg a.s./kg). See Tables B.8.91 and B.8.92 for details of the generated PEC values used in this risk assessment.

The NOEC for both emergence and development of *Chironomus riparius* based on measured concentrations of proquinazid in the water phase at day 0 was 0.456 mg a.s./l. and on the 28 day concentration in sediment was 4.35 mg a.s./kg. It is acceptable to use the sediment NOEC value as this was likely to have been the maximum concentration of proquinazid in the sediment phase during the study, based on the consistent decrease (day 0 to day 28) in mean proquinazid concentrations seen in the overlying water (Table B.9.35) and the final measured concentrations in sediment on day 28 (Table B.9.36). The TER_{lt} values for *Chironomus riparius* are summarised in Table B.9.54.

Table B.9.54 Long-term risk to *Chironomus riparius* from proquinazid using „Total Loading' exposure estimates (FOCUS Step 1)

Test organism	Water phase			Sediment phase			Annex VI trigger
	NOEC (mg a.s./l)	[1]PECsw (mg a.s./l)	TER	NOEC (mg a.s./kg)	[2]PECsed (mg a.s./kg)	TER	
[3]***Cereals (2 applications of 50 g a.s./ha)***							
C. riparius	0.456	0.03412	13.36	4.35	0.2408	18.1	10
[4]***Vines (4 applications of 75 g a.s./ha)***							
C. riparius	0.456	0.1076	**4.24**	4.35	0.75945	**5.73**	10

[1]Maximum „total loading' PECsw values (based on FOCUS Step 1, but assuming no partition to sediment occurs)
[2]Maximum PECsed values used (using normal FOCUS Step 1 assumptions)
[3]Spray drift contamination at 1 metre. [4]Spray drift contamination at 3 metres
Figures in bold indicate breaches of Annex VI trigger value.

Only the cereal use TERlt water and sediment phase scenarios for *Chironomus riparius* did not breach the Annex VI trigger of 10. Therefore, further refinement of the risk was required for both vine use scenarios, using FOCUS Step 2 to produce a „total loading' maximum PECsw (again assuming that the total load was present in the water phase.) and the maximum actual concentration in the sediment (the maximum PECsed using FOCUS Step 2) for proquinazid (see Section B.8.6.2.1). The „total loading' FOCUS Step 2 maximum PECsw value for proquinazid in vines was 17.98 µg a.s./l (0.01798 mg a.s./l) for late use in Southern Europe. The FOCUS Step 2 maximum PECsed value for proquinazid was 122.95 µg a.s./l (0.12295 mg a.s./kg) for the early use on vines (multiple applications of 75 g a.s./ha) see Table B.8.107. The FOCUS Step 2 TER_{lt} values for *Chironomus riparius* are presented in Table B.9.55.

Table B.9.55 Long-term risk to *Chironomus riparius* from proquinazid use in vines using „Total Loading' exposure estimates (FOCUS Step 2)

Test organism	Water phase			Sediment phase			Annex VI trigger
	NOEC (mg a.s./l)	[1]PECsw (mg a.s./l)	TER	NOEC (mg a.s./kg)	[2]PECsed (mg a.s./kg)	TER	
[3]***Vines (4 applications of 75 g a.s./ha)***							
C. riparius	0.456	0.01798	25.36	4.35	0.12295	35.38	10

[1]Maximum „total loading' PECsw values (based on FOCUS Step 2, but assuming no partition to sediment occurs)
[2]Maximum PECsed values (using normal FOCUS Step 2 assumptions)
[3]Spray drift contamination at 3 metres

As the TER_{lt} values for *Chironomus riparius* were above the Annex VI trigger value of 10 for the proposed use patterns of proquinazid on cereals and vines, both for risk in the overlying water (using „total loading' PECsw exposure values at FOCUS Step 1 or Step 2) and in sediment (using either FOCUS Step 1 or Step 2 exposure values) the long-term risk to sediment dwellers is considered to be acceptable.

Open Point 5.10 in the Evaluation Table. Correction.

The following is replacement text for Section B. 9.4.1.1 in the original DAR.

This is provided to address Open point 5.10 in the Evaluation Table. In the original DAR the reference was incorrect. This is now corrected and the correction highlighted.

B.9.4.1.1 Active substance

Details for honeybee acute oral and contact toxicity studies conducted with technical proquinazid are summarised in Table B.9.62.

Table B.9.62 The acute oral and contact toxicity of proquinazid (purity 96.4%) to honeybees

Test type	LD50 (µg a.s./bee)	Test Guideline	GLP	Reference
48 hr acute oral*	>125	EPPO 170	Yes	(Engelhard and Ott, 1998)
48 hr acute contact*	>125	EPPO 170	Yes	

*Values were also provided for 72hr acute oral and contact toxicity (both >125 µg a.s./bee).

References

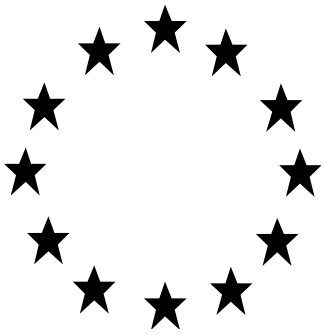
Active substance

Annex point	Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
B.9.2.1 IIA 8.2.6	Hoberg J R	2007	Proquinazid (DPX-KQ926) Technical: 72-Hour Acute Toxicity Test with Freshwater Green Alga, *Pseudokirchneriella subcapitata* Springborn Smithers Laboratories Du Pont number 21531 GLP Unpublished	Y	

Plant protection product

Annex point	Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
B.9.2.2 IIA 10.2.1	Hoberg J R	2007a	Proquinazid (DPX-KQ926) 200 g/L EC: 72-Hour Acute Toxicity Test with Freshwater Green Alga, *Pseudokirchneriella subcapitata* Springborn Smithers Laboratories Du Pont number 21739 GLP Unpublished	Y	

Council Directive 91/414/EEC



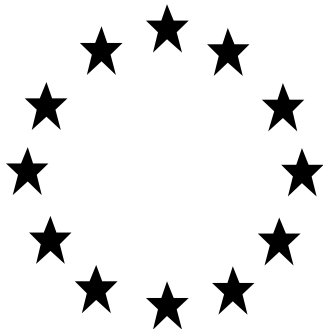
Proquinazid

Addendum 2
to
Annex C
to the Report and Proposed Decision of the United Kingdom made to the European Commission under Article 8 of 91/414/EEC

Confidential

March 2009

Council Directive 91/414/EEC



Proquinazid

Addendum 3
to
Annex B
to the Report and Proposed Decision of the United Kingdom made to the European Commission under Article 8 of 91/414/EEC

B.7 Residues

July 2009

CONTENTS

Introduction

This Addendum has been prepared to the following Open Points remaining after the PRAPeR expert meetings held in May 2009:

- Open point 3.6 - RMS to evaluate the apple metabolism study or to compare the investigation of the lignin fraction in the grape study with the procedure described in literature (Bjorkman).

- Open point 3.6 - Method validation data (method used in grape residue trials) to be reported by RMS in an addendum

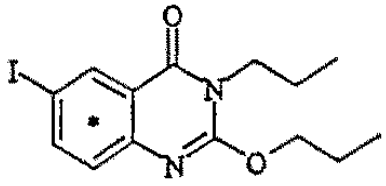
- Open point 3.8 - RMS to calculate the actual N rate on the basis of the residues in soil and re-evaluate on this basis the rotational crop study.

B.7.1 Metabolism, distribution and expression of residues in plants (IIA 6.1, IIIA 8.1)

In the original DAR March 2006 a grape metabolism was evaluated. In this study the un-extractable residues in fruit were subjected to reflux with strong alkali that released 22.7 % TRR. This extract formed a precipitate on acidification and was postulated by the Notifier to be lignin. The Notifier further clarified that un-extractable residues in an apple metabolism study were submitted to similar tests that gave base soluble residues which formed a precipitate upon acidification. The identification of this fraction as lignin was confirmed by isolation using dioxane/water (Bjorkman procedure) and dioxane/acid as part of the apple metabolism study.
The notifier has subsequently submitted the apple metabolism study on request, which is evaluated below.

B.7.1.1 Apple

In a GLP study conducted in 1997 apple trees (var. Winesap) were treated with phenyl-^{14}C-proquinazid (radiochemical purity >97%) formulated as an EC. Two applications at a rate of 225g ai/ha were made as a foliar spray with an interval of 40 days between the applications. Samples of apple fruit were taken 0, 15 and 28 days after the last application. Samples of leaves were taken after the first application, before the 2nd application and again after the 2nd application (Ca 1.5N based on maximum total dose for grapes).



* Position of Radiolabel

Total residues in the samples were determined by combustion LSC. To determine the amount of surface-associated radioactivity, several treated apples from the 15- and 28-day samplings were peeled and aliquots of the pulp and peel were combusted to determine the distribution of radioactivity.

Fruit samples were extracted three times with ethyl acetate and centrifuged. The extracts were combined and concentrated before measurement by LSC. The remaining solids were further extracted with three aliquots of methanol: 0.1% formic acid (50: 50 v/v). The extracts were combined and filtered before measurement by LSC. Radioactivity in the solid material remaining after extraction (post extraction solids, PES) was determined by combustion followed by LSC.

Leaf samples were extracted as for the fruit samples, except that methanol: 0.1% phosphoric acid was used instead of methanol: 0.1% formic acid.

Post extraction solids were subjected to enzyme digestion with cellulase, amylase, and amyl o-glucosidase, followed by digestion with 1 N sodium hydroxide at room temperature and acidification with hydrochloric acid. The remaining solid was refluxed with 1 N sodium hydroxide at 80°C and then acidified with hydrochloric acid. A separate sample of PES was twice subjected to acid hydrolysis by reflux at 110°C with 6N hydrochloric acid.

Identification and characterisation of the extracted radioactivity was by TLC and HPLC with UV, DAD or radiochemical detection systems.

Levels of radioactivity detected in the samples are shown in Tables 7.1 and 7.2 of this Addendum. TRR were 0.13 - 0.18 mg/kg in apple fruit. The majority of the radioactivity was associated with the peel (89-90%). For leaves TRR were 15.4 mg/kg after the 1st application, 9.1mg/kg before the 2nd application and 30.4 mg/kg after the 2nd application. Extractability of residues was high (> 70%) for leaves and Day 0 apples, but decreased in apple fruit with increasing harvest interval.

Further treatment of the apple fruit PES with enzymes released 2-4% TRR. Reflux with 1N sodium hydroxide at room temperature released 17-36% TRR and reflux at 80°C released a further 5-10% TRR. Acidification of the basic extracts resulted in precipitation which indicated the radioactivity was associated with lignin. Additional analytical work to investigate residues thought to be associated with lignin is described below.

Table 7.3 of this Addendum shows the identification and distribution of metabolites in the organic and aqueous extracts.

Proquinazid was the major component identified residue in apple fruit, declining from 61% of the TRR (0.11 mg/kg) for the day 0 samples to 22% of the TRR (0.03 mg/kg) for the 28-day samples. Small amounts of metabolites IN-MM671, IN-MM986, and IN-MM991 were also identified in apple fruits, each metabolite accounted for less than 5% TRR (<0.01 mg/kg) in the samples.

Two broad regions of radioactivity assigned as “polar regions” 1 and 2 were found in the 15 and 28 day samples; polar region 1 accounted for up to 16% TRR in the 28 day sample. These regions were found to consist of multiple components, each individually less than 5% TRR or 0.01 mg/kg.

The metabolism in apples has been demonstrated to be similar to that in grapes (considered in the original DAR March 2006).

Table 7.1 Total Radioactive residues and extractability of residues in apples treated with [^{14}C] - phenyl labelled proquinazid

	Apple fruit						**Leaves**					
Sample	0 days		15 days		28 days		After 1st application		Before 2nd application		After 2nd application	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR	**0.18**	**-**	**0.15**	**-**	**0.13**	**-**	**15.4**	**-**	**9.1**	**-**	**30.4**	**-**
Ethyl acetate	0.14	77.7	0.067	45.5	0.05	39.2	13.7	89.2	4.98	54.7	24.5	80.5
Aqueous (MeOH/acid)	0.005	2.9	0.011	7.5	0.011	8.2	0.88	5.7	1.51	16.6	2.3	7.5
Total extracted	**0.145**	**80.6**	**0.078**	**53.0**	**0.061**	**46.9**	**14.58**	**94.7**	**6.49**	**71.3**	**26.8**	**88.2**
PES	**0.044**	**23.6**	**0.069**	**47.0**	**0.068**	**52.6**	**0.79**	**5.1**	**2.61**	**28.7**	**3.6**	**12.0**
Enzymes	0.003	1.8	0.005	3.5	0.005	3.9						
1N NaOH ambient temp.	0.031	17.0	0.049	33.4	0.046	35.8						
Acidified aqueous	0.002	1.2	0.004	2.6	0.004	3.1						
Acidified precipitate	0.029	15.8	0.045	30.8	0.042	32.7						
1N NaOH reflux at 80°C	0.010	5.2	0.012	8.0	0.012	9.4						
Acidified aqueous	0.002	1.2	0.003	1.7	0.003	2.0						
Acidified precipitate	0.007	4.0	0.009	6.3	0.009	7.4						

Table 7.2 Distribution of radioactive residues in apple peel and pulp.

Sample	**Matrix**	**Weight (g)**	**% of total fruit weight**	**dpm/g**	**Total dpm/ matrix**	**% of total dpm**
15 day	Peel	149.5	26	19661	2939320	10
	Pulp	416.7	74	775	322943	90
28 day	Peel	142.4	23	20669	2943266	89
	pulp	477.3	77	756	360839	11

dpm = decays per minute

Table 7.3 Characterisation of Radioactive residues in apple fruit treated with [^{14}C] - phenyl labelled proquinazid

	Total radioactivity		Proquinazid		MM671		MM986		MM991		Rt 4-6 min (Polar region 1)		Rt 7-9 min (Polar region 2)		Other unidentified radioactivity	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Day 0 apple, 0.18 mg/kg TRR																
Ethyl acetate	0.14	77.7	0.11	61.3	0.006	3.3	0.006	3.1	<0.005	1.2	-	-	-	-	0.005	2.6
Aqueous[1]	0.008	4.7	-	-	-	-	-	-	-	-	-	-	-	-	0.005	3.0
Total identified	-	-	0.11	61.3	0.006	3.3	0.006	3.1	<0.005	1.2	-	-	-	-	0.010	5.6
Day 15 apple, 0.15 mg/kg TRR																
Ethyl acetate	0.067	45.5	0.041	27.5	0.007	4.8	-	-	-	-	<0.005	3.0	<0.005	1.1	0.012	8.0
Aqueous[1]	0.016	11.0	-	-	-	-	-	-	-	-	0.007	4.9	-	-	0.007	5.0
Total identified			0.041	27.5	0.007	4.8	-	-	-	-	0.011	7.9	<0.005	1.1	0.019	13.0
Day 28 apple, 0.13 mg/kg TRR																
Ethyl acetate	0.05	39.2	0.028	22.2	<0.005	3.2	<0.005	1.3	<0.005	1.7	0.005	4.1	0.006	5.0	-	-
Aqueous[1]	0.016	12.0	-	-	-	-	-	-	-	-	0.015	11.9[2]	-	-	-	-
Total identified	-	-	0.028	22.2	<0.005	3.2	<0.005	1.3	<0.005	1.7	0.020	16.0	0.006	5.0	-	-

[1]Aqueous extract = methanol/aqueous acid extract and enzyme digest combined.

[2]Further characterisation suggested the composition of this region was different from the ethyl acetate fraction

Confirmation of Incorporation of Radioactivity into Plant Lignin

In order to confirm that the acid precipitate of the basic hydrolysis extracts indicated incorporation of radioactivity into the plant matrix a larger sample of the 28 day fruit was extracted with ethyl acetate and methanol: 0.1% formic acid (50: 50 v/v) as described previously, to generate a sufficient amount of PES. The Bjorkman and dioxane acidolysis lignins were isolated from the PES as follows:

Bjorkman lignin was obtained by repetitive extraction (6 aliquots) with dioxane-water (9:1 v/v). The extracts were combined and concentrated under vacuum until a brown viscous residue remained. This residue was re-dissolved in dioxane/water (9:1) to allow an aliquot to be counted by LSC, and then the remainder was re-concentrated and water added to precipitate Bjorkman Lignin. The mixture was centrifuged and the supernatant decanted. The solids (Bjorkman lignin) were washed again with water, centrifuged and the supernatant decanted. The water washes were analyzed for soluble radioactivity by LSC. The amount of Bjorkman lignin precipitated was determined by difference from the radioactivity determined in the extracts and water washes.

Dioxane acidolysis lignin was obtained by twice refluxing the PES with dioxane/2N HCl (9:1, 50 mL) under nitrogen at 87°C for 30 min. The extracts were cooled to room temperature, centrifuged, and the supernatant decanted. The combined acidic dioxane extracts were evaporated under vacuum until a viscous brown residue remained. Water was added to the residue with stirring until a suspension of fine particles was obtained. The suspension was centrifuged, the water decanted and counted by LSC. The remaining solid was re-dissolved in dioxane-water (9:1), centrifuged to remove insoluble matter, and concentrated under vacuum to a viscous brown residue. The dioxane acidolysis lignin was precipitated by the addition of water with stirring. The precipitate was centrifuged and the water decanted. The dioxane acidolysis lignin precipitate was washed a total of 3 more times with water. The amount of dioxane acidolysis lignin precipitated was determined by difference from the radioactivity determined in the extracts and water washes.

The Bjorkman extraction procedure released was 3.67% TRR and the dioxane acidolysis procedure released 27.6% TRR, giving a total amount of lignin as 31.3% TRR. This value would support the proposition that the base hydrolysis acid precipitate was associated with lignin (for the day 28 sample ca 33% TRR was precipitated by acid from the base extraction.)

(Schneiders, G.E; Irelan, M.J. 2002)

Conclusion and comparison with grape metabolism study

The metabolism study in apple is considered acceptable and it is the RMS opinion that the information provided on characterisation of the base hydrolysis extracts demonstrates the radioactivity found in the apple metabolism study is indeed associated with lignin.

In the grape metabolism study evaluated in the original DAR dated March 2006 (Section B.7.1.2), post extraction solids (PES) were refluxed with 1N sodium hydroxide. The acid treatment of this basic extract caused precipitation of the base soluble fraction which amounted to 22.7% (0.06 mg/kg). The notifier postulated that this radioactivity was therefore lignin incorporated. Based on

comparison with the extraction procedures used in the apple metabolism study the RMS agrees with this assumption.

B.7.1.4 Metabolism, distribution and expression of the residue in rotational crops

During the PRAPeR 70 residues meeting of experts (May 2009) concern was raised that rotational crop metabolism study may not take into account the accumulation of proquinazid and its metabolite IN- MM671 in soil, as both these compounds were found to be persistent in soil. The meeting concluded that: "Because of the very long DT90, we need maximum concentration of parent and metabolite in the soil considering potential accumulation to calculate the actual N rate".

In Section B.8.3 of the original DAR dated March 2006, PECs in soil based on accumulation were calculated for both the grape and cereal uses for proquinazid and metabolite MM671. These figures were calculated on the assumption of a 5 cm soil depth. Fort eh use on grapes a peak was estimated to have been reached in the 14th year of continuous application, and for the use on cereals a peak was estimated to have been reached in the 4th year of continuous application. The PECs calculated were as follows:

Compound	**PEC (mg/kg)**	
	Grape use	**Cereal use**
Proquinazid	0.164	0.062
IN-MM 671	0.113	0.024

When considering residues in following crops, grapes are considered to be a permanent crop i.e. not rotated, therefore the PECs relating to cereals use are considered the most relevant.

Data on the total radioactive residues in soil were provided in the study report for the rotational crop metabolism study considered in the original DAR. In this study two applications at a rate of 150g/ha were made at 30 day intervals to bare soil, which was considered to be 3N in relation to the notified GAP on cereals. Soil samples were taken after the first application of test substance, before and after the second application of text substance and prior to the planting of the following crops. By comparing these values with the PECs for the cereal use the level of overdosing in the rotational crop metabolism study can be estimated (assuming that the TRR value consists completely of either proquinazid or IN-MM671, itself an overestimation of the likely residue situation) as follows:

$$\frac{\text{TRR in soil}}{\text{PEC for cereal use}}$$

Total residues found in the treated soil samples and the estimated dose rates are given in Table 7.4 of this Addendum below.

Table 7.4 Total residues found in the treated soil samples and the estimated dose rates

Time interval	TRR in soil (mg/kg)	N rate based on cereal use PECs	
		Proquinazid	MM 671
After 1st application	0.273	4.4	11.4
30 days after 1st application (= before 2nd application)	0.211	3.4	8.8
After 2nd application	0.555	9.0	23.2
45 days after 2nd application	0.173	2.8	7.2
210 days after 2nd application	0.224	3.6	9.3

The lowest N rate estimated is therefore 2.8N. The evaluation in the original Dar was considered on the basis of 3N dose rate therefore a further reconsideration of the rotational crop metabolism study is not required.

B.7.6 Residues arising from supervised trials (IIA 6.3; IIIA 8.2)

As a result of comments received from EFSA and the MS it was requested that the validation data for the pre-registration method used in the grape residue trials should be reported. This information is presented in Table 7.5 below:

Table 7.5 Method Validation Data

Component	**Linearity**	**Fortification level (mg/kg)**	**Precision**		**% Recovery**		**Specificity**	**LOQ (mg/kg)**
			Apples	**Grapes**	**Apples**	**Grapes**		
Proquinazid	0.01-0.5 mg/kg Coefficient = 0.999	0.02	% RSD 3.4 (n=3)	% RSD 6.7 (n=3)	Range: 86-92%, mean = 89	Range: 81-92%, mean = 88	GC-MS chromatograms submitted and acceptable.	0.02
		0.1	% RSD 16.2 (n=3)	% RSD 1.8 (n=3)	Range: 97-130%, mean = 110	Range: 93-96%, mean = 95		
		0.5	% RSD 6.8 (n=3)	% RSD 1.7 (n=3)	Range: 90-101%, mean = 94	Range: 88-91%, mean = 90		
IN-MW671	0.01-0.5 mg/kg Coefficient = 0.999	0.02	% RSD 7.1 (n=3)	% RSD 16.9 (n=3)	Range: 77 – 92%, mean = 93%	Range: 75-107%, mean = 88	GC-MS chromatograms submitted and acceptable.	0.02
		0.1	% RSD 1.8 (n=3)	% RSD 3.6 (n=3)	Range: 85 – 88%, mean = 87	Range: 93-99%, mean = 95		
		0.5	% RSD 16.8 (n=3)	% RSD 0.7 (n=3)	Range: 69 -97%, mean = 83	Range: 83-84%, mean = 83		

(Linkerhägner, M., Jernberg, K.M.1998; Steinhauer, S.,Hornshuh, M.J.2002; Steinhauer, S.,Hornshuh, M.J.2003)

References

Annex point	Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
IIA 6.1	Schneiders, G. E. & Irelan, M. J.	2002	Metabolism of 14C-DPX-KQ926 in apples E I du Pont de Nemours and Company Wilmington AMR 4313-97 GLP: Yes Published: No	Y	DuPont
IIA, 6.3./11	Linkerhägner, M., Jernberg, K.M.	1998	Magnitude of residues of DPX-KQ926 and DPX-KZ165 in grapes (berries and small fruits) - Europe, season 1997 Dr. Specht & Partner Chemische Laboratorien GmbH AMR 4243-96 GLP: Yes Published: No	Y	DuPont
IIA, 6.3./13	Steinhauer, S., Hornshuh, M.J.	2002	Combined decline and magnitude of residues of DPX-KQ926 in grapes (berries and small fruit) following applications of DPX-KQ926 20EC - Europe, season 2001 Dr. Specht & Partner Chemische Laboratorien GmbH DuPont-5859 GLP: Yes Published: No	Y	DuPont

IIA, 6.3./14	Steinhauer, S., Hornshuh, M.J.	2003	Combined decline, magnitude, and comparison of residues of DPX-KQ926 in grapes (berries and small fruit) following six or four applications of two DPX-KQ926 20EC formulations, Europe, season 2002 Dr. Specht & Partner Chemische Laboratorien GmbH DuPont-9717 GLP: Yes Published: No	Y	DuPont