ECHA
EUROPEAN CHEMICALS AGENCY

Committee for Risk Assessment
RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

Proquinazid

EC number: n.a.

CAS number: 189278-12-4

ECHA/RAC/CLH-O-0000002607-72-01/A1

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of the original CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted
9 March 2012

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Proquinazid
EC number:	None
CAS number:	189278-12-4
Annex VI Index number:	Not yet assigned
Degree of purity:	The minimum purity of proquinazid is 95 %
Impurities:	Confidential

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	No entry	No entry
Current proposal for consideration by RAC	Carc 2; H351 Aquatic Acute 1; H400 Aquatic Chronic 1; H410	Carc Cat 3; R 40 N: R50-53
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Carc 2; H351 Aquatic Acute 1; H400 Aquatic Chronic 1; H410	Carc Cat 3; R 40 N: R50-53

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	Not applicable	Not classified	Conclusive but not sufficient for

					classification
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
	Acute toxicity - dermal	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	**Carc 2; H351**	**None**	**None**	
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	**H400** **H410**	**M factor: 10**	**none**	
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Pictogram: GHS08, GHS09

Signal word: Warning

Hazard statement codes:

H351: Suspected of causing cancer

H410: Very toxic to aquatic life with long lasting effects

Precautionary statements : Not required as PS are not included in Annex VI.

Proposed notes assigned to an entry:

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Oxidising properties	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Flammability	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Other physico-chemical properties *[Add rows when relevant]*	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Thermal stability	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Acute toxicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Acute toxicity – irreversible damage after single exposure	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Repeated dose toxicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Irritation / Corrosion	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Sensitisation	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Carcinogenicity	**Carc Cat 3; R40**	**None**	**None**	
Mutagenicity – Genetic toxicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Toxicity to reproduction – fertility	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Toxicity to reproduction – development	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Toxicity to reproduction – breastfed babies. Effects on or via lactation	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Environment	**N: R50-53**	**none**	**None**	

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Indication of danger: Xn, N

Risk phrases : R40, R50-53

Safety phrases : S36-37- 60-61

Proposed specific concentration limits (if any):

M-factor according to Directive 67/548/EEC and Regulation EC 1272/2008:

The M factor is 1 based on a 96-h EC50 value of 0.11 mg/l obtained for the marine crustacean *Americamysis bahia* in a flow-through study.

Proposed notes (if any):

None

BACKGROUND TO THE CLH PROPOSAL

1.4 History of the previous classification and labelling

Proquinazid is a new active substance in the scope of Directive 91/414/EEC. There have been no previous classification and labelling discussions for this substance.

1.5 Short summary of the scientific justification for the CLH proposal

Proquinazid is a fungicide and belongs to the quinazolinone group. It acts by blocking secondary appressorial development in powdery mildew, but not germ tube growth. In 2010, a positive opinion was given at the Standing Committee on the Food Chain and Animal Health (SCoFCAH) to include the new active substance in Annex I of Council Directive 91/414/EEC, with the UK as rapporteur Member State (2010/25/EU). In accordance with Article 36(2) of the CLP Regulation, Proquinazid should now be considered for harmonised classification and labelling. Therefore, this proposal considers all human health and environmental endpoints.

At the time of submission, no registration dossiers were available for this substance.

1.6 Current harmonised classification and labelling

Not applicable

1.6.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Not listed

1.6.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Not listed

1.7 Current self-classification and labelling

1.7.1 Current self-classification and labelling based on the CLP Regulation criteria

Carc 2; H351

1.7.2 Current self-classification and labelling based on DSD criteria

Carc Cat 3; R 40

2 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Proquinazid is a fungicide and belongs to the quinazolinone group. It acts by blocking secondary appressorial development in powdery mildew, but not germ tube growth. In 2010, a positive opinion was given at the Standing Committee on the Food Chain and Animal Health (SCoFCAH) to include the new active substance in Annex I of Council Directive 91/414/EEC, with the UK as rapporteur Member State (2010/25/EU). In accordance with Article 36(2) of the CLP Regulation, Proquinazid should now be considered for harmonised classification and labelling. Therefore, this proposal considers all human health and environmental endpoints.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

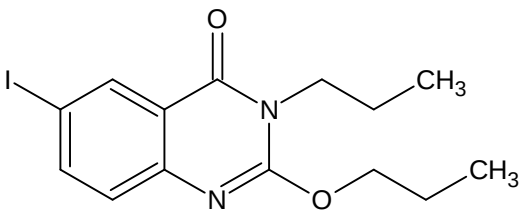
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	None
EC name:	None
CAS number (EC inventory):	189278-12-4
CAS number:	189278-12-4
CAS name:	4(3H)-Quinazolinone, 6-iodo-2-propoxy-3-propyl-
IUPAC name:	6-iodo-2-propoxy-3-propylquinazolin-4(3*H*)-one
CLP Annex VI Index number:	Not currently assigned
Molecular formula:	$C_{14}H_{17}IN_2O_2$
Molecular weight range:	372.21 g/mole

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Proquinazid	95 %		

Current Annex VI entry: Not Applicable

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks

The manufacturer has requested that all impurities remain confidential therefore information on the impurities is presented in the technical dossier only. There are 7 process impurities present in proquinazid They have been taken into consideration in the classification and are not considered to be of additional concern.

Current Annex VI entry: Not Applicable

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks

Current Annex VI entry: Not applicable

1.2.1 Composition of test material

Proquinazid manufactured for use as a pesticide has a minimum purity of 95 % with 7 identified impurities, none of which appear to be of additional toxicological concern. Three separate batches of proquinazid have been used. Batch DPX-KQ926-45 (96 % minimum purity, containing 4 of the 6 identified impurities) produced using an old production method, and batches DPX-KQ926-75 and DPX-K926-85 produced using the current production method. Both DPX-KQ929-75 and DPX-K926-85 have a higher content of proquinazid (98 % and all 6 impurities) and have been shown to be of an equivalent or lesser toxicity than the original batch. All batches are judged adequate for the substance that is marketed.

1.3 Physico-chemical properties

The physico-chemical properties of proquinazid have been well investigated, as summarised in the Pesticide Assessment Report attached to the IUCLID 5 dossier. Some of the key information is provided in the table below. In all the studies below the purity of the test substance was ≥ 97 %.

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Pure analytical grade – crystalline Solid Technical grade – waxlike crystalline solid	Moore, 1997 Brown, 2004a in reference 1	Visual assessment
Melting/freezing point	61.5 °C – 62.0 °C	Moore, 1997 in reference 1	EEC method A1,
Boiling point			No boiling point was observed at temperatures up to 360 °C. The substance was found to decompose at 367 °C.
Relative density	1.57 @ 20 °C	Moore, 1997 in reference 1	EEC method A3,
Vapour pressure	9 x 10^{-5} Pa at 25°C	Moore and Schmuckler, 1998 in reference 1	EEC method A4,
Surface tension	73.9 mN/m at 19.8 oC.	Huntley, 2002 in reference 1	OECD 115
Water solubility	At 25 oC: 0.97 mg/l HPLC grade 0.93 mg/l pH 7 phosphate buffer 0.73 mg/l filtered sea water	Moore, 1997 in reference 1	OECD 105 (EEC A6), shake flask method
Partition coefficient n-octanol/water	Log Kow = 5.5 at 25 °C	Moore, 1997 in reference 1	OECD 107 (EEC A8), Shake flask method
Flash point			Not applicable as the substance is a solid with a melting point > 60 °C.
Flammability	Flammability: Proquinazid did not support combustion in an initial screening test. Experience in handling and use indicates that the substance will not spontaneously ignite on contact with air or water.	Gravell 1997 in reference 1	Flammability: EEC method A 10,
Explosive properties	No explosions were observed with regard to both thermal and mechanical sensitivity.	Gravell 1997 in reference 1	EEC method A14
Self-ignition temperature	Negative, the test		EEC, A16

	substance gave no exothermic indication up to it's melting point when the test was concluded (62 °C)		
Oxidising properties	Not oxidising	See reference 1	Examination of the chemical structure indicates that proquinazid does not possess and chemical groups typical of oxidising agents.
Granulometry	Not conducted		
Stability in organic solvents and identity of relevant degradation products	Solubilities at 25°C: Acetone >250 g/kg Acetonitrile 154 g/l Dichloromethane >250 g/kg Dimethylformamide >250 g/kg Ethyl acetate >250 g/kg n-hexane >250 g/kg methanol 136 g/l 1-octanol >250 g/kg o-xylene >250 g/kg	Moore, 1997 in reference 1	Technical grade proquinazid commonly has a purity of around 97 %. Therefore 99.5% is uncharacteristic of the technical material. This difference in purity is not considered to significantly affect the solubility.
Dissociation constant	Proquinazid does not dissociate between pH 2.4 and 11.6. The dissociation constant is not relevant as proquinazid is not a salt.	Moore 1997 in reference 1	OECD Test Guideline 112

2 MANUFACTURE AND USES

2.1 Manufacture

Proquinazid is manufactured and placed on the market as a fungicide.

2.2 Identified uses

Proquinazid is manufactured and placed on the market as a fungicide.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Refer to table 9			

3.1 *[Insert hazard class when relevant and repeat section if needed]*

3.1.1 Summary and discussion of physico-chemical properties

Refer to table 9.

3.1.2 Comparison with criteria

3.1.3 Conclusions on classification and labelling

The substance does not meet the criteria for classification for physico-chemical properties

4 HUMAN HEALTH HAZARD ASSESSMENT

Proquinazid manufactured for use as a pesticide has a minimum purity of 95 % with 7 identified impurities, none of which appear to be of additional toxicological concern. Three separate batches of proquinazid have been used in the following studies. A complete battery of mammalian toxicity studies were conducted using batch DPX-KQ926-45 (96 % minimum purity, containing 4 of the 7 identified impurities), produced by an old production method. All of the studies, apart from the acute studies, were submitted by the applicant and are summarised in this proposal. In addition, some toxicity studies have been submitted on two other batches (DPX-KQ926-75 and DPX-K926-85) produced using the current production method. These batches have a higher content of proquinazid (98 % and all 6 impurities). The results of these studies indicate that these batches are of an equivalent or lesser toxicity than the original batch and all batches are judged adequate for the substance that is marketed.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The following summary is derived from the Pesticide Assessment Report made for the review under Directive 91/414/EEC.

All the following toxicokinetic information on proquinazid was acquired from rat studies. One study investigated ADME following a oral single dose of 1 or 20 mg/kg bw. A second study primarily investigated tissue levels of radioactivity and metabolism during and/or after exposure to 1 mg/kg bw/day for 7 days. The findings were similar in both sexes.

In the single dose study, proquinazid was well absorbed following a dose of 1 mg/kg bw/day (86-89 % within 48 h, based on a biliary cannulation experiment). The peak plasma concentration was

reached after 4-8 h (low dose) or 6-8 h (high dose). Radiolabel was widely distributed in the body. At T_{max} and $T_{1/2\ max}$, excluding the GI tract and contents, the highest tissue levels of radiolabel were observed in the adrenals, liver, kidneys, fat, pituitary, thyroid (also uterus and ovary at the high dose). Excretion was rapid and extensive (86-89 % within 48 h) and was equally important via the urine and feaces, with biliary excretion accounting for nearly all of the fecal excretion. By 7 days post dosing, no tissue sampled contained more than 5 ppm, and total body burden was < 1 % of the dose administered.

In the repeat dose study, highest tissue levels of radiolabel were in liver, kidneys and fat. Terminal half-life for these tissues was 36-89 h, and 37-49 h for plasma. These values were much longer than the terminal elimination half-life from plasma of 8.5-11 h following a single dose of 1 mg/kg bw but there was no evidence of bioaccumulation. Tissue levels measured at similar times after the end of dosing with 1 mg/kg bw were mostly similar after dosing for 1 day or for 7 days. Lack of significant bioaccumulation is also supported by no tissue (apart from the gastrointestinal tract and contents) containing more than 0.1 % of the cumulative dose at 48-49 h after the end of repeated dosing and by total body burden being only 0.2% of the cumulative dose at 169-170 h post dose.

There was extensive metabolism of proquinazid (> 98 % of the dose). The major metabolic reactions were phenyl ring hydroxylation and hydroxylation at the propyl and propoxy side chains, as well as some hydrolysis of side chains.

4.1.2 Human information

Non-available

4.1.3 Summary and discussion on toxicokinetics

The toxicokinetics of proquinazid was investigated orally in one single dose and one repeat dose study in rats. Following single and repeat administration, proquinazid was well absorbed and widely distributed. Proquinazid was extensively metabolised and was rapidly excreted in the urine and faeces. Biliary excretion accounted for nearly all of the faecal excretion. There was no evidence of bioaccumulation.

4.2 Acute toxicity

Table 11: Summary table of relevant acute toxicity studies

Method	LD_{50}	Remarks	Reference
Oral OECD 401 Sprague-Dawley rat 5/sex/dose DPX-KQ926-75	> 2000 mg/kg bw	Black ocular discharge was observed in one female. Other effects (e.g. hunched posture, and bodyweight loss) indicative of general toxicity were observed. No gross lesions indicative of organ toxicity were observed at necropsy	Filliben; 1999a in reference 2
Inhalation OECD 403 (1981) Sprague- Dawley rat 5/sex DPX-KQ926-85A	> 5.2 mg/l (4 hour exposure)	Mean particle size of the dust tested was 3.3 µm; 36-46 %, < 3 µm (% w/w). No mortalities were observed. Ocular and/or oral discharges were noted in one rat immediately after exposure and one day later. Apart from slight to severe weight losses on the day following exposure, no other signs of toxicity or gross lesions were observed.	Kegelman (2003) in reference 2
Dermal OECD 402 Sprague-Dawley rat 5/sex/group DPX-KQ926-75	> 2000 mg/kg bw	No deaths were observed following occluded exposure to 5000 mg/kg bw. Apart from very slight erythema observed in one rat, no treatment–related signs of toxicity were observed.	Filliben, 1999b in reference 2

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

An oral LD_{50} value of > 2000 mg/kg bw was derived from a study conducted with rats.

4.2.1.2 Acute toxicity: inhalation

An inhalation LC_{50} of > 5.2 mg/l for 4 hours was derived from a study conducted with rats.

4.2.1.3 Acute toxicity: dermal

A dermal LD_{50} of > 2000 mg/kg bw was derived from a study conducted with rats.

4.2.1.4 Acute toxicity: other routes

No data available

4.2.2 Human information

No data available

4.2.3 Summary and discussion of acute toxicity

See Section 4.2.1

4.2.4 Comparison with criteria

Under Directive 67/548/EEC and the CLP Regulation, substances should be classified for the oral and dermal routes if the LD_{50} values are ≤ 2000 mg/kg. Since the LD_{50}s are > 2000 mg/kg bw via either route, no classification is required.

Via the inhalation route, classification is only required if the LC_{50} is ≤1 mg/l for aerosols and particulates under Directive 67/548/EEC and ≤ 5 mg/l for dusts and mists under the CLP Regulation. Since the LC_{50} is > 5.2 mg/l, no classification is required.

4.2.5 Conclusions on classification and labelling

Directive 67/548/EEC: Not classified based on available data

CLP: Not classified based on available data

RAC evaluation of Acute toxicity

Summary of dossier submitter's proposal

The dossier submitter proposed not to classify Proquinazid for acute toxicity. Dossier submitter's proposal not to classify Proquinazid for acute toxicity was based on three studies where rats were exposed via oral, inhalation and dermal routes. All the reported studies were performed according to OECD test protocols.

Comments received during public consultation

No comments were received regarding this classification during public consultation.

Outcome of RAC assessment - comparison with criteria and justification

According to a protocol OECD Guideline No. 401 (Filiben, 1999a), the oral LD50 value for male and female rats is above 2000 mg/kg bw and, therefore, no classification or labelling is required for acute oral toxicity.

According to a protocol OECD Guideline No. 402 (Filiben, 1999b), the dermal LD50 value for male and female rats is above 2000 mg/kg bw and, therefore, no classification or labelling is required for acute dermal toxicity.

According to a protocol OECD Guideline No. 403 (Kegelman, 2003), the inhalation LC50 value for male and female rats is above 5.2 mg/l (rats, 4 hour), above threshold levels for aerosols and particulates (≤1 mg/l) and for dusts and mists (≤ 5 mg/l). Therefore, no classification or labelling is required for acute inhalation toxicity.

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

Black ocular discharge was observed in one top dose female from both the oral and inhalation studies. All other clinical signs were considered to be non-specific signs of general acute toxicity.

4.3.2 Comparison with criteria

Classification in category 1 is not justified since there is no evidence of proquinazid causing specific target organ toxicity in humans. Classification with category 2 should be considered if significant toxic effects occur at moderate concentrations. The only potential effect of concern was black ocular discharge observed in one female from both the oral and inhalation studies. Other clinical signs of toxicity were considered to be non-specific signs of general acute toxicity and, therefore, not relevant for classification. The discharge was only observed at high dose levels (although only one dose was tested in the inhalation study, the low incidence suggests it would not be observed at lower levels) and, as such, classification with category 2 is not considered appropriate. No narcotic or respiratory tract irritation was observed and therefore classification with category 3 is not necessary.

4.3.3 Conclusions on classification and labelling

Directive 67/548/EEC: Not classified based on available data

CLP: Not classified based on available data

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of dossier submitter's proposal

The dossier submitter proposed not to classify Proquinazid for specific target organ toxicity – single exposure (STOT SE).

Comments received during public consultation

No comments were received regarding this classification

Outcome of RAC assessment - comparison with criteria and justification

Black ocular discharge was observed in female rats from the oral study and in one rat in the inhalation study. All other clinical signs were considered to be non-specific signs of general acute toxicity. Based on the results of the acute toxicity, no classification or labelling is required for acute toxicity according to Directive 67/548/EEC and the CLP Regulation.

4.4 Irritation

4.4.1 Skin irritation

Table 12: Summary table of relevant skin irritation studies

Method	Results: Average Scores	Remarks	Reference
OECD 404 (1992) New Zealand White rabbits DPX-KQ926-75	Erythema: 0.33 (max score: 2) Oedema : 0 (max score: 0)	Six animals were tested	Filliben (1999c) in reference 2

4.4.1.1 Non-human information

The skin irritation potential of proquinazid has been investigated in one standard guideline study. Erythema, but not oedema, was observed.

4.4.1.2 Human information

No data available

4.4.1.3 Summary and discussion of skin irritation

The skin irritation potential of proquinazid has been investigated in one standard guideline study. Erythema, but no oedema, was observed.

4.4.1.4 Comparison with criteria

The average erythema and oedema scores were < 2, therefore no classification is required under Directive 67/548/EEC.

Since this study was conducted with six animals, the criteria laid out in the CLP Regulation are not directly applicable. However, in accordance with the "Guidance on the Application of the CLP Criteria", for tests conducted with more than three animals, if either the overall average is above 2.3 or the mean score per animal is above 2.3 in 4 out of the 6 animals, classification as Category 2 is required. No scores above 2.3 were observed and, therefore, classification is not justified.

Desquamation and erythema were observed in the 28-dermal study (section 4.7.1.3). As these effects were only observed from day 24, they are considered indicative of proquinazid's weak irritating potential and are not considered relevant for classification.

4.4.1.5 Conclusions on classification and labelling

Directive 67/548/EEC: Not classified based on available data

CLP: Not classified based on available data

RAC evaluation of skin irritation
Summary of dossier submitter's proposal The dossier submitter proposed not to classify Proquinazid for skin irritation. The proposal was based on one study in white rabbits which was performed according to the OECD Guideline 404. ***Comments received during public consultation*** No comments were received regarding this classification. ***Outcome of RAC assessment - comparison with criteria and justification*** In the reported study on white rabbits, erythema, but not oedema, was observed. The average erythema and oedema scores were < 2, therefore no classification is required under Directive 67/548/EEC. Desquamation and erythema were observed in the 28-dermal study. As these effects were only observed from day 24, they are considered indicative of proquinazid's weak irritating potential and are not considered relevant for classification. Based on the results, no classification or labelling is required according to Directive 67/548/EEC and CLP Regulation (EC) 1272/2008 as regards the irritation of skin.

4.4.2 Eye irritation

Table 13: Summary table of relevant eye irritation studies

Method	Results: Average scores	Remarks	Reference
OECD 405 (1987) New Zealand White rabbits DPX-KQ926-75B	Cornea: 0 Iris: 0 Conjunctivae – redness: 1.33 (max score 3) Conjunctiva – chemosis: 0.28 (max score 1)	Six animals were tested	Filliben (1999d) in reference 2

4.4.2.1 Non-human information

The eye irritation potential of proquinazid has been investigated in a standard guideline study. No effects on the cornea or iris were noted. Effects on the conjunctivae were limited to erythema and mild oedema. Clear conjunctival discharge was noted after 1 h, but not at later time points.

4.4.2.2 Human information

No data available

4.4.2.3 Summary and discussion of eye irritation

The eye irritation potential of proquinazid has been investigated in a standard guideline study. No effects on the cornea or iris were noted. Effects on the conjunctivae were limited to redness and mild oedema. Clear conjunctival discharge was noted after 1 h, but not at later time points.

4.4.2.4 Comparison with criteria

No effects on the cornea or iris were noted. The average scores for effects on the conjunctivae were < 2; therefore, no classification is required under Directive 67/548/EEC.

Since this study was conducted on six animals, the criteria within the CLP Regulation are not directly applicable. However, the "Guidance on the Application of the CLP Criteria" states that classification is required if the individual average is greater than the cut off in 4 out of the 6 animals. No effects on the cornea or iris were observed. The relevant average score for conjunctival redness and oedema is 2. Only one animal had a conjunctival redness score of ≥ 2 and therefore classification is not required under the CLP Regulation.

4.4.2.5 Conclusions on classification and labelling

Directive 67/548/EEC: Not classified based on available data

CLP: Not classified based on available data

RAC evaluation of eye irritation

Summary of dossier submitter's proposal

The dossier submitter proposed not to classify Proquinazid for eye irritation. The eye irritation potential of proquinazid was investigated in a standard guideline study.

Comments received during public consultation

No comments were received regarding this classification.

Outcome of RAC assessment - comparison with criteria and justification

No effects on the cornea or iris were noted in the reported study. Effects on the conjunctivae were limited to erythema and mild oedema. Clear conjunctival discharge was noted after 1 h, but not at later time points. Based on the results, no classification or labelling is required according Directive 67/548/EEC and CLP Regulation (EC) 1272/2008 as regards eye irritation.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

This endpoint was not investigated directly; however, no signs of respiratory irritation were observed in the acute inhalation study (see section 4.2).

4.4.3.2 Human information

No information available

4.4.3.3 Summary and discussion of respiratory tract irritation

This endpoint was not investigated directly; however, no signs of respiratory irritation were observed in the acute inhalation study (see section 4.2).

4.4.3.4 Comparison with criteria

No signs of respiratory tract irritation were observed as outlined in either Directive 67/548/EEC or the CLP Regulation.

4.4.3.5 Conclusions on classification and labelling

Directive 67/548/EEC: Not classified based on available data **CLP: Not classified based on available data**

RAC evaluation of respiratory tract irritation
Summary of dossier submitter's proposal The dossier submitter proposed not to classify Proquinazid for respiratory track irritation. ***Comments received during public consultation*** No comments were received regarding this classification. ***Outcome of RAC assessment - comparison with criteria and justification*** Although not experimentally tested, proquinazid was assumed not to be a respiratory irritant from acute toxicity experiments. No specific information is given, RAC agrees that no classification is needed.

4.5 Corrosivity

Table 14: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
Refer to table 12			

4.5.1 Non-human information

Proquinazid is not irritating to skin (see section 4.4)

4.5.2 Human information

No data available.

4.5.3 Summary and discussion of corrosivity

See section 4.5.1

4.5.4 Comparison with criteria

No signs of corrosivity were observed in an *in vivo* skin irritation study.

4.5.5 Conclusions on classification and labelling

Directive 67/548/EEC: Not classified based on available data

CLP: Not classified based on available data

RAC evaluation of corrosivity

Summary of dossier submitter's proposal

The dossier submitter proposed not to classify Proquinazid for corrosivity.

Comments received during public consultation

No comments were received regarding this classification.

Outcome of RAC assessment - comparison with criteria and justification

Dossier submitter stated that no signs of corrosivity were observed in an *in vivo* skin irritation study of Proquinazid. Given the available data, RAC agrees with the DS proposal that no classification or labelling is required for corrosivity.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 15: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
OECD 406 (1992) – maximization study Guinea-pig/ Hartley albino DPX-KQ926-75	Negative 3/18 test (2 animals died) 1/10 control	Induction: Intradermal: 3% + FCA Skin responses not reported Topical: 0.5 g in 0.5 ml propylene glycol + SLS Skin responses not reported Challenge: 0.5 g in 0.5 ml propylene glycol (considered 100 %) Positive control behaved as expected	Hershma (1999) in reference 2

4.6.1.1 Non-human information

The skin sensitisation potential has been investigated in a standard maximisation study. Positive responses were observed in 3/18 animals compared to 1/10 in the control.

4.6.1.2 Human information

No data available

4.6.1.3 Summary and discussion of skin sensitisation

The skin sensitisation potential has been investigated in a standard maximisation study. Positive responses was observed in 3/18 animals compared to 1/10 in the control.

4.6.1.4 Comparison with criteria

The sensitisation response was < 30 % in a guinea-pig maximisation study. Therefore, no classification is required under Directive 67/548/EEC or the CLP Regulation.

4.6.1.5 Conclusions on classification and labelling

Directive 67/548/EEC: Not classified based on available data

CLP: Not classified based on available data

RAC evaluation of skin sensitisation
Summary of dossier submitter's proposal The dossier submitter proposed not to classify Proquinazid for skin sensitisation. The proposal not to classify Proquinazid for skin sensitisation was based on a standard maximisation study performed according to the OECD 406 test Guideline. ***Comments received during public consultation*** No comments were received regarding this classification. ***Outcome of RAC assessment - comparison with criteria and justification*** According to the Guinea pig maximisation test (OECD Guideline No. 406), Proquinazid induced skin sensitisation in 3/18 animals compared to 1/10 in the control. Given that less than the 30% positive responses were obtained in the test, RAC agrees that no classification for skin sensitisation is required under Directive 67/548/EEC or the CLP Regulation.

4.6.2 Respiratory sensitisation

Table 16: Summary table of relevant respiratory sensitisation studies

Method	Results	Remarks	Reference

4.6.2.1 Non-human information

No data available

4.6.2.2 Human information

No data available

4.6.2.3 Summary and discussion of respiratory sensitisation

Not applicable

4.6.2.4 Comparison with criteria

Not applicable

4.6.2.5 Conclusions on classification and labelling

There is no available information on the potential of the test substance to induce respiratory sensitisation.

Directive 67/548/EEC: Not classified - data lacking

CLP: Not classified - data lacking

RAC evaluation of respiratory sensitisation
Summary of dossier submitter's proposal
The dossier submitter proposed not to classify Proquinazid for respiratory sensitisation.
Comments received during public consultation
No comments received.
Outcome of RAC assessment - comparison with criteria and justification
Data is lacking and RAC concludes that no classification is required.

4.7 Repeated dose toxicity

Table 17: Summary table of relevant repeated dose toxicity studies

Method	Results	Reference
90-day study OECD 408 (1981) Rat Sprague-Dawley 22/sex/group Daily in the diet 0, 30, 100, 300 or 2000 ppm in males corresponding to 2, 6, 19 and 135 mg/kg bw/day 0, 30, 100, 300 or 600 ppm in females corresponding to 2, 8, 23 and 50 mg/kg bw/day in females DPX-KQ926-45	***General toxicity (10 rats/sex)*** <u>2000 ppm (males only)</u> 28 % ↓ bodyweight, 47 % ↓ bodyweight gain, 14 % ↓ food consumption, 40 % ↓ food efficiency *Anemia:* 15 % ↓ haemoglobin (day 45) *Liver:* 23 % ↑ relative to bodyweight, 14 % ↓ relative to brain weight, 132 % ↑ ALP, 57 % ↑ bilirubin, 63 % ↑ cholesterol, alteration, periportal hepatocytes (10/10), fatty change, midzonal (7/10), hyperplasia (oval cell) (2/10), hyperplasia, bile duct (1/10), increased Kupffer cell pigment (2/10), 40 % ↓ hepatic deiodinase, 299 % ↑ hepatic UDP- glucoronyltransferase *Thyroid:* 50 % ↑ thyroid weight relative to body weight, follicular hypertrophy (7/10), 46 % ↓ T_4, 10 % ↓ T_3, 22 % ↑ rT_3, 75 % ↑ TSH, *Kidney:* Tubular pigment (4/10) <u>600 ppm (females only)</u> 18 % ↓ bodyweight, ↓ 38 % bodyweight gain, 11 % ↓ food consumption, 30 % ↓ food efficiency *Liver:* 21 % ↑ relative to body weight, 183 % ↑ ALP, alteration, periportal hepatocytes (6/10), fatty change, midzonal (5/10), hyperplasia (oval cell) (2/10), hyperplasia, bile duct (7/10), increased Kupffer cell pigment (1/10), 43 % ↓ hepatic deiodinase, 161 % ↑ hepatic UDP- glucoronyltransferase *Thyroid:* 17 % ↑ absolute, 56/25 % ↑ relative to body/brain weight, follicular hypertrophy (2/10), 47 % ↓ T_4, 38 % ↓ T_3, 30 % ↑ rT_3, 43 % ↑ TSH *Kidney*: tubular pigment (4/10) <u>300 ppm</u> *Anemia:* 28 % ↓ red blood cells (day 45), 26 % ↓ haemoglobin (day 45), 26 % ↓ haematocritt (day 45) in males only *Liver:* 10 % ↑ relative to body weight (males), 30 % ↑ cholesterol (males), periportal hepatocytes alteration (3/10 males), fatty change, midzonal (1/10 male), hyperplasia, bile duct (1/10 female), 17 % ↓ hepatic deiodinase (females), 63/ 95 % ↑ hepatic UDP- glucoronyltransferase (males/females) *Thyroid:* 17/ 19 % ↓ T_3 (males/females), 27/53 % ↑ rT_3 (day 45 only in males/females), 38/21 % ↑ TSH (males/females), follicular hypertrophy (7/ 10 males and 4/10 females) *Kidney:* tubular pigment (4/10 males) <u>100 ppm</u> *Anemia:* 26 % ↓ red blood cells (day 45) (males) , 23 % ↓ haemoglobin (day 45) (males), 26 % ↓ in haematocrit (day 45) (males) *Liver:* 36 % ↑ hepatic UDP- glucoronyltransferase (males) *Thyroid*:16 % ↑ rT_3 (males), follicular hypertrophy (8/10 males) <u>30 ppm</u> *Anemia:* 6 % ↓ in haemoglobin (day 45) - the magnitude of this effect was such that it was not considered adverse ***Neurotoxicity (12 rats/sex)*** No neurotoxicological effects were observed at any dose level in either sex. NOAEL 30/100 ppm in males/females based on hormonal and morphological changes in the thyroid	Malley (2003a) in reference 2

90-day study OECD 408 (1981) Daily in the diet Sprague-Dawley 10/sex/group 0, 30, 100, 300 or 2000 ppm in males corresponding to 2, 6, 19 and 127 mg/kg bw/day in males 0, 30, 100, 300 or 600 ppm in females corresponding to 2, 8, 24 and 50 mg/kg bw/day in females DPX-KQ926-75	***General toxicity (10 rats/sex)*** <u>2000 ppm (males only)</u> *Liver:* 22 % ↑ absolute weight, hepatocellular hypertrophy (6/10), 53 % ↓ hepatic deiodinase, 141 % ↑ hepatic UDP- glucoronyltransferase *Thyroid:* 32 % ↑ absolute weight, follicular hypertrophy (10/10), 26 % ↑ T_4, 23 % ↓ T_3, 48 % ↑ rT_3, 44 % ↑ TSH <u>600 ppm (females only)</u> 25 % ↓ bodyweight gain, 21 % ↓ food efficiency, 46 % ↓ white blood cells, 46 % ↓ in lymphocytes *Liver*: 17 % ↑ relative to body weight, hepatocellular hypertrophy 7/10, 148 % ↑ hepatic 5'-UDP- glucoronyltransferase *Thyroid*: follicular hypertrophy (7/10) <u>300 ppm</u> 30 % ↓ white blood cells (females) *Liver:* 83 % ↑ hepatic 5'-UDP- glucoronyltransferase (females) *Thyroid:* follicular hypertrophy (9/10 males), 82 % ↑ TSH (males), <u>100 ppm</u> *Thyroid*: follicular hypertrophy (7/10 males), 38 % ↑ TSH (day 14only in males) <u>30 ppm:</u> No effects NOAEL – 30 ppm and 100 ppm in males and females, respectively based on thyroid hypertrophy and hormonal changes	Malley (2002b) in reference 2
90-day study OECD 409 (1981) Daily in diet Dog Beagle 4/sex/group 0, 500, 2000 or 4000/3000 ppm equivalent to 0, 17, 62 and 87 mg/kg bw/day in males and 0, 18, 56 and 95 mg/kg bw/day in females Due to weight loss and decreased food consumption, the high dose group received no test substance on week 5 and resumed on week	<u>4000/3000 ppm</u> *Eyes and ears*: clear ocular discharge most notable at time of feeding (total number of times observed - number of animals: 32-2 in males and 82-4 in females)* Bilateral epiphora (mucoid and serous discharge) was seen in 4 dogs (sex not given); one also showed unilateral conjunctivitis. Brown/green material around eye, reddened ears also noted at time of feeding (total number of times observed-number of animals: 17-2 in males and 9-2 in females) *Bodyweight:* 22/24 %↓ body weight (males/females), 73/94 % ↓ bodyweight gain (males/females), 43/41 % ↓ food consumption (males/females) (palatability effects?) *Liver*: 50/64 % ↑ relative liver weight to body weight (males/females) <u>2000 ppm</u> *Eyes and Ears:* clear ocular discharge most notable at time of feeding (total number of times observed - number of animals: 17-1 in right eye and 8-2 in left eye in males and 66-3 in females), reddened ears also noted 1 to 2 hrs after feeding (total incidence/number of animals: 3-2 in males and 4-3 in females) *Bodyweight*: 29 % ↓ bodyweight gain (males), 24 % ↓ food consumption (males) *Liver:* 57 % ↑ relative liver weight to brain weight (females) <u>500 ppm</u> *Eyes and ears:* clear ocular discharge most notable at time of feeding (total number of times observed-number of animals: 36-1 in males and 42-2 in females), reddened ears also noted 1 to 2 hrs after feeding (total number of times observed-number of animals: 23-4 in males and 11-2 in females) *Liver*: 24 % ↑ relative liver weight to body weight (females), 42 % ↑ relative liver weight to brain weight (females) Controls	Mertens (1997) in reference 2

6 at 3000 ppm DPX-KQ926-45	Clear ocular discharge most notable at time of feeding (total number of times observed-number of animals: 0-0 in males and 15-1 (left eye only) in females) Reddened ears also noted 1 to 2 hrs after feeding (total incidence/number of animals: 0/0 in males and 2-2 (right ear only) in females) No NOAEL derived	
One year study Oral (capsules) Dogs Beagles 5/sex/dose OECD 452 (1981) 0, 15, 60, 180 mg/kg bw/day High dose was 0 for study week 1, 120 mg/kg bw/day for study week 1 and 180 mg/kg bw/day for the remainder DPX-KQ926-45	<u>180 mg/kg bw/day</u> *Clinical signs:* included increased emesis (particularly in males) *Eyes and Ears*; clear ocular discharge most notable at time of dosing (total number of times observed-number of animals: 265-4 in males and 452-3 in females)* Nasal discharge after 1-2 hr (4-3 in males and 35-4 in females) *Bodyweight:* 11 % ↓ bodyweight (males NS), 43/43 % ↓ body weight gain (males/females NS) Seminiferous tubules: severe atrophy/degeneration (1 dog), Moderate atrophy/degeneration (1 dog – accompanied by minimal inflammation). Severe oligospermia/germ cell debris (bilateral) (2 dogs – accompanied by minimal inflammation in 1 dog) <u>60 mg/kg bw/day</u> *Clinical signs:* included increased emesis (particularly in males) *Eyes and ears*: clear ocular discharge most notable at time of dosing (total number of times observed-number of animals: 6-3 in males and 154-2 in females)* Nasal discharge after 1 to 2 hr (8-4 in males and 1-1 in females) *Bodyweight:* 10 % ↓ bodyweight (males NS), 31% ↓ body weight gain (males NS) Seminiferous tubules: mild atrophy/degeneration (1 dog – accompanied by mild inflammation). moderate oligospermia/germ cell debris (bilateral) (1 dogs – accompanied by minimal inflammation) <u>15 mg/kg bw/day</u> *Eyes and ears*: clear ocular discharge most notable at time of dosing (total number of times observed-number of animals: 37-3 in males and 41-3 in females)* Nasal discharge after 1-2 hr (1-1 in males and 1-1 in females) <u>Control</u> *Eyes and ears*; clear ocular discharge most notable at time of dosing (total number of times observed-number of animals: 4-3 in males and 26-3 in females)* Nasal discharge after 1-2 hr (0-0 in males and females) Seminiferous tubules: minimal atrophy/degeneration (2 dogs) Minimal oligospermia/germ cell debris (bilateral) (2 dogs) A NOAEL of 15 m/kg/day for males based on reduced bodyweight gain observed at 60 mg/kg bw/day. A NOAEL of < 15 mg/kg bw/day proposed for females based on increased incidence of ocular discharge.	Mertens (2002) in reference 2
28-day study Rat OECD 410 (28-day dermal) 6 h per day Sprague-Dawley	<u>2000 mg/kg bw/day</u> 13 % ↓ body weight (females), 68 % ↓ body weight gain (females), 77 % ↓ food efficiency (females), desquamation and local erythema *Liver*: 15 % ↑ absolute weight (males), 20/11 (non statistically significant) % ↑ relative to body weight (males/females), 15 % ↑ relative to brain weight (males), hypertrophy (10/9 in males/females) *Thyroid:* follicular hypertrophy (4/1 in males/females) <u>1000 mg/kg bw/day</u> 10 % ↓ body weight (females), 57 % ↓ body weight gain (females), 57 % ↓	Finlay (2002) in reference 2

10/sex/dose 100, 500, 1000, 2000 mg/kg bw/day DPX-KQ926-45	food efficiency (females) Desquamation and local erythema *Liver:* 12 % ↑ absolute weight (males), 16/17 % ↑ relative to body weight (males/females), 13/15 % ↑ relative to brain weight (males/females), hypertrophy (4/9 in males/females) *Thyroid:* follicular hypertrophy (4/1 in males/females) <u>500 mg/kg bw/day</u> 32 % ↓ body weight gain (females), 32 % ↓ food efficiency (females) *Liver:* 15 % relative to body weight (females), hypertrophy (6 females) <u>100 mg/kg bw/day</u> No adverse effects observed The NOAEL is 500 mg/kg bw/day in males based on thyroid hypertrophy and 100 mg/kg bw/day for females based on decreased bodyweight, nutritional parameters, increased liver weight and hepatic hypertrophy	

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed at a PRAPER expert meeting

** The presence of the ocular discharge differed between eyes; the data presented in the table represent the eye with the highest incidence (expressed as no of times observed – number of animals).*

4.7.1 Animal information

4.7.1.1 Repeated dose toxicity: oral

There are two 90-day studies and one chronic study (see section 4.10) available in the rat, one chronic study in mice (see section 4.10), and one 90-day study and a one-year study available in the dog.

Rat studies

Two 90-day rat studies are available. The dose levels chosen for females were lower than for males based on higher toxicity observed in females in the acute toxicity studies. As the effects observed in top dose males (127 mg/kg bw/day) were similar to those observed in females at 50 mg/kg bw/day, a sex difference in the sensitivity to proquinazid is confirmed. In both 90-day studies, the liver and thyroid were identified as target organs and effects on bodyweight were also observed. The thyroid was the most sensitive organ with adverse effects observed from 6 mg/kg bw/day (follicular hypertrophy and changes in thyroid hormones). It is possible these changes were due to the elevated hepatic UDP-glucuronyltransferase observed at this dose level. In males, in one study (Malley 2003b), relative liver weight was increased (> 10 %) from 19 mg/kg bw/day and histopathological changes (fatty change, hyperplasia of the oval cell and bile duct) indicative of organ dysfunction were also observed at this dose level. Whereas, in the other study (Malley, 2002b), relative liver weight was not increased until 127 mg/kg bw/day (although this may partly be due to dose spacing), and was accompanied by adaptive, rather than adverse changes. In females, reductions in food consumption/efficiency and bodyweight/bodyweight gain (> 10 %) were observed in both studies from 50 mg/kg bw/day. Since, similar effects on bodyweight were observed in the dermal rat study (see section 4.7.1.3), these reductions would not appear to be due to unpalatability of the test substance.

In one of the 90-day studies (Malley, 2003b), effects suggestive of anaemia were observed in males (characterised by ↓ red blood cells, ↓ haemoglobin, ↓ haematocrit) in all doses apart from the high dose. A lack of effects in females and in high dose males, in combination with an absence of effects in the spleen (e.g. haemosiderosis) and a failure to observe similar effects in the chronic studies

(conducted on the same batch), suggests that the effects observed may be spurious and not a direct consequence of proquinazid administration.

In the available rat chronic study (see section 4.10), adverse effects were observed from 12 mg/kg bw/day (see section 4.10). At this dose level, brown-stained teeth, increases in liver enzymes (↑sorbitol dehydrogenase) and histopathological changes in the liver (fatty changes and degeneration heptocytosis) and thyroid (follicular cell hypertrophy, cystic hyperplasia), as well as perturbations in thyroid hormone levels were observed. At the next dose level in males (43 mg/kg bw/day) and females (35 mg/kg bw/day), additional adverse effects included dark red eyes, reductions in bodyweight (> 10 % in females) and food consumption, increases in relative liver weight with accompanying clinical chemistry changes (↑ alanine aminotransferase, ↓ total protein) and histopathological changes (cholangiofibrosis, biliary cyst, bile duct and oval cell hyperplasia). Histopathological affects in the thyroid were also observed (large size and masses). At higher doses (≥ 76 mg/kg bw/day), bodyweight was reduced (> 10 %) in males, thyroid weights were increased in both sexes, and increases in absolute testes and ovary weights were observed. No histopathological changes were noted in the testes, but the increase in ovary weight was likely to be due to the increased number of ovarian cysts observed (16 compared to 9 in the controls).

Mouse studies

In an 18-month chronic study, a reduction in bodyweight gain was observed in females during the first year of the study at 27 mg/kg bw/day (see section 4.10). At this dose level, effects were also observed in the liver (hepatocyte alteration, hypertrophy and pigment accumulation). At higher doses (≥ 282 m/kg/day), reductions in bodyweight gain were also observed in males and liver effects were more pronounced (↑ liver weight, necrosis, hypertrophy, hyperplasia, fatty change and pigment accumulation). At this dose level, effects in the thyroid (enlarged size, follicular cell hypertrophy, cysts and inflammation) were also observed.

Dog studies

In the 90-day study effects were observed from the lowest dose tested (17 mg/kg bw/day). At this dose level, relative liver weight was considerably increased in females (> 20 %). Ocular discharge and reddened ears were also observed in both sexes and to a lesser extent in the control animals. The cause of the ocular discharge is unknown. At the higher dose levels (≥ 56 mg/kg bw/day), reduced body weight gain (> 20 %), bodyweight and food consumption was observed.

In the one-year study, clear ocular discharge was noted in all dose groups (from 15 mg/kg bw/day) and to a lesser extent in the controls. Bodyweight in males and bodyweight gain in males/females was reduced from 60 mg/kg bw/day. Effects on sperm parameters (atrophy and bilateral oligospermia) were also observed from this dose level. The severity of some of the lesions in the testes and epididymides worsened at 60 and 180 mg/kg bw/day, but there was no increase in incidence compared to the control. These effects are common in the testes of beagle dogs of all ages and may have been influenced by the reductions in body weight gain. In addition, similar effects were not noted in the rat studies. Overall, the testicular findings are considered spurious or secondary to the effects on body weight gain.

4.7.1.2 Repeated dose toxicity: inhalation

No data available

4.7.1.3 Repeated dose toxicity: dermal

There is one 28-day dermal study available in the rat.

In this study, an adverse reduction in bodyweight gain and effects on the liver (↑ relative weight and hypertrophy) started to occur at 500 mg/kg bw/day. At higher doses (≥ 1000 mg/kg bw/day), reduced bodyweight, increased absolute liver weight and thyroid effects (follicular hypertrophy) were observed.

Desquamation and erythema were observed in animals treated with either 1000 or 2000 mg/kg bw/day proquinazid from day 24. These effects are considered consistent with the weak irritant potential observed in the acute studies (see section 4.4)

4.7.1.4 Repeated dose toxicity: other routes

No data available

4.7.1.5 Human information

No data available

4.7.1.6 Other relevant information

Not applicable

4.7.1.7 Summary and discussion of repeated dose toxicity

The repeat dose toxicity of proquinazid has been investigated in two 90-day and one chronic study in the rat, one chronic study in the mouse, and one 90-day and one 1-year study in the dog.

Rat studies

Oral

In the available sub-chronic and chronic studies in the rats, adverse effects were observed. From 6 mg/kg bw/day effects in the thyroid (relative weight changes, follicular hypertrophy and thyroid hormone level alterations) were observed. From 19 mg/kg bw/day, reductions in bodyweight and effects in the liver (e.g. ↑ liver weight, fatty change and hyperplasia) were evident. The available data identified both the liver and thyroid as target organs of proquinazid toxicity.

Dermal

In the 28-day dermal study in rats, an adverse reduction in bodyweight gain and effects on the liver (↑ relative weight and hypertrophy) started to occur at 500 mg/kg bw/day. At higher doses (≥ 1000 mg/kg bw/day), reduced bodyweight, increased absolute liver weight and thyroid effects (follicular hypertrophy) were observed.

Mouse studies

In an 18-month chronic study (see section 4.10), a reduction in bodyweight gain was seen during the first year only and effects on the liver (hepatocyte alteration, hypertrophy and pigment accumulation) were observed at 27 mg/kg bw/day. At higher doses (≥ 282 m/kg/day), effects on

bodyweight and liver were more pronounced and effects in the thyroid (enlarged size, follicular cell hypertrophy, cysts and inflammation) were observed.

Dog studies

In the available 90-day study in the dog, adverse effects started to occur from a dose of 18 mg/kg bw/day. At this dose level, effects consisted of an increase in relative liver weight. At higher doses (62 mg/kg bw/day), reductions in bodyweight gain and food consumption were observed.

In a subsequent 12 month study, adverse effects started to occur from 60 mg/kg bw/day. At this dose level, a reduction in bodyweight and bodyweight gain was observed in males. At the next dose level (180 mg/kg bw/day), a reduction in bodyweight gain was also observed in females.

In both studies, ocular discharge and reddened ears were observed at all dose levels. Although the number of observations of these effects increased with dose, neither effect is considered sufficiently adverse to warrant classification.

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

The repeat dose toxicity of proquinazid has been investigated in two 90-day studies and one chronic study in the rat, one chronic study in the mouse, and one 90-day and one 1-year study in the dog. There was no evidence that the dog or mouse were more sensitive to proquinazid than the rat. Therefore, the following summary focuses on the effects that are relevant for classification based mainly on the data from the two rat sub-chronic studies.

Oral

Bodyweight

Reduced bodyweight were observed at the cut-off for classification under the DSD of 50 mg/kg bw/day. Although adverse, this effect is not severe enough to support classification by itself.

Thyroid

Effects in the thyroid (relative weight changes, follicular hypertrophy and thyroid hormone alterations) were observed below the cut-off for classification under the DSD of 50 mg/kg bw/day. These effects are not considered relevant to humans (see section 4.10.1.1) and are, therefore, not relevant for classification.

Liver

Relative liver weight was increased in one study at > 19 mg/kg bw/day (Malley, 2003) and changes indicative of metabolic perturbation (fatty change, biliary tract hyperplasia) were observed below the classification cut-off of 50 mg/kg bw/day. Although these effects are consistent with metabolic perturbation, the magnitude was not such to be considered marked. In addition, similar effects were not observed in the second rat study (Malley, 2002b), conducted at the same dose levels, nor in repeat dose studies conducted on mice or dogs. In addition, these effects are consistent with the type of effects observed with substances that cause carcinogenicity (see section 4.10). Therefore, overall no classification is required.

Eyes

In the two dog studies, ocular discharge was observed at all dose levels. Although the number of observations of this effect increased with dose, the effect is not considered to represent serious damage to health. No classification proposed.

Dermal

The classification cut-off for harmful (Xn) effects in rat dermal sub chronic studies under the DSD is 100 mg/kg bw/day. No adverse effects were observed at this dose level or below.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

See section 4.7.1.8

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

Directive 67/548/EEC: Not classified based on available data

RAC evaluation of repeated dose toxicity (DSD)
See the RAC evaluation under section 4.8

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

The repeat dose toxicity of proquinazid has been investigated in two 90-day studies and one chronic study in the rat, one chronic study in the mouse, and one 90-day and one 1-year study in the dog. There was no evidence that the dog or mouse were more sensitive to proquinazid than the rat. Therefore, the following summary focuses on the effects that are relevant for classification based mainly on the data from the two rat sub-chronic studies.

Oral

Bodyweight

Effects on bodyweight were observed at dose levels below the cut-of for classification under the CLP Regulation of 100 mg/kg bw/day. Although adverse, this effect is not severe enough to support classification by itself.

Thyroid

Effects in the thyroid (relative weight changes, follicular hypertrophy and thyroid hormone alterations) were observed below the cut-off for classification under the CLP Regulation of 100

mg/kg bw/day. These effects are not considered relevant to humans (see section 4.10.1.1) and are, therefore, not relevant for classification.

Liver

Relative liver weight was increased in one study at > 19 mg/kg bw/day (Malley, 2003) and changes indicative of metabolic perturbation (fatty change, biliary tract hyperplasia) were observed below the classification cut-off of 100 mg/kg bw/day. Although these effects are consistent with metabolic perturbation, the magnitude was not such to be considered marked. In addition, similar effects were not observed in the second rat study (Malley, 2002b), conducted at the same dose levels, nor in repeat dose studies conducted on mice or dogs. In addition, these effects are consistent with the type of effects observed with substances that cause carcinogenicity in chronic studies (see section 4.10). Therefore, overall no classification is required.

Eyes

In the two dog studies, ocular discharge was observed at all dose levels. Although the number of observations of this effect increased with dose, the effect is not considered to represent serious damage to health. No classification proposed.

Dermal

The classification cut-off for STOT RE category 2 for effects in rat dermal sub chronic studies under the CLP is 200 mg/kg bw/day. No adverse effects were observed at this dose level and below.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

See section 4.8.1

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

CLP: Not classified based on available data

RAC evaluation of repeated dose toxicity (DSD) and specific target organ toxicity – repeated exposure (STOT RE) (CLP)

Summary of dossier submitter's proposal

The dossier submitter proposed not to classify Proquinazid for repeated dose toxicity (DSD) or specific target organ toxicity – repeated exposure (STOT RE) (CLP). The proposal not to classify proquinazid for this hazard class was based on several studies where repeated dose toxicity of proquinazid was tested in two 90-day studies and one chronic study in the rat, one chronic study in the mouse, and one 90-day and one 1-year study in the dog.

Comments received during public consultation

Specific comments on repeated dose toxicity (DSD) or specific target organ toxicity – repeated exposure (STOT RE) (CLP) were not received. However, effects on thyroid and liver were commented.

Outcome of RAC assessment - comparison with criteria and justification

The repeated dose toxicity of proquinazid was investigated in two 90-day and one chronic study in rats, one chronic study in mice, and one 90-day and one 1-year study in dogs. Liver and thyroid were considered target organs of proquinazid toxicity, whereas reductions in bodyweight gain and food consumption, and ocular discharges were observed in dogs. The latter effects were not considered relevant for classification, thyroid toxicity not relevant for humans and hepatic effects were considered secondary to the liver carcinogenic activity. The observed liver effects are consistent with the carcinogenic effect, and warrant the Carc. classification (see below).

Classification according to DSD criteria

Only thyroid and liver toxicity occurred below the 50 mg/kg bw/day limit for classification. The observed liver effects are consistent with the carcinogenic effect, and warrant the Carc. classification (see the section concerning carcinogenicity). Thyroid effects in rats occur just below the cut-off dose (20 mg/kg bw/day, and the proposed MoA (the same as for thyroid tumours) is assumed not to apply to humans. In addition, DAR explicitly reports no effects in the thyroid gland in dogs. Therefore, no repeated dose toxicity classification according to DSD is proposed.

Classification according to CLP criteria

No dermal effects were observed below the CLP cut-off dose. Effects on bodyweight and eyes were observed below the cut-off dose 100 mg/kg bw/day, but not considered severe enough to support classification. Relative liver weight increase and other negative effects (fatty change, biliary tract hyperplasia) were considered as related to the carcinogenic activity. Effects in the thyroid (relative weight changes, follicular hypertrophy and thyroid hormone alterations) were not considered relevant to humans and therefore not relevant for classification.

Whereas the effects in rats (and with less extend, in mice) may warrant a STOT RE classification for thyroid, the Mode of Action (MoA) of Proquinazid for observed thyroid effects in rodents is considered not applicable to humans according to the existing information, a position favored in the comments given during the public consultation. Therefore, RAC agrees with the dossier submitter's proposal not to classify for STOT RE according to the CLP Regulation.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 18a: Summary table of relevant *in vitro* mutagenicity studies

Method	Results	Remarks	Reference
Ames OECD 471 (1997) *Salmonella typhimurium* TA97a, TA98, TA100, TA1535 and *E.coli* WP2 uvrA (pKM101) Seven concentrations between 10 – 5000 µg/plate DPX-KQ926-75	- S9: Negative + S9: Negative	Precipitation observed > 100 µg/plate No cytotoxicity observed Positive controls included	Cox (1998) in reference 2
Ames OECD 471 *Salmonella typhimurium* TA100, TA1535, TA 97a and TA 98 and *E.coli* WP2 uvrA (pKM101) Seven concentrations between 10-5000 µg/plate DPX-KQ926-45	- S9: Negative + S9: Negative	Positive controls included Precipitate observed 500 µg/plate No cytotoxicity observed	Mathison (1997) in reference 2
Chromosome aberration study OECD 473 (1983) Human peripheral blood lymphocytes Seven – eight doses between 40 – 5000 µg/ml DPX-KQ926-45	- S9: Negative + S9: Negative	Deviation: no investigation of the effect of continuous exposure –S9 Positive controls included, but responses were relatively low Cytotoxic levels recommended by the guideline were reached in this study	Gudi and Schadly (1999) in reference 2
Mammalian cell gene mutation OECD 476 Chinese Hamster Ovary cells Six – eight doses between 1.5 – 100 µg/ml DPX-KQ926-45	- S9: Negative + S9: Negative	Positive controls included Cytotoxicity was around or greater than recommended by the guideline	San and Clarke (1997) in reference 2
Mammalian cell gene mutation	- S9: Negative + S9: Negative	Positive controls included Cytotoxic levels recommended by the guideline	Ballantyne, (2005) in reference 2

OECD 476 L5178Y mouse lymphoma cells Doses between 10 - 180 µg/ml DPX-KQ926-75		were reached in this study	
Unscheduled DNA synthesis OECD 482 (1986) Rat hepatocytes Five to eight doses between 3.9 – 500 µg/ml DPX-KQ926-45	Negative	Positive controls included Precipitate observed ≥ 250 µg/ml. Excessive toxicity was also observed at these dose levels At the next dose level (125 µg/ml), 74 % and 19 % was observed in the initial and repeat dose assay	San (1999) in reference 2

Table 18b: Summary table of relevant *in vivo* mutagenicity studies

Method	Results	Remarks	Reference
Micronucleus study (bone marrow) OECD 474 Single oral dose Mouse, CD-1, 5/sex/dose 0, 720, 1440 or 2000 mg/kg in males 0,360, 720, 1440 or 2000 mg/kg in females DPX-KQ926-75	Negative	Positive controls included Signs of toxicity included lethargy, salivation, hunched over posture and/or abnormal gait in the mid and high dose groups. Statistically significant decreases in mean body weight were observed in the high dose groups at the 24, but not the 48 h time point in both sexes. No effects on the P/N ratio was observed	Wun-Kim (1999a) in reference 2
Micronucleus Study (bone marrow) OECD 474 Single dose Mouse, CD-1, 5/sex/group 0, 360, 720, 1400 mg/kg oral gavage DPX-KQ926-45	Negative	One female died at the top dose. Slight body weight loss was observed at 720 mg/kg bw. Other signs of toxicity included lethargy and diarrhea at the top two doses No effects on the P/N ratio was observed	Gudi (1999) in reference 2

4.8.4 Non-human information

4.8.4.1 *In vitro* data

The genotoxicity of proquinazid was tested in two Ames tests, two mammalian cell gene mutation assays, a chromosome aberration assay and an unscheduled DNA synthesis assay. Positive controls were included in all assays. No evidence of mutagenicity was observed in any assay.

4.8.4.2 *In vivo* data

Two studies have evaluated the potential for proquinazid to induce cytogenetic damage in the bone marrow of mice. No evidence of micronucleus formation was found in either study. In both studies, the test substance was judged to have reached the target organ.

Overall, the results of these studies provide reassurance that proquinazid has no *in vivo* mutagenic potential.

4.8.5 Human information

No information available

4.8.6 Other relevant information

No information available

4.8.7 Summary and discussion of mutagenicity

Data indicate that proquinazid is not mutagenic *in vitro* or *in vivo*.

4.8.8 Comparison with criteria

Data indicate that proquinazid is not mutagenic *in vitro* or *in vivo* and, therefore, does not require classification.

4.8.9 Conclusions on classification and labelling

No classification for mutagenicity is required.

RAC evaluation of germ cell mutagenicity (Mutagenicity)
Summary of dossier submitter's proposal The dossier submitter proposed not to classify Proquinazid for germ cell mutagenicity. The proposal was based on two Ames tests, two mammalian cell gene mutation assays, a chromosome aberration assay and an unscheduled DNA synthesis assay. ***Comments received during public consultation*** No comment specifically addressed to this hazard class was received. However, one comment explicitly accepts the lack of genotoxic/mutagenic potential for proquinazid. ***Outcome of RAC assessment - comparison with criteria and justification*** The results of any of the reported studies indicated mutagenicity of proquinazid. The data shows that proquinazid is not mutagenic *in vitro* or *in vivo* and, therefore, RAC agrees that classification according to Directive 67/548/EEC and CLP Regulation (EC) 1272/2008 for mutagenicity is not required.

4.9 Carcinogenicity

There is one carcinogenicity study available in the rat and one study available in the mouse.

Table 19: Summary table of relevant carcinogenicity studies

Method	Results Remarks	Reference
Oral OECD 453 Rat: Sprague-Dawley Daily in diet 60/sex/group exposed for 2 years 5/sex/group sacrificed after 1 week and 15/sex/group sacrificed after 1 year 0, 10, 30, 300, 1000 and 2000 ppm in males equivalent to 0, 0.4, 1.2, 12, 43 and 92 mg/kg bw/day 0, 10, 30 , 300, 600 and 1200 ppm in females equivalent to 0, 0.5, 1.4, 16, 35 and 76 mg/kg bw/day DPX-KQ926-45	***Non-neoplastic findings*** 2000 ppm (males only): Brown stained teeth, 16 % ↓ bodyweight, 26 % ↓ bodyweight gain between d0-693, 7 % ↓ in food consumption, max 12.5 % ↓ total protein (due to ↓ albumin and globulin), 19 % ↑ in testes weight (absolute), kidney tubular pigment (12) *Liver:* 156 % ↑ total hepatic P-450 content after 1 week, 31 % ↑ relative to bodyweight, discoloration of the liver, alteration/degeneration hepatocytosis (34), Fatty change (24), centrilobular fatty change (13), fatty change (midzonal) (24), focus of cellular alteration, eosinophilic (18), focus of cellular alteration (mixed) (4), Hyperplasia of the oval cell (7), hypertrophy (5) *Thyroid:* 14 % and 58 % ↑ rT_3 at 1 week and 1 year, respectively, 61 % ↑ TSH after one week, 27 % and 10 % ↓ T_3 at 1 week and 1 year, respectively. 46 % and 29 % ↓ T_4 at 1 week and 1 year, respectively. 33 % ↑ relative to bodyweight, 6 large thyroids and 3 with masses compared to 0 in the control, folicullar hypertrophy (30), follicular hyperplasia (16) 1200 ppm (females only): Brown stained teeth and dark red eyes, 35 % ↓ bodyweight; 60 % ↓ bodyweight gain between d0-693; 16 % ↓ in food consumption, 13 % ↑ in ovary weight relative to bodyweight, ↑ ovary cysts (16 compared to 9), kidney tubular pigment (35) *Liver:* ↑ alanine animotransferase (max 140 %), ↑ asparate aminotransferase (max 167 %) and sorbitol dehydrogenase (max 231 %), max 17.7 % ↓ total protein (due to ↓ albumin), 157 % ↑ total hepatic P-450 content after 1 week, 33 % ↑ absolute liver weight, alteration/degeneration hepatocytosis (59), biliary chloangiofibrosis cyst (10), biliary cyst (8) fatty change, individual cell (4), fatty change (midzonal) (37), focus of cellular alteration, eosinophilic (36), focus of cellular alteration (mixed) (6), hyperplasia bile duct (29), hyperplasia of the oval cell (52) *Thyroid*: 23 % ↑ and 46 % ↓ rT_3 at 1 week and 1 year, respectively, 18 % and 25 % ↓ T_3 at 1 week and 1 year, respectively. 47 % and 77 % ↓ T_4 at 1 week and 1 year, respectively. 26 thyroids had masses compared c.f 1 control, follicular hypertrophy (45) 1000 ppm (males only) Brown stained teeth. *Liver:* 143 % ↑ total hepatic P-450 content after 1 week, 21 % ↑ relative to bodyweight, alteration/degeneration hepatocytosis (22), cholangiosis (5), fatty change (24), fatty change (midzonal) (7), hyperplasia of the oval cell (9), kidney tubular pigment (7) *Thyroid*: 21 % and 60 % ↑ rT_3 at 1 week and 1 year, respectively, 41 % ↑ TSH after one week, 21 % and 11 % ↓ T_3 at 1 week and 1 year, respectively. 18 % ↓ T_4 at 1 week, 3 large thyroids and 2 with masses compared to 0 in the control, folicullar hypertrophy (23), follicular hyperplasia (9) 600 ppm (females only): brown stained teeth and dark red eyes. 18% ↓ bodyweight, 32 % ↓ bodyweight gain between d0-693, 8 % ↓ in food consumption, kidney tubular pigment (29) *Liver:* ↑ alanine animotransferase (max 200%), ↑ asparate	Malley (2002a) in reference 2

aminotransferase (max 125 %) and sorbitol dehydrogenase (max 306 %), max 14 % ↓ total protein (due to ↓ albumin), 138% ↑ total hepatic P-450 content after 1 week, 20 % ↑ absolute weight, alteration/degeneration hepatocytosis (46), cholangiofibrosis (4), biliary cyst (3), fatty change individual cell (17), fatty change (midzonal) (22), focus of cellular alteration, basophilic (25), focus of cellular alteration, eosinophilic (48), hyperplasia of the oval cell (42), hypertrophy (4)

Thyroid: 30 % ↑ rT_3 at 1 week, 20 % and 16 % ↓ T_3 at 1 week and 1 year, respectively. 59 % ↓ T_4 at 1 year, 14 large thyroids c.f. 1 in control, follicular hypertrophy (36)

<u>300 ppm</u> Brown stained teeth, kidney tubular pigment (5 in females)

Liver: ↑ sorbitol dehydrogenase (max 100 %) in females, 120/121 % ↑ total hepatic P-450 content in males/females after 1 week, alteration/degeneration hepatocytosis (16/13 in males/females), Fatty change individual cell (6 in females), fatty change (midzonal) (5 in either sex), focus of cellular alteration, eosinophilic (20 in females),

Dose Level (ppm)						
Males						
Dose	**0**	**10**	**30**	**300**	**1000**	**2000**
Thyroid: Follicular cell						
Animal no	60	59	61	58	59	**60**
Adenoma	1	0	1	3	**6# (10 %)**	**8# (13 %)**
Carcinoma	0	0	0	0	1	**1**
Liver: Hepatocellular						
Animal no	60	60	61	60	60	**60**
Adenoma	2	5	3	3	1	**1**
Carcinoma	0	2	2	1	2	**0**
Females						
Dose	**0**	**10**	**30**	**300**	**600**	**1200**
Thyroid: Follicular cell						
Animal no	30	61	60	59	57	**59**
Adenoma	2	0	0	0	2	**1**
Carcinoma	0	0	0		0	**0**
Liver: Hepatocellular						
Animal no	60	61	60	59	57	**59**
Adenoma	1	0	0	2	**11# (19 %)**	**29# (49 %)**

Carcinoma	0	0	0	0	2	**1**
Liver: Cholangiocarcinoma						
Intestinal type	**0**	**0**	**0**	**0**	**8#** **(14 %)**	**12#** **(20 %)**

Thyroid: 17 % and 70 % ↑ rT_3 at 1 week and 1 year, respectively (males), 18 % and 7 % ↓ T_3 at 1 week and 1 year, respectively (males). 20 % and 16 % ↓ T_3 at 1 week and 1 year (males), 59 % ↓ T_4 at 1 year (females), folicullar hypertrophy (14/10 in males/females), follicular hyperplasia (7 males)

30 ppm No adverse findings

10 ppm No adverse findings

Neoplastic findings **(descedants and terminal)**

statistically significant by Cochran test, $p< 0.05$

NOAEL 30 ppm in males/females based on non-neoplastic liver lesions and changes in thyroid hormones

<table>
<tr>
<td>Oral

OECD 451 (1981)

Mouse: CD-1

Daily in diet

55/sex/group exposed for 18 months

15/sex/group used in mechanistic liver investigations

0, 5, 30, 200, 2000 ppm, equivalent to 0, 0.7, 4, 27, 282 mg/kg bw/day in males and 0, 1, 6, 38 and 415 mg/kg bw/day in females

DPX-KQ926-45</td>
<td><u>Non-neoplastic lesions</u>
2000 ppm
22 and 13 % ↓ body weight gain (males/females) at 1 year, no difference at 18 months
Liver: 49/53 % ↑ absolute weight in males/females, 52/62 % ↑ in relative body weight, hepatic alteration (10/40 in males/females), hepatocyte hypertrophy (42/44 in males/females), oval cell hyperplasia (2/7 in males/females), bileduct hyperplasia (12/4 in males/females), hepatocyte necrosis (15/27 in males/females), intracytoplasmic erythrocytes (10 females), ↑ hepatocellular pigment (6 males), ↑ kupffer cell pigment (13/8 in males/females), fatty change (2/24 in males/females), ↑ mitotic figures (7 females)
142/208 and 127/111 % ↑ β – oxidation (measure of peroxisome proliferation) and 97/169 and 55/104 % ↑ cytochrome 450 content at 1 week and 6 months in males/females, respectively

Thyroid: enlarged thyroid gland (2/7 in males/females), follicular cell hyperplasia (7 females), follicular cell hypertrophy (6 females) , follicular cyst (2 females), subacute/chronic inflammation (15/2 males/females)

200 ppm: 10 % ↓ body weight gain at 1 year in females.
Liver: Hepatocyte alteration (all zones) (4/5 in males/females), Hepatocyte hypertrophy (11/ 11 in males/females), ↑ pigment Kupffer cells (6 females)

30 and 5 ppm: No significant adverse effects

<u>Neoplastic effects(descedants and terminal)</u>
<table>
<tr><th colspan="6">Dose Level (ppm)</th></tr>
<tr><th colspan="6">Males</th></tr>
<tr><td>Dose</td><td>0</td><td>5</td><td>30</td><td>200</td><td>2000</td></tr>
<tr><td>Animal no</td><td>55</td><td>55</td><td>55</td><td>56</td><td>55</td></tr>
<tr><td colspan="6">Liver, hepatocellular</td></tr>
<tr><td>Adenoma</td><td>12</td><td>4</td><td>6</td><td>8</td><td>10</td></tr>
<tr><td>Carcinoma</td><td>1</td><td>-</td><td>2</td><td>-</td><td>4
(7.3 %)</td></tr>
<tr><td colspan="6">Thyroid; follicular cell</td></tr>
<tr><td>Animal no</td><td>51</td><td>54</td><td>55</td><td>53</td><td>54</td></tr>
<tr><td>Adenoma</td><td>-</td><td>-</td><td>-</td><td>-</td><td>1</td></tr>
<tr><th colspan="6">Females</th></tr>
<tr><td colspan="6">Liver, hepatocellular</td></tr>
<tr><td>Animal no</td><td>55</td><td>55</td><td>55</td><td>55</td><td>55</td></tr>
<tr><td>Adenoma</td><td>1</td><td>1</td><td>-</td><td>-</td><td>3
(5.5 %)</td></tr>
<tr><td>Carcinoma</td><td>-</td><td>-</td><td>1</td><td>-</td><td>-</td></tr>
<tr><td>Hemangioma</td><td>-</td><td>-</td><td>-</td><td>1</td><td>1</td></tr>
<tr><td>Hemangiosarcoma</td><td>-</td><td>-</td><td>1</td><td>-</td><td>-</td></tr>
<tr><td colspan="6">Thyroid; follicular cell</td></tr>
<tr><td>Animal no</td><td>55</td><td>52</td><td>51</td><td>53</td><td>55</td></tr>
<tr><td>Adenoma</td><td>-</td><td>-</td><td>-</td><td>-</td><td>2
(3.6 %)</td></tr>
<tr><td>Adenoma c-cell</td><td>-</td><td>-</td><td>-</td><td>-</td><td>1</td></tr>
</table>
</td>
<td>Donner (2002) in reference 2</td>
</tr>
</table>

Historical control data

Tumour	Sex	Population mean	Range
Hepatocellular carcinoma	Male	2.6 %	0- 6.3 %
Hepatocellular adenoma	Female	0.5 %	0-2.6 %
Thyroid follicular cell adenoma	Male	0.7 %	0 – 2.9 %
Thyroid follicular cell adenoma	Female	0.4 %	0-1.3 %

Note, dates for this historical control data were 1991-1996; selected because of limited historical control data for test laboratory.

NOAEL is 30 ppm in males/females based on liver lesions at 200 ppm

4.9.1 Non-human information

4.9.1.1 Carcinogenicity: oral

Rats

As shown in the table, in the available rat study, increased incidences of tumour findings were seen in the liver and thyroid. A detailed analysis and discussion of these tumour findings is presented below.

Discussion

Liver

In Sprague-Dawley rats, significant increases in cholangiocarcinoma and hepatocellular adenomas were observed in females at the 600 ppm (14 and 19 %, respectively) and 1200 ppm (20 and 49 %, respectively) dose levels in the presence of significant generalised toxicity (considerable ↓ bodyweight).

There is no information on a potential mechanism that would exclude relevance of these tumours to humans.

Overall, there is a carcinogenic effect in the liver of female Sprague-Dawley rats (hepatocellular adenoma and cholangiocarcinoma) of potential relevance to humans.

Thyroid

An increase in the incidence of benign follicular cell adenomas was observed in males at the 1000 ppm (10 %) and 2000 ppm (13 %) dose levels. Significant generalised toxicity (considerable ↓ bodyweight) was only evident at the top dose. The increase observed at 300 ppm (5.2 %) was reported to be at the top of the historical control range (0-5 % for male SD rats between 1989-1996).

It has been demonstrated that the thyroid follicular tumours observed in male rats with proquinazid are the result of a perturbation of hypothalamus, pituitary and thyroid (HPT) axis caused by an increase in UDP-gluconronyltransferase (UGT) activity (see section 8). Since rats and humans respond differently to substances that cause hypothyroidism, these effects are not considered to relevant to human health.

Overall, the increase in thyroid adenomas in males are not considered of potential concern to humans.

Mice

As shown in table 19, in the available mouse study an increased tumour incidence was observed in the liver and the thyroid.

Discussion

Liver

In CD-1 males, a weak increase in hepatocellular carcinoma (but not adenoma) was observed in the high dose group (7.3 %) in the absence of significant generalised toxicity. The carcinoma incidence was close to, but slightly higher than, the historical control range for this strain (0-6.3 % range) and, therefore, it is not clear whether the finding is treatment related or, given the high adenoma rate, occurred by chance. In the absence of further information, a treatment related effect cannot be ruled out.

In CD-1 females, a slight increase in hepatocellular adenoma (but not carcinoma) was observed in high dose females (5.5 %) in the absence of significant generalised toxicity. Hepatocellular adenomas in the three affected high dose females were multiple, whereas the occurrence in one female in each the control and the 5 ppm was singular. The 5.5 % incidence was outside the laboratory historical control range of 0-2.6 % for mice of this strain. In addition, the adenomas occurred in association with a slight increase in the incidence of eosinophilic foci of cellular alteration.

There is no data on a potential mechanism that would exclude the relevance of these tumours to humans.

Overall, the increase in adenomas in female CD-1 mice and carcinomas in male CD-1 mice are considered treatment-related and of potential relevance to humans

Thyroid

An increase in the incidence of follicular cell adenoma was observed in high dose females (3.6 %), but not males, in the absence of significant generalised toxicity. This increase was outside of the Haskell laboratory historical control range (0-1.3 %).

The increased tumour incidence was accompanied by histopathological changes (follicular cell hypertrophy and hyperplasia) and may be consistent with prolonged TSH stimulation as, similar to rats, mice also lack thyroid hormone globulin protein (Hurley, 1998). However, as the tumours were observed in females and the mode of action for mice is not as well established as in the rats, human relevance cannot be ruled out.

Overall, the increase incidence of follicular cell adenoma in female CD-1 mice is treatment related and of potential relevance to humans

4.9.1.2 Carcinogenicity: inhalation

No data available

4.9.1.3 Carcinogenicity: dermal

No data available

4.9.2 Human information

No data available

4.9.3 Other relevant information

No data available

4.9.4 Summary and discussion of carcinogenicity

There is one carcinogenicity study available in the rat and one study available in the mouse. Carcinogenic effects were observed in the liver and thyroid of both species.

Liver

In rats, proquinazid was shown to have a carcinogenic effect in the liver of female (not male) Sprague-Dawley rats (hepatocellular adenomas and cholangiocarcinomas). In mice, a weak carcinogenic effect was observed in the liver of high dose male (carcinomas only) and female (adenomas only) CD-1 mice.

Overall, proquinazid caused carcinogenic effects in the liver of rats and mice of potential relevance for classification.

Thyroid

A carcinogenic response of possible relevance to humans was also observed in the thyroid of female CD-1 mice (adenomas only).

4.9.5 Comparison with criteria

In accordance with the criteria in the CLP Regulation, classification in category 1A for carcinogenicity is not justified as there is no evidence of proquinazid having caused cancer in humans. It is therefore necessary to decide whether to classify proquinazid in category 1b or category 2.

Since increased tumours have been seen in two species, a simple argument for category 1B classification can be made. However, on consideration of all the available data, there are a number of factors that indicate classification in category 2 is more appropriate. Most significantly, there is the lack of genotoxicity seen with proquinazid in *in vitro* and *in vivo* studies. In addition, the carcinogenic response in mice is very weak and sex specific. The findings in the liver of females rats (cholangiocarcinoma and adenoma), whilst clear, were also only observed at doses causing significant generalised toxicity and the neoplastic nature of the cholangiocarcinomas has been questioned by an expert committee on carcinogenicity (see section 6.8.2 of the Pesticide Assessment Report).

In view of these considerations, the available evidence is deemed to match the criteria for classification as a category 2 carcinogen. There are no grounds to draw attention to a particular route of exposure on the label.

Similarly, according to Directive 67/548/EEC, classification as a category 3 carcinogen is considered to be appropriate.

4.9.6 Conclusions on classification and labelling

CLP Regulation: propose Carc 2; H351

Directive 67/548/EEC Criteria: propose Carc cat 3; R 40

RAC evaluation of carcinogenicity

Summary of dossier submitter's proposal

The dossier submitter proposed to classify Proquinazid as Carc 2 (H351) according to CLP and Carc cat 3 (R40) according to DSD. The proposal was based on one carcinogenicity study in the rat and one study in the mouse. Human information on Proquinazid's carcinogenicity was not available.

Comments received during public consultation

All comments referred to this issue, and three MS explicitly agreed with the proposed classification.

Outcome of RAC assessment - comparison with criteria and justification

Proquinazid caused carcinogenic effects in the liver of rats (hepatocellular adenomas and cholangiocarcinomas, only females) and mice (carcinomas in males and adenomas in females). The observed thyroid tumours in rats are considered not relevant for humans (Part II RIVM report 601516009/2002), whereas follicular cell adenomas observed in mice are considered of potential relevance for humans.

Carcinogenic effects were seen in two species (rat and mouse) and in two tissues (liver and thyroid). This would warrant a Cat 1B. However, three circumstances indicate the CLP Carc. 2 labelling as more adequate: 1) Liver carcinogenicity is only observed at very high doses (600-1200 ppm); 2) Thyroid adenomas appears to be related to a MoA not applicable to humans; 3) Proquinazid demonstrated no mutagenic potential.

From the data and arguments of the dossier submitter, RAC considers adequate the proposed classification. Whereas the carcinogenic effects are well established for two model species (rat and mouse), which would argue for a Cat 1B classification, the high doses required for liver carcinogenicity and the doubts about the applicability of the proposed MoA for thyroid carcinogenicity to humans (see chapter 8 *Annexes* in the background document) justify the proposed CLP classification Carc 2; H351 and DSD classification Carc cat 3; R40.

4.10 Toxicity for reproduction

Table 20: Summary table of relevant reproductive toxicity studies

Method	Results	Reference
2-generation study OECD 416 (2001) Rat, Sprague-Dawley 30/sex/dose 0, 10, 30, 150 or 600 ppm equivalent to 0, 0.6, 1.8, 9, 35 mg/kg bw/day in males and 0, 0.7, 2.1-2.3, 10-11 and 40-44 m/kg/day in females of the P1 generation Equivalent to 0.9, 2.7, 14 and 54 mg/kg bw/day in males and 0.7-1.0, 2.2-3.2, 12-16 and 43-60 mg/kg bw/day in females from the F1 generation DPX-KQ926-85	*Parental toxicity* 600 ppm: F0 generation: ↓ bodyweight gain during pre-mating (11 % in females) and gestation (7 % in females). ↓ food efficiency during pre-mating and 1st week gestation (9 % in females), ↓ food consumption during gestation (6 % in females). *Liver*: 25 % ↑ relative to bodyweight, hypertrophy (14/29 of 30 males/ females). *Thyroid:* 8 % ↑ relative to brain weight (males), minimal hypertrophy (7/8 of 30 males/females) F1 generation: ↓ bodyweight gain (7 % in males). *Liver:* ↑ relative to bodyweight (13/14 % in males/females); hypertrophy (17/12 of 30 in males/females). *Thyroid:* minimal hypertrophy in 12/29 males and 9/30 females 150 ppm: F0 generation: 7 % ↓ bodyweight gain (females) and ↓ food consumption. *Thyroid:* hypertrophy (9/2 of 30 in males/females) F1 generation: minimal thyroid hypertrophy (3of 30 males and 5/29 females) A NOAEL of 30 ppm based on thyroid hypertrophy. *Reproductive parameters* No effect on reproductive parameters was observed at any dose in either generation A NOAEL of 600 ppm was derived (the highest dose tested) *Offspring effects* F1: 600 ppm: ↓ pup weight (6 % day 0 and 8/9 % from day 4 – day 21); ↓ relative spleen weight (8-12 %) F2: No adverse treatment related effects were observed. A NOAEL of 150 ppm for offspring toxicity based on a marginal reduction in total litter weight of F1 pups	Mylchreest (2003) in reference 2
2-generation study OECD 416 Rat, Sprague-Dawley 30/sex/group Oral, diet 0,150, 300 or 600 ppm equivalent to 0, 9, 17, or 35 mg/kg bw/day in males and 0, 10-11, 19-21, and 39-44 mg/kg bw/day in females of	*Parental toxicity* 600 ppm: F0 generation: 13 % ↓ pre-mating bodyweight (females), 16 % ↓ gestation bodyweight (females), 19 % ↓ lactation bodyweight (females), 11-21 % ↓ food consumption (females), 39-40 % ↓ food efficiency (females), No microscopic investigations were conducted. F1 generation: 13 % ↓ bodyweight (males), 29 % ↓ pre-mating bodyweight, 26 % ↓ gestation bodyweight (females), 26 % ↓ lactation bodyweight (females) 16/ 21-23 % ↓ food consumption (males/females), 7-22 % ↓ food efficiency (females) *Liver:* discolouration of liver (20 females), altered hepatocytes (27 females) fatty change (15/19 males/females), cholangiofibrosis (1/6 male/females) *Thyroid:* follicular cell hyperplasia (13 /6 males/females) , follicular cell	Krams (2002) in reference 2

the P1 generation Equivalent to 0, 12, 24, and 52 mg/kg bw/day in males and 0, 10-14, 21-27, and 46-63 mg/kg bw/day in females from the F1 generation DPX-KQ926-45	hypertrophy (25/27 males/females) 300 ppm: P0 generation: No effect on bodyweight, but 27 % ↓ bodyweight gain females (day 0-7 gestation), 8 % ↓ food consumption (females), F1 generation ~ 7- 10 % ↓ bodyweight days 0-91 (males), 7 – 12 % ↓ body weight days 0-105 (females), ~ 7 % ↓ bodyweight throughout lactation (females), 8/10 % ↓ pre-mating food consumption (males/females) *Liver:* altered hepatocytes (11 females), *Thyroid*: follicular cell hypertrophy (5 females) 150 ppm: F1 generation: Thyroid follicular cell hypertrophy (2 females) No NOAEL was established due to thyroid effects observed at 150 ppm *Reproductive parameters* 600 ppm F1: 14 % ↓ in implantation number (females). 23 % ↓ ovary weights, 13 % ↓ in epididymides and 17 % ↓ testes weight. No changes relative to body weight No other effects on reproductive parameters was observed at any dose in either generation A NOAEL of 300 ppm based on reduction in implantation number at 600 ppm *Offspring effects* 600 ppm: F1: 17 % ↓ pup viability (day 0-4), 17 % ↓ pup weight on day 0, 34 % ↓ by day 21 F2: 600 ppm ↓ pups born (22 %), born alive (22 %) and alive on day 4 (23 %), 10 % ↓ pup weight on day 0, 30 % ↓ by day 21 300 ppm: F1: 10 % ↓ pup weight on day 4, 11.3 % ↓ by day 21 F2: 10 % ↓ pup weight by day 21 150 ppm: No toxicologically relevant effects A NOAEL of 150 ppm was derived based on reductions on pup weight at 300 ppm	
Developmental toxicity OECD 414 Rat, Sprague-Dawley 25 dose/group Oral (gavage) 0.5 % methyl Cellulose	<u>Maternal Toxicity:</u> 60 mg/kg bw/day: No deaths were observed and clinical signs were limited to salivation, stained fur on chin and piloerection. 19 % ↓ bodyweight gain; 53 % ↓ uterine adjusted bodyweight gain (7-22 gestation) 30, 10 and 5 mg/kg bw/day: No toxicologically relevant effects <u>Foetal effects</u> No malformations were noted at any dose tested 60 mg/kg bw/day: 10 % reduction in mean foetal weight. Increased incidence of retarded sternal ossification: Litter incidence (13 litters out of 25 compared to 2 litters out of 23 in controls) and foetal incidence (28 out of 385 pups compared to 2 out of 339 pups in controls). Within historical	Munley (1997); Brown (2004h) in reference 2

Days 7-21 of gestation 0, 5, 10, 30 or 60 mg/kg bw/day DPX-KQ926-45	control data Increased incidence of patent ductus arteriosis: Litter incidence (5 litters out of 25 – outside historical control (0-2 litters)) and foetal incidence (13 out of 196 pups). 30, 10 and 5 mg/kg bw/day: No toxicologically relevant effects NOAEL 30 mg/kg bw/day for maternal and foetal effects	
Developmental Toxicity New Zealand white rabbit OECD 414 (1981) 22/dose group Oral (gavage) 0.5 % methyl Cellulose Days 7-28 of gestation 0, 1, 2.5, 5 or 10 mg/kg bw/day DPX-KQ926-45	<u>Maternal toxicity:</u> There were no substance-related deaths or clinical signs of toxicity Non significant 19 and 25 % ↓ bodyweight gain and 6 and 6.3 % ↓ food consumption at 10 and 5 mg/kg bw/day, respectively. 2 out of 22 females aborted at 10 mg/kg bw/day <u>Foetal effects</u> No substance related malformations noted at any dose level Significant decreases in foetal weight of 9 and 11 % were observed at 5 and 10 mg/kg bw/day. Reductions observed at the lower dose level were not considered toxicologically significant based on the small level of reduction (4 and 7 % at 1 and 2.5 mg/kg bw/day, respectively) and because all weights fell within the concurrent control range. NOAEL 2.5 mg/kg bw/day for both maternal and foetal effects	Munley (1998) in reference 2

4.10.1 Effects on fertility

4.10.1.1 Non-human information

The effects of proquinazid on fertility have been investigated in two 2-generation studies in rats.

In one 2-generation study (Mylecreest, 2003), bodyweight gain was lower during pre-mating (11 %) and gestation (7 %) and there were also effects on the liver and thyroid at 600 ppm. No effect on any reproductive parameter was observed. Effects on offspring were noted at 600 ppm (reduced pup weight) in F1 animals, but not F2. Given the extent of the general toxicity observed at this dose in the F0 generation (bodyweight gain was reduced by 11 % at time of mating), it is likely these effects were a secondary, non specific consequence of maternal toxicity and not a specific effect on reproduction.

In an earlier 2-generation study, conducted on a different batch of proquinazid produced by an old synthesis process, bodyweight was decreased throughout pre-mating, gestation and lactation in both the F0 and F1 generations at 600 ppm. Similar but less severe effects were observed in the 300 ppm dose groups. No effects on mating performance or the number of pregnant animals was observed, but the number of implantations was reduced in the F1 generation at the top dose. The weight of the ovaries, epididymides and testes were also reduced, but only in the F1 generation with no associated histopathology and therefore are most likely the consequence of the reduced bodyweight. In the offspring, effects were noted in the top and mid doses (reduced pup weight and decreased viability). Due to the extent of the general toxicity observed at these doses (bodyweight was reduced by > 13

% at 600 ppm and either lower bodyweights or reductions in bodyweight gain of > 7 % at 300 ppm), it is likely the effects seen were a secondary, non-specific consequence of maternal toxicity and not a specific effect on reproduction.

Overall, the results show that proquinazid does not affect fertility and reproductive performance.

4.10.1.2 Human information

No information available

4.10.2 Developmental toxicity

Developmental toxicity was investigated in a developmental study in rats and a developmental study in rabbits.

4.10.2.1 Non-human information

The developmental toxicity of proquinazid has been investigated in one study in rats and one study in rabbits.

In the rat study, at the top dose, marked maternal toxicity was observed, manifested as a marked reduction in uterine adjusted bodyweight gain (53 %) over the treatment period. No malformations were observed and the foetal findings (patent ductus arteriosis[1] and retarded sternal ossification) were considered indicative of developmental delay, as a consequence of marked maternal toxicity, and not a direct effect on development.

In the rabbit study, a non-significant but marked decrease in bodyweight gain was observed in the top and mid doses. Two females aborted at the top dose. The abortions are likely to be a non-specific, stress-related, maternal response typical of the rabbit. No malformations were observed. The reduction in offspring bodyweight observed at the mid and top dose was considered to be a non-specific consequence of the maternal toxicity and not a direct effect on development.

Overall, the results of the developmental studies show that proquinazid does not cause specific developmental toxicity in rats or rabbits.

4.10.2.2 Human information

No data

4.10.3 Other relevant information

No data

[1] Background note about patent ductus arteriosus. The UK rapporteur was provided with the following information (Brown 2004h in reference 2). "The ductus arteriosus is a continuation of the pulmonary trunk that ends in the dorsal aorta. Prenatally, this vessel is patent and serves to reduce the total workload of the ventricles by ensuring that most of the blood flow is diverted away from the lungs and to the placenta by the way of the right ventricle. At parturition, aortic pressure gradually exceeds pulmonary pressure and the shunt in the ductus arteriosis shifts. The ductus then constricts and functional closure occurs. If persistent patency is observed in fetuses at scheduled near-term caesarean-sections, the fetuses are generally considered to be slightly delayed developmentally since historical control data indicate that closure of the ductus is typically grossly observable at this time."

4.10.4 Summary and discussion of reproductive toxicity

Fertility

Effects on fertility were investigated in two 2–generation studies (different batches of proquinazid were used).

In both studies, administration of proquinazid resulted in reduced pup size. In the older study, there was also a reduction in pup viability and in the number of implantations. These effects were observed at a dose level at which significant maternal toxicity was observed (bodyweight reductions of > 10 % in the top dose and > 7 % in the mid dose). As such, it is considered that these effects are likely to be a non-specific secondary consequence of general toxicity and not a direct consequence of administration of proquinazid.

Overall, the results show that proquinazid does not affect fertility and reproductive performance.

Development

Developmental toxicity of proquinazid has been investigated in one study in rat and one study in rabbits.

In neither study were any malformations of concern noted and the foetal findings observed were considered to be a secondary non specific consequence of the maternal toxicity and not a direct effect on development.

Overall, the results show that proquinazid does not affect development.

4.10.5 Comparison with criteria

No effects were observed in the absence of marked toxicity that provide sufficient evidence to cause a strong suspicion of impaired fertility or developmental toxicity.

4.10.6 Conclusions on classification and labelling

Directive 67/548/EEC: Not classified based on available data

CLP: Not classified based on available data

RAC evaluation of reproductive toxicity

Summary of dossier submitter's proposal

The dossier submitter proposed not to classify Proquinazid for reproductive toxicity. The proposal was based on results from two fertility studies in rats and two developmental toxicity studies, one in rats and one in rabbits.

Comments received during public consultation

No comments specifically addressed to this issue

Outcome of RAC assessment - comparison with criteria and justification

The effects of proquinazid on fertility have been investigated in two 2-generation studies in rats.
In both studies, administration of proquinazid resulted in reduced pup size. In the older study, there

was also a reduction in pup viability and in the number of implantations. These effects were observed at a dose level at which significant maternal toxicity was observed (bodyweight reductions of > 10% in the top dose and > 7% in the mid dose). As such, it is considered that these effects are likely to be a non-specific secondary consequence of general toxicity and not a direct consequence of administration of proquinazid.

The developmental toxicity of proquinazid was investigated in one study in rats and one study in rabbits. No relevant malformations were observed.

Overall, the results show that proquinazid does not affect fertility, reproductive performance or development. No effects providing sufficient evidence to cause a strong suspicion of impaired fertility or developmental toxicity were observed in the absence of marked toxicity.

RAC thus concludes that classifications for fertility effects or toxicity for development are not required under Directive 67/548/EEC and Regulation (EC) 1272/2008.

4.11 Other effects

No relevant data

4.11.1 Non-human information

No relevant data

4.11.1.1 Neurotoxicity

No neurotoxicity was observed in a 90-day study conducted up to 135 mg/kg bw/day in males and 50 mg/kg bw/day in females (Malley (2003b)) (see section 4.7).

4.11.1.2 Immunotoxicity

No data

4.11.1.3 Specific investigations: other studies

No data

4.11.1.4 Human information

No data

4.11.2 Summary and discussion

No neurotoxicity was observed in a 90-day study conducted up to a dose of 135 mg/kg bw/day (males) and 50 mg/kg bw/day (females) (Malley, 2003b)

4.11.3 Comparison with criteria

No neurotoxicity was observed in a 90-day study conducted up to 135 mg/kg bw/day (males) and 50 mg/kg bw/day (females) (Malley (2003b))

4.11.4 Conclusions on classification and labelling

Directive 67/548/EEC: Not classified based on available data

CLP: Not classified based on available data

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 21: Summary of relevant information on degradation

Method	Results	Remarks	Reference
OECD 111	<10% degradation in 30 days at pH 4, 7 and 9 at 20°C	Hydrolytically stable	Hatzenbeler, 2002a
OECD 301B	1% biodegradation in 28 days	Not readily biodegradable	Barnes, 2002
SETAC 2005	Aqueous photolysis DT50 = 0.03 days	Proquinazid is unstable to photolysis under laboratory conditions	Hatzenbeler, 2002/Umstätter, 2003

5.1.1 Stability

Hydrolysis

An OECD 111 hydrolysis study conducted using radio-labelled test substance found no significant degradation at 20°C over 30 days at pH 4, 7 and 9. Since there was no significant degradation by the completion of the study, it was not possible to calculate degradation rates for proquinazid, and the substance was considered to be stable to hydrolysis. (Reference in DAR: Hatzenbeler, 2002a)

Aqueous photolysis

An aqueous photodegradation study according to SETAC (1995)[2] using radio-labelled proquinazid was run for 15 days in artificial sunlight at pH 7 in sterile aqueous buffered solution (pH 7). The test temperature was 20°C, and a xenon arc lamp used with a similar intensity and wavelength distribution to natural sunlight at midday in Concord, Ohio. The artificial sunlight was estimated to be equivalent to 30 days of midday sunlight in Ohio.

The parent substance had a photolytic half life of 0.03 days producing two products initially, IN-MM671 and IN-MM986 which also degraded further to IN-MT884 and IN-MM991 respectively. IN-MM884 was the most significant degradant. Further degradation did occur, as at the end of the test 21% of the radiation was found as $^{14}CO_2$. In contrast the control samples run in the dark did not show significant degradation. (Reference in DAR: Hatzenbeler, 2002/Umstätter, 2003)

[2] SETAC, 1995. Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides

Soil photolysis
A radio-labelled soil photodegradation study in artificial sunlight using a sandy loam soil was run for 15 days according to EPA guidelines (subdivision N, No 161-3, 1982). The laboratory DT50 for proquinazid was estimated to be 15.5 days. The results for the samples kept in the dark gave a DT50 of 82 days. (Reference in DAR: Misra, 1997)

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No estimation of biodegradation using QSARs is available in the Pesticide Assessment Report.

5.1.2.2 Screening tests

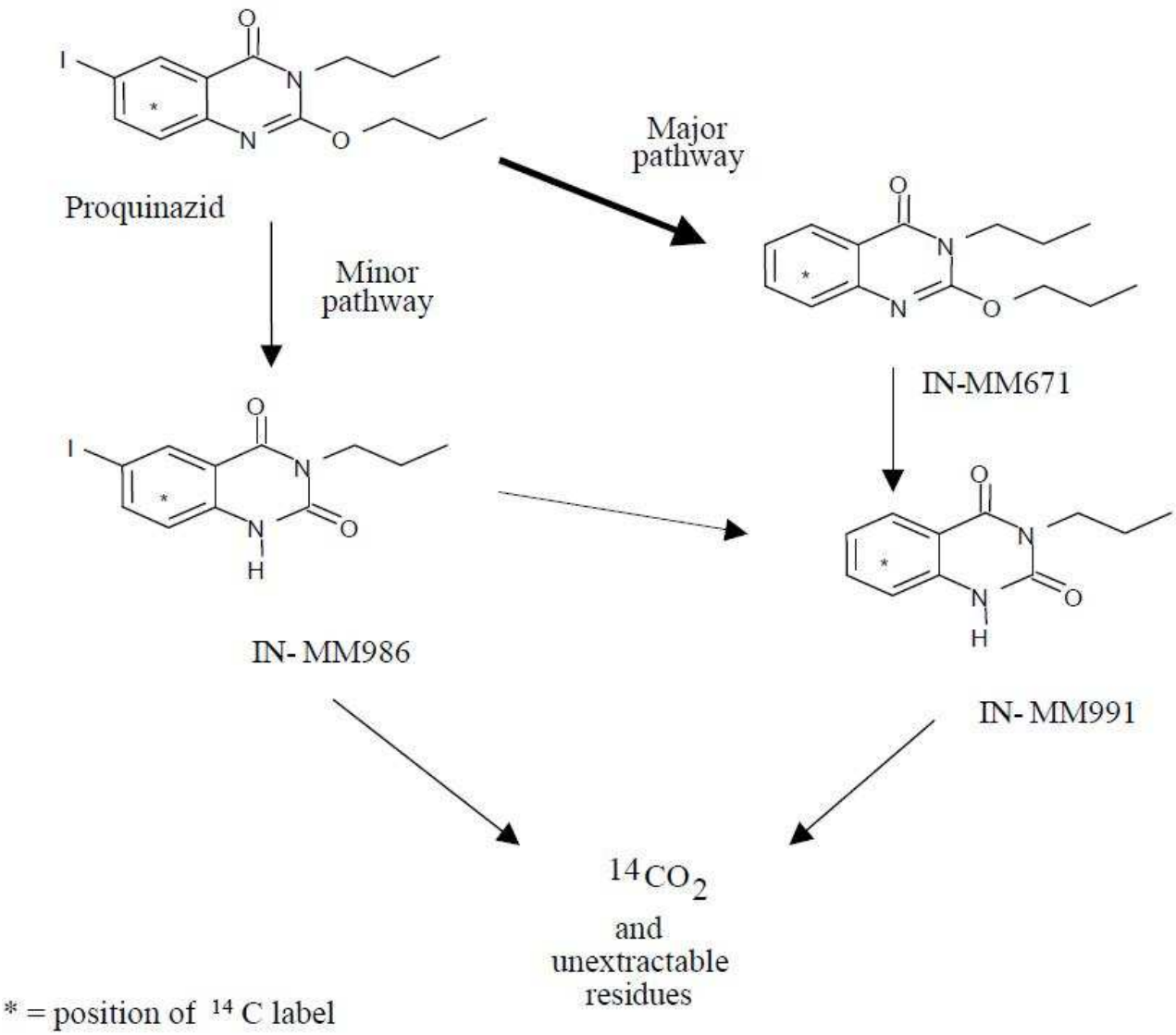
An OECD 301B CO_2 evolution study was run using 10 mg/l of proquinazid. This above the measured water solubility of the substance (0.97 mg/l). The result of 1% biodegradation after 28 days indicated that proquinazid was not readily biodegradable. The reference substance, sodium benzoate, degraded 68% in 7 days. The toxicity control showed that the substance was not inhibitory to the microbial inoculum in the test. (Reference in DAR: Barnes, 2002)

5.1.2.3 Simulation tests

An aerobic water/sediment study was conducted according to SETAC 1995 using radio-labelled proquinazid (radio-chemical purity >95%). This used two fresh-water/sediment systems, one derived from a pond, the other from a stream. At the end of the test (100 days), little mineralisation had occurred (0.2 and 1.4%, respectively).

The large majority of the radioactivity was found in the sediment, with DT50s for water of < 1 day. Degradation occurred in the study, with the principle metabolite being IN-MM671. Analysis of the sediment showed that in the pond sediment 68% of the radio-activity was accounted for by the main metabolite at the end of the test, whilst in the stream sediment system it accounted for up to 32% - day 60 (30% - day 100). Significant degradation of IN-MM671 did not occur during the study, with only 1.1% of the radioactivity in sediment accounted for by a second metabolite (IN-MM991) by day 100. Negligible amounts of a further metabolite (IN-MM986) were detected in the water (0.2%). Sediment DT50 values were 191 days for the stream sediment and 38 days for pond sediment. (Reference in DAR: Spare, 1999). These mainly represent primary degradation, although some unextractable residues were also noted.

A radio-labelled aerobic soil degradation study in sandy loam was run for one year in the dark. This used proquinazid with radio-chemical purity >97%. The DT50 for proquinazid was estimated as 345 days. A further aerobic study, also run for one year, used three other soils and estimated DT50s of between 58 and 204 days. Both studies were run according to SETAC 1995 / EPA subdivision N, No 162-1, 1982. (References in DAR: Spare, 1999a, Spare, 1999b). The proposed degradation pathway for proquinazid in aerobic soil is shown below.
Proposed degradation pathway for proquinazid in aerobic soil



5.1.3 Summary and discussion of degradation

In screening studies, proquinazid was found to be hydrolytically stable. Rapid aquatic photolytic degradation was shown to occur, although this is not considered relevant for classification purposes (since it is only likely to be significant in the top layer of water bodies, and other natural substances can reduce the reaction rate). The substance is not readily biodegradable. Long-term degradation studies indicate primary degradation to form one main metabolite (IN-MM671) through loss of the iodine atom. Two minor metabolites IN-MM986 (primary metabolite) and IN-MM991 (secondary metabolite) were also observed. Metabolite IN-MM884 was only observed as a product in the photodegradation test.

The substance is therefore not readily biodegradable and not rapidly biodegradable for the purposes of classification and labelling.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

An OECD 106 study carried out using radio-labelled test substance and four soils found the K_{oc} to range between 9,091 and 14,126 ml/g. The substance therefore has a relatively high adsorption potential (Reference in DAR: Schmuckler, 2003)

5.2.2 Volatilisation

The substance has a vapour pressure of 9 x 10^{-5} Pa at 25°C and a Henry's Law constant of 3 x 10^{-2} Pa m^3mol^{-1}. Based on the value of the Henry's Law constant the substance is described as *moderately volatile*. However, no significant losses by this route are suggested in the degradation or ecotoxicity tests.

5.2.3 Distribution modelling

Not relevant to classification

5.3 Aquatic Bioaccumulation

Table 22: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
OECD 305	Whole fish BCF = 821		Hoke, 2003a

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The DAR does not contain an estimated BCF. The log K_{ow} of the substance is 5.5, which suggests a high bioaccumulation potential.

5.3.1.2 Measured bioaccumulation data

An OECD 305 fish bioaccumulation study was run at two test substance concentrations (0.41 and 4.08 µg/l) using bluegill sunfish (*Lepomis macrochirus*) and 98% purity radio-labelled proquinazid. The test was conducted using flow-through conditions. The uptake phase was 15 days and the depuration phase 14 days. Steady state (whole fish) was reached after 4 days for both concentrations. Bioconcentration factor (BCF) values were calculated from the mean-measured water concentration and whole fish concentration. The maximum steady state BCF value was 821 based on the fish sampled at day 15, 4 hours after the start of depuration. This is likely to be a worst case value, since it would include the contribution of metabolites; the actual BCF of the parent substance is not known. According to the DAR, BCF was also measured for carcass and fillet, but not lipid (lipid content was not specified in the DAR). (Reference in DAR: Hoke, 2003a)

Under Directive 91/414/EEC requirements for the metabolites, only the log K_{ow} of IN-MM671 triggered the need for a measured bioaccumulation study. A flow-through study using radio-labelled material determined a BCF of 483 for this metabolite.

5.3.2 Summary and discussion of aquatic bioaccumulation

Proquinazid exhibits moderate bioaccumulation in fish. The BCF of 841 exceeds the two classification criteria of 100 and 500. Of the four known degradants, only one (IN-MM671) had a log K_{ow} that triggered a need for a bioaccumulation test. The result for this was lower than for the parent substance.

5.4 Aquatic toxicity

Based on the aqueous photolysis test result, it is possible that rapid photodegradation could have occurred during the ecotoxicity studies. Therefore for a test to be considered fully valid it should be conducted using flow-through conditions and results based on mean-measured concentrations.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Three acute fish studies are available. These were all conducted using standard 16 hours light, 8 hours dark conditions. All tests were conducted under flow-through conditions. Comparison of measured concentrations during the test indicated stability of the test solutions.

Table 23: short-term toxicity to fish

Purity	Species	Test guideline	Endpoint	Toxicity value in mg a.s./l	Conditions	Ref.
98%	Rainbow trout *Oncorhynchus mykiss*	OECD 203	96-h LC50 96-h NOEC	0.349 0.211 Mean measured concentrations (70-75% of nominal)	Flow-through	Boeri et al, 1997a
98%	Bluegill sunfish *Lepomis macrochirus*	OECD 203	96-h LC50 96-h NOEC	0.454 0.189 Mean measured concentrations (64-79% of nominal)	Flow-through	Boeri et al, 1997b
97%	Sheepshead minnow *Cyprinodon variegates*	EPA 72-3a	96-h LC50 96-h NOEC	>0.58 0.35 Mean measured concentrations (79-81% of nominal)	Flow-through	Boeri et al, 1998a

The quoted fish results for the four degradants (all static tests) were:

- IN-MM671 (*Oncorhynchus mykiss*): 96-h LC50 = 2.2 mg/l; 96-h NOEC <0.56 mg/l (sub-lethal effects)
- IN-MM671 (*Lepomis macrochirus*): 96-h LC50 = 4.2 mg/l; 96-h NOEC = 1.4 mg/l

- IN-MM884: No results.
- IN-MM986 (*Oncorhynchus mykiss*): 96-h LC50 >1.03 mg/l; 96-h NOEC = 1.03 mg/l
- IN-MM991 (*Oncorhynchus mykiss*): 96-h LC50 = 28.4 mg/l; 96-h NOEC <2.5 mg/l (sub-lethal effects)

Based on these test results, the parent substance is more ecotoxic to fish than the degradants.

5.4.1.2 Long-term toxicity to fish

Table 24: long-term toxicity to fish

Purity	Species	Test Guideline	Endpoint	Toxicity value in mg a.s./l	Conditions	Ref.
96.4%	Rainbow trout *Oncorhynchus mykiss*	OECD 210 EPA 72-4a	90-day NOEC (abnormalities) 90-day NOEC (survival, length, weight)	0.0030 0.022 (mean-measured concentrations)	Flow-through	Kreamer, 1998a
97%	Sheepshead minnow *Cyprinodon variegates*	EPA 72-4a	36-day NOEC (survival) 36-day NOEC (length, weight)	0.00872 0.0189	Flow-through	Boeri et al, 1998b

Early life stage toxicity of proquinazid to *Oncorhynchus mykiss* (Kreamer, 1998a)

A 90 day (34 day pre-hatch and 56 days post-hatch) early life stage (ELS) flow-through study was conducted on rainbow trout (Oncorhynchus mykiss) using technical proquinazid (96.4% purity).

Newly fertilised eggs (4 replicates of 20 eggs) were exposed to mean measured concentrations of: 0.0012, 0.0030, 0.0082, 0.022, 0.058 and 0.13 mg a.s./l (81-100% of nominal) dispersed in diluent water using the solvent dimethylformamide (DMF) as a vehicle. DMF solvent (dispersed in diluent water) and untreated control groups were also included. The test was conducted at a mean water temperature of 10.9°C (10.5-11.1°C) under dynamic conditions.

Hatching commenced on day 29 and was completed 34 days after initiation of the study (designated day 0 post-hatch). The hatchlings were 'thinned' to 15 per replicate (2 replicates) on day 46 (after swim-up had begun in the controls).

The NOECs for this study were; 0.022 mg a.s./l for percentage fish survival, fish length and fish wet weight, and 0.0030 mg a.s./l for 'abnormalities'. Abnormalities were: loss of equilibrium, one fish lying on the bottom and one fish smaller in size than the associated control (to the end of the study). These values are based on mean measured concentrations.

Environmental parameters were within acceptable limits throughout the study. The study was GLP compliant and undertaken according to OECD 210 and EPA 72-4(a) guidelines. A deviation was noted from the OECD 210 guideline, in that the study duration post-hatch was 56 days, compared to 60 days, as recommended.

Early life stage toxicity of proquinazid to *Cyprinodon variegates* (Boeri et al, 1998b)

A 36 day (4 day pre-hatch and 32 days post-hatch) early life stage (ELS) flow-through study was conducted on Sheepshead minnow (Cyprinodon variegates) using technical proquinazid (97% purity). Newly fertilised eggs were exposed to mean measured concentrations of 0.00872, 0.0189, 0.0365, 0.0721 and 0.146 mg a.s./l (79-86% of nominal) dispersed in diluent water using the solvent dimethylformamide (DMF) as a vehicle. DMF solvent (dispersed in diluent water) and untreated control groups were also included. The test was conducted at 30°C (± 1°C) under dynamic conditions.

Hatching was completed four days after initiation of the study (designated day 0 post-hatch). At 0.0189 mg a.s./l there was also an increase in total length and wet weight of fish exposed for 32 days post-hatch compared to both the water and solvent controls. This was possibly due to the lower number of fish surviving in that treatment (10%) as compared to the controls (97%). The NOECs for this study were 0.00872 mg a.s./l for percentage fish survival (7-32 days post-hatch) and 0.0189 mg a.s./l for fish length and weight (32 days post-hatch). These values were based on mean measured concentrations. Environmental parameters were within acceptable limits throughout the study. The study was undertaken according to EPA guideline 72-4(a) and was GLP compliant.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Three acute aquatic invertebrate tests are available. These were all conducted under flow-through conditions.

Table 25: short-term toxicity to aquatic invertebrates

Purity	Species	Test Guideline	Endpoint	Toxicity value in mg a.s./l	Conditions	Ref.
98%	Waterflea *Daphnia magna*	OECD 202 FIFRA 72-2	48-h EC50 48-h NOEC	0.287 0.149 Mean-measured concentrations (70-77% of nominal)	Flow-through[3]	Boeri et al, 1997c
97%	Oyster *Crassostrea virginica*	EPA 72-3c	96-h EC50 96-h NOEC	0.219 0.074 Mean-measured concentrations (78-88% of nominal)	Flow-through	Boeri et al, 1997d
97%	Saltwater Mysid *Americamysis bahia*	EPA 72-3b	96-h EC50 96-h NOEC	0.11 0.021 Mean-measured concentrations (81-89% of nominal)	Flow-through	Boeri et al, 1997e

The key aquatic toxicity study for the acute classification and labelling is the saltwater mysid test. The acute toxicity of technical proquinazid (97% purity) to *Americamysis bahia* was assessed during a 96-hour exposure test under flow-through conditions. The study was run according to guideline EPA 72-3b. Juvenile mysids less 24 hours old were exposed to mean measured concentrations of 0.0021, 0.039, 0.061, 0.10 and 0.17 mg/l. Control and solvent (DMF) controls were also run. Two replicates per concentration were run, each containing 10 animals. Animals were inspected every 24 hours. No effects were observed in the control and solvent control animals. Mean-measured concentrations were between 81 and 89% of nominal, and were used to calculate the results. The 96-h EC50 was 0.11 mg/l, and the 96-h NOEC was 0.021 mg/l.

The quoted *Daphnia magna* results for the four degradants (all static tests) were:

- IN-MM671: 48-h EC50 = 5.4 mg/l; 48-h NOEC = 0.99 mg/l
- IN-MM884: 48-h EC50 >114 mg/l; 48-h NOEC <7.70 mg/l (sub-lethal effects)
- IN-MM986; 48-h EC50 >0.791 mg/l; 48-h NOEC = 0.791 mg/l
- IN-MM991; 48-h EC50 >45.5 mg/l; 48-h NOEC = 22.3 mg/l

Based on these test results, the parent substance is more ecotoxic to *Daphnia magna* than the degradants.

[3] Note there is a typo in the DAR as this test is stated to be a static test.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Table 26: Long-term toxicity to aquatic invertebrates

Purity	Species	Test Guideline	Endpoint	Toxicity value in mg a.s./l	Conditions	Ref.
96.4%	Waterflea *Daphnia magna*	OECD 202 pt 2	21-day NOEC (reproduction)	0.0018 (mean measured concentrations)	Semi-static (48-hour renewal)	Kreamer, 1998b
97%	Saltwater Mysid *Americamysis bahia*	EPA 72-4c	28-day NOEC (reproduction and adult mortality)	0.0105 (mean measured concentrations)	Flow-through	Boeri et al, 1998c
99.2%	*Chironomus riparius*	BBA method 1995	28-day EC50 28-day NOEC (emergence and development)	>1.06 0.456 (initial measured concentration)	Water-sediment system using spiked water	Haworth, 1999

The key aquatic toxicity study for the chronic classification and labelling is the 21-day *Daphnia magna* reproduction test.

Long-term toxicity of proquinazid to *Daphnia magna* (Kreamer, 1998b)

The effect of technical proquinazid (96.4% purity) on the survival and reproduction of *Daphnia magna* was assessed during a 21-day exposure test under semi-static conditions. The study design included ten replicates, seven replicates comprising individually housed Daphnid and three replicates comprising five Daphnids. All *Daphnia* were less than 24 hours old at the start of the test and were exposed to mean measured concentrations of 0.00068, 0.0018, 0.0046, 0.013, 0.033, 0.083 and 0.21 mg a.s./l. Two controls were included; water (the dilution medium) alone and water plus DMF (the solvent for proquinazid). Measured concentrations of proquinazid ranged between 71 and 88% of nominal concentrations in samples of freshly prepared media and were maintained at between 55 and 92% of their initial values in the expired media.

Test media were renewed every 48 hours. Concentrations of proquinazid were verified by analysing samples of the control and test media on days 0, 6, 14 and 18. Stability was confirmed by analysing samples of expired media taken on days 2, 8, 16 and 20 from the contents of three replicate vessels for both controls and each test concentration. Absorbance of test material was indicated to be a problem for stability of the lower concentrations in the test. Results are based on mean-measured concentrations,

The reproductive NOEC for proquinazid from this study was calculated as 0.0018 mg a.s./l, based on effects at the next higher dose on total numbers of live neonates (young) per adult surviving to the end of the study (day 21) as this was the most sensitive end point assessed. The NOEC for adult survival was 0.033 mg a.s./l proquinazid. The study was conducted according to OECD 202 (pt 2) and in compliance with GLP.

Long-term toxicity of proquinazid to *Americamysis bahia* (Boeri et al, 1998c)

The effect of proquinazid on the survival and reproduction of *Americamysis bahia* (Saltwater mysid) was assessed during a 28-day flow-through chronic toxicity test. The study design included two replicates per treatment, comprising 30 mysids per replicate. All mysids were less than 24 hours old at the start of the test and were exposed to mean measured concentrations of proquinazid were 0.0105, 0.0210, 0.0386, 0.0845 and 0.169 mg a.s./l technical proquinazid (97% purity). Two controls were included; seawater (the dilution medium) alone and seawater plus dimethylformamide (the solvent for proquinazid). Test media were exchanged equivalent to a rate of 14 total volume renewals per 24 hours. Concentrations of proquinazid were verified by analysing samples of the control and test media on days 0, 7, 14, 21 and 28. Measured concentrations of proquinazid ranged between 79 and 85% of nominal concentrations.

The reproductive NOEC for proquinazid was calculated as 0.0105 mg a.s./l for both adult mortality and the reproductive performance (based on adult survival on day 28 and numbers of live young per female by day 28). The study was conducted in compliance with GLP, according to guideline EPA 72-4c.

Long-term toxicity of proquinazid to *Chironomus riparius* (Haworth, 1999)

A 28-day static study using newly hatched *Chironomus riparius* larvae (less than 36 hours old) was undertaken using radiolabelled technical proquinazid (purity 99.2%). Groups of six replicates of 20 animals were exposed to initial concentrations of 0.011, 0.036, 0.10, 0.32 and 1.0 mg a.s./l in a water sediment system (spiked water system). A water control, an acetone solvent control (six replicates) were also run. The test medium was not renewed during the study. Loss of test compound from the overlying water during the study period was in excess of 80% by day 28, with the majority of the test substance found in the sediment.

There were no significant differences in mean percentage emergence between the controls and any of the test concentrations, except for the highest test concentration (1.0 mg a.s./l) where a 27.6% reduction occurred, compared to the solvent control. There were no substantial differences (<6%) in the mean rate of development between the controls and any of the treatments. There was a slight difference observed in the numbers of male and female midges emerging from the 0.036 mg a.s./l treatment. Male emergence (34%) was noted as being low, compared to the other treatment groups, but as a similar trend was not followed by the higher doses tested, the applicant believed that this effect was not treatment related.

The EC50 for emergence and development was determined to be >1.0 mg a.s./l and the NOEC for both emergence and development was 0.32 mg a.s./l (nominal) equivalent to 0.456 mg a.s./l (initial measured).

The study was conducted in accordance with the BBA method (1995) for sediment dwelling organisms and in compliance with GLP.

A 21-day *Daphnia magna* reproduction study using semi-static renewal is also available for IN-MM671. This determined a NOEC for adult growth and reproduction of 0.519 mg/l. Again this indicates that the parent substance is more toxic than the degradant.

5.4.3 Algae and aquatic plants

Table 27: Toxicity to algae and aquatic plants

Test no.	Purity	Species	Test Guideline	Endpoint	Toxicity value in mg a.s./l	Conditions	Ref.

1	97%	Algae *Anabaena flos-aquae*	EPA 123-2	120-h ErC50 120-h NOEC	>0.884 0.884 (initial measured concentrations)	Static	Boeri et al, 1997f
2	98%	Algae *Pseudokirch-neriella subcapitata*	OECD 201 EPA 123-2	72-h ErC50 120-h ErC50 120-h NOErC	>0.74 >0.74 0.478 (initial measured concentrations)	Static	Boeri et al, 1999
3	Not stated	Algae *Pseudokirchneriella subcapitata*	OECD 201 (2006)	72-h ErC50 72-h NOErC	>0.12 0.12 (geometric mean measured concentrations)	Static	Hoberg, JR 2007b
4	97%	Diatom *Navicula pelliculosa*	EPA 123-2	72-h ErC50 120-h ErC50 120-h NOErC	0.36 0.48 0.25 (initial measured concentrations)	Static	Boeri et al, 1998d
5	97%	Duck weed *Lemna gibba*	ASTM E1415-91	14-day EC50 14-day NOEC	>0.2 0.2 (initial measured concentrations)	Static. Limit test	Solman and Leva, 1997

Algal and *Lemna* studies are run using static conditions. The photo-instability of proquinazid means that under these conditions the organisms will have been exposed to degradants as well as parent substance. In addition, the solubility of proquinazid was around 1 mg/l in the algal media, so the higher concentrations in the tests were at or close to the limit of solubility. Again this is likely to affect the dissolved concentrations of parent substance. The K_{oc} value of proquinazid also means that it is absorptive, and was noted as having stuck to test vessel walls in some studies.

Results for tests 1, 2, 4 and 5 were quoted based on initial measured concentrations. Available analytical data for these studies showed measured concentrations of proquinazid declined significantly during the tests. In test 1 concentrations at 72-hours were <40% of the initial concentrations. For test 2, concentrations at 72 hours were between 39 and 52% of the initial concentration. At the end of test 5 (the *Lemna* study), concentrations on day 14 were around 10% of the initial concentration.

Test 3 was added as part of an addendum to the DAR and the results are quoted as geometric mean-measured concentrations. These were noted as being within 77-81% of nominal concentrations

The ErC50 was above the maximum concentration tested in tests 1, 2, 3 and 5. Therefore even if the results based on mean-measured concentrations were lower, these would still be “greater than” ErC50 results.

The *Navicula pelliculosa* study (test 4) was the only test where effects were seen to the extent that an EC50 could be derived. The quoted 72-hour ErC50 of 0.36 mg/l was based on initial measured concentrations. Analytical data for the two concentrations either side of the ErC50 are shown in the first three columns in the table below. There were no other analytical measurements made between 0 and 72 hours. The column on the right has the geometric mean of the 0 and 72 hour measurements calculated by the UK CA for the purposes of this dossier.

Table 28: Analytical measurement of parent substance for relevant replicates in the *Navicula pelliculosa* study

Nominal concentration, mg/l	Measured concentration at 0 hours, mg/l	Measured concentration at 72 hours, mg/l	Geometric mean concentration, mg/l
0.25	0.25	0.087	0.15
0.50	0.48	<0.20	0.22 [a]

a: assuming that the <0.20 value can be represented by half the limit of analytical detection

The result of the data in Table 29 suggest that the mean-measured concentration for the 72-hour ErC50 for *Navicula pelliculosa* would be between 0.15 and 0.22 mg/l.

The quoted algal results for the four degradants are:

- IN-MM671: 72-h ErC50 >0.725 mg/l (6% inhibition); 72-h NOEC <0.725 mg/l
- IN-MM884: No results.
- IN-MM986; 72-h ErC50 >0.96 mg/l (-0.04% inhibition); 72-h NOEC <0.96 mg/l
- IN-MM991; 72-h ErC50 = 4.0 mg/l; 72-h NOEC = 0.475 mg/l

Based on these test results, the parent substance is more ecotoxic to algae and aquatic plants than the degradants.

The result of the data in Table 28 suggest that the mean-measured concentration for the 72-hour ErC50 for *Navicula pelliculosa* would be between 0.15 and 0.22 mg/l. It can also be seen that the NOEC derived as a geometric mean is 0.15 mg/l.

The NOEC for test 2 is also based on initial measured test concentrations. The table below recalculates the NOEC using mean measured concentrations. There is no need to recalculate the EC50 for this test as it is a greater than value.

Table 29: Analytical measurement of parent substance for relevant replicates in the Pseudokirch-neriella subcapitata study (Boeri et al, 1999)

Nominal concentration, mg/l	Measured concentration at 0 hours, mg/l	Measured concentration at 72 hours, mg/l	Geometric mean concentration, mg/l
0.60	0.478	ND	0.219

Limit of quantitation indicated to be 0.200 mg/l - assume that ND value can be represented by half the limit of LOQ

5.4.4 Other aquatic organisms (including sediment)

None

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Proquinazid is not readily biodegradable, and is hydrolytically stable. In the laboratory, the substance was found to be photolytically unstable, with a DT50 of 0.03 days, but this is not considered relevant for classification. Overall, the substance is considered not to be readily or rapidly degradable. The fish BCF value (based on total radioactivity) is >500.

The three trophic levels represented in the data set are of similar sensitivity to proquinazid in acute ecotoxicity tests. Tests for fish and invertebrates were all carried out using flow-through conditions, with concentrations reported as mean-measured concentrations. Such conditions should help maximise exposure of the animals to the parent substance and minimise the effect of photodegradation. The most sensitive acute result was from the mysid shrimp study using flow-through conditions, which gave a 96-hour EC50 of 0.11 mg/l (based on mean measured concentrations).

The test conditions required for the algae and aquatic plant studies potentially mean that photodegradation is an issue. Concentrations of parent substance clearly decline in a number of these studies and results were quoted based on initial concentrations. A 50% inhibition of growth was only observed in one test, using the diatom *Navicula pelliculosa*. If the results of that test are considered using mean-measured concentrations, the EC50 would still be higher than the EC50 of the mysid shrimp study. Re-calculation of the results for the remaining algae/aquatic plant tests would not provide a more sensitive result than the *Navicula pelliculosa* test because 50% inhibition was not reached.

A further supporting argument is that proquinazid is a fungicide, so it would not be expected that algae and aquatic plants would be more sensitive than other trophic groups.

The most sensitive chronic result for proquinazid is from the *Daphnia magna* reproduction test. The 21-day reproductive NOEC from this study was calculated as 0.0018 mg/l. Both long-term fish results are of a similar order of magnitude to the *Daphnia* result. All three tests used either flow-through or semi-static regimes, and results were derived from mean-measured concentrations. Results for other taxa and trophic levels indicate that these are less sensitive.

Based on the available data, all the identified degradants are less acutely toxic to fish, invertebrates and algae than the parent compound. A long-term *Daphnia magna* study and a fish bioaccumulation study on the main metabolite from the water-sediment degradation test indicated less toxicity and lower bioaccumulation than the parent proquinazid.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Conclusion of environmental classification according to CLP

Based on the CLP Regulation, proquinazid should be classified as:

Aquatic Acute 1 and Aquatic Chronic 1 with the following labelling:

H410 ‘Very toxic to aquatic life with long lasting effects’,

‘Warning’ signal word and environmental warning label.

An M factor of 10 is applicable based on 0.001 <NOEC ≤0.01 mg/l and the substance being not readily biodegradable.

An M factor of 1 is applicable based on 0.1<L(E)C_{50}≤1 mg/l

Conclusion of environmental classification according to Directive 67/548/EEC

Proquinazid should be classified Dangerous for the Environment with the following risk and safety phrases:

N Dangerous for the Environment

R50 Very toxic to aquatic organisms

R53 May cause long term effects in the environment

S60 This material and its container must be disposed of as hazardous waste

S61 Avoid release to the environment. Refer to special instructions/Safety Data Sheet

RAC evaluation of environmental hazards

Summary of dossier submitter's proposal

The dossier submitter proposed to classify Proquinazid as Aquatic Acute 1 and Aquatic Chronic 1 with an acute M-factor 1 and a chronic M-factor 10 according to CLP, and R50/53 according to DSD. The proposed classification was based on studies on hydrolysis, ready biodegradability, bioaccumulation and both acute and chronic aquatic toxicity tests on three different trophic levels of aquatic organisms.

Comments received during public consultation

Two MS supported the proposed classification.

Outcome of RAC assessment - comparison with criteria and justification

Proquinazid is hydrolytically stable and not readily biodegradable. The result of 1% biodegradation after 28 days is clearly lower than the 70% reference value (CLP Regulation) for biodegradable substances. Proquinazid is moderately bioaccumulative: BCF=821 (fish), Kow=5.5. These values meet the CLP criteria of BCF=500 for bioaccumulative substances.

Several proquinazid degradation products have been described and analysed. They are considered less toxic and bioaccumulative than the parental compound.

Acute toxicity: The three trophic levels represented in the data set (fish, invertebrates and algae) showed similar sensitivity to proquinazid in acute ecotoxicity tests. The mysid shrimp (*Americamysis bahia)* study (flow-through conditions, 96-hour exposure) showed the lowest EC50 (0.11 mg/L) and this value was chosen for classification in CLP as Aquatic Acute Cat 1 (EC50<1 mg/L). As 0.1<EC50<1 mg/L, an M factor of 1 should apply for acute toxicity.

Chronic toxicity: The most sensitive chronic result is from the *Daphnia magna* reproduction test showing the highest sensitivity to long-term (21 d) exposure to proquinazid, with a NOEC of 0.0018 mg/l. Long-term fish results showed a similar toxicity within an order of magnitude, whereas results from other taxa and trophic levels, albeit less sensitive, were in line with fish data. Therefore, the *Daphnia* results were chosen as criteria for classification in CLP as Aquatic Acute Cat 1 (NOEC<0.01 mg/L, not readily biodegradable). As 0.001<NOEC<0.01 mg/L, an M factor of 10 should apply for chronic toxicity.

RAC concludes that classification according to the CLP criteria as Aquatic Acute 1 (H400) with an

M-factor 1), and Aquatic chronic 1 (H410) with an M-factor 10 is warranted.

As proquinazid shows EC50<1mg mg/L for aquatic species and it is not biodegradable, the classification N; R50/R53 is warranted according to the criteria in Directive 67/548/EEC.

6 OTHER INFORMATION

This substance has been reviewed under Council Directive 91/414/EEC, with the rapporteur Member State being the United Kingdom. The studies evaluated in this dossier were taken from the pesticide assessment report; where necessary, the full study reports were consulted, but these are generally not publically available. Where other information from additional references has been sources, this is indicated.

7 REFERENCES

Human Health Hazard references

1. Pesticide Assessment Report (DAR) – public Version – initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance proquinazid of the third stage (part A) of the review programme referred to in Article 8 (2) of the Council Directive 91/414/EEC. Volume 3, Annex B. 1-5 (B.2), 2006

2. Pesticide Assessment Report (DAR) – public Version – initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance proquinazid of the third stage (part A) of the review programme referred to in Article 8 (2) of the Council Directive 91/414/EEC. Volume 3, Annex B.6, parts 1 and 2, 2006

3. Hurley, P.M., Hill, R.N., and Whiting, R.J. (1998). Mode of carcinogenic action of pesticides includeing thyroid follicular-cell tumors in rodents. Environ. Health Perspect. 106(8): 437-445.

Environmental hazard references

The studies referenced below are summarised in the Pesticide Assessment Report (DAR) – public Version – initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance proquinazid of the third stage (part A) of the review programme referred to in Article 8 (2) of the Council Directive 91/414/EEC. Volume 3, Annexes B.8 and 9, 2006;

Environmental Fate references

Barnes, S.P. 2002 DPX-KQ926: Assessment of ready biodegradability by modified Sturm test Huntingdon Life Sciences Ltd. DuPont-9782

Hatzenbeler, C.J. 2002 Photodegradation of [^{14}C]DPX-KQ926 in pH 7 buffer by simulated sunlight Ricerca Biosciences, LLC. DuPont-9998

Hatzenbeler, C.J. 2002a Hydrolysis of [^{14}C]DPX-KQ926 in buffer solutions of pH 4, 7, and 9 Ricerca Biosciences, LLC DuPont-9785

Lee, D.Y., Corrigan, N.M. 2000 Degradation rate of DPX-KQ926 (one soil), and IN-MM671, IN-MM986, and IN-MM991 in three soils DuPont Experimental Station

Misra, B. 1997 Photodegradation of (phenyl-14C(U))DPX-KQ926 on sandy loam soil Pittsburgh Environmental Research Laboratory, Inc. AMR 3716-95

Schmuckler, M.E. 2003 Batch equilibrium (absorption/desorption) study of [phenyl-^{14}C (U)]DPX-KQ926 on soil DuPont Experimental Station AMR 3976-96, Revision No. 1

Smyser, B.P. 2003 Calculated half-lives of proquinazid and its metabolites in environmental fate studies. Critical Path Services. DuPont-13715.

Spare, W.C. 1999 Degradability and fate of ^{14}C-DPX-KQ926 in water/sediment systems Agrisearch, Inc. AMR 3860-96, Revision No. 1

Spare, W.C. 1999a Aerobic soil metabolism of 14C-DPX-KQ926 Agrisearch, Inc
AMR 3858-96, Revision No. 1

Spare, W.C. 1999b Degradation rate of 14C-DPX-KQ926 in three soils. Agrisearch, Inc
AMR 3859-96, Revision No. 1

Umstätter, S. 2003 Calculation of the quantum yield and aquatic lifetime of proquinazid (DPX-KQ926) and its metabolites (IN-MM671, IN-MM986, IN-MT884 and IN-MM991) in water DuPont-Stine-Haskell Research Center. DuPont-13326

Ecotoxicity references

Barefoot, A.C. 2004 Estimates of Log Kow values for metabolites of proquinazid. (e-mail of 21/7/2004)

Boeri, R.L., Magazu, J.P., Ward, T.J. 1997a DPX-KQ926 (technical): Acute toxicity to the rainbow trout, *Oncorhynchus mykiss.* T.R. Wilbury Laboratories, Inc. AMR 4165-96

Boeri, R.L., Magazu, J.P., Ward, T.J. 1997b DPX-KQ926 (technical): Acute toxicity to the bluegill sunfish, *Lepomis macrochirus* T.R. Wilbury Laboratories, Inc. AMR 4166-96

Boeri, R.L., Magazu, J.P., Ward, T.J. 1997c DPX-KQ926 (technical): Acute toxicity to the daphnid, *Daphnia magna* T.R. Wilbury Laboratories, Inc. AMR 4167-96

Boeri, R.L., Magazu, J.P., Ward, T.J. 1997d DPX-KQ926 technical: Flow-through mollusc shell deposition test using the eastern oyster, *Crassostrea virginica.* T.R. Wilbury Laboratories, Inc. AMR 4484-97

Boeri, R.L., Magazu, J.P., Ward T.J. 1997e DPX-KQ926 technical: Flow-through acute toxicity to the mysid, *Mysidopsis bahia* T.R. Wilbury Laboratories, Inc. AMR 4481-97

Boeri, R.L., Magazu, J.P., Ward, T.J. 1997f DPX-KQ926 technical: Growth and reproduction test with the freshwater alga, *Anabaena flos-aquae* T.R. Wilbury Laboratories, Inc. AMR 4480-97

Boeri, R.L., Magazu, J.P., Ward, T.J. 1998a DPX-KQ926 technical: Flow-through acute toxicity to the sheepshead minnow, *Cyprinodon variegatus*T.R. Wilbury Laboratories, Inc.
AMR 4482-97

Boeri, R.L., Magazu, J.P., Ward, T.J. 1998b DPX-KQ926 technical: Flow-through early life stage toxicity to sheepshead minnow, *Cyprinodon variegatus.* T.R. Wilbury Laboratories, Inc
AMR 4486-97

Boeri, R.L., Magazu, J.P., Ward, T.J. 1998c DPX-KQ926 technical: Flow-through chronic toxicity to the mysid, *Mysidopsis bahia* T.R. Wilbury Laboratories, Inc. AMR 4485-97

Boeri, R.L., Magazu, J.P., Ward, T.J. 1998d DPX-KQ926 technical: Growth and reproduction test with the freshwater alga, *Navicula pelliculosa* T.R. Wilbury Laboratories, Inc. AMR 4478-97

Boeri, R.L., Ward, T.J. 2000 IN-MM671: Static renewal 21-day chronic (life cycle) toxicity test with *Daphnia magna* T.R. Wilbury Laboratories, Inc. DuPont-1996

Boeri, R.L., Magazu, J.P., Ward, T.J. 1999 DPX-KQ926 (technical): Growth and reproduction

test with the freshwater alga, *Selenastrum capricornutum* T.R. Wilbury Laboratories, Inc. AMR 4168-96, Revision No. 1

Haworth, S. 1999 [^{14}C] DPX-KQ926: To assess the toxicity to the sediment dwelling phase of the midge *Chironomus riparius* Huntingdon Life Sciences Ltd. DuPont-2042

Hoke, R.A. 2003a ^{14}C-Proquinazid (DPX-KQ926) technical: Bioconcentration in bluegill sunfish, *Lepomis macrochirus* DuPont Haskell Laboratory. DuPont-8197

Hoke, R.A. 2003b ^{14}C-IN-MM671: Bioconcentration in bluegill sunfish, *Lepomis macrochirus* DuPont Haskell Laboratory. DuPont-10460

Kreamer, G-L.C. 1998a DPX-KQ926 technical: Early life-stage toxicity to rainbow trout, *Oncorhynchus mykiss.* DuPont Haskell Laboratory. HL-1998-00638

Kreamer, G-L.C. 1998b DPX-KQ926 technical: 21-Day chronic, static-renewal toxicity to *Daphnia magna* DuPont Haskell Laboratory HL-1998-00628

Kreamer, G-L.C. 1999a IN-MM671 (metabolite of DPX-KQ926): Static, acute, 96-hour LC_{50} to rainbow trout, *Oncorhynchus mykiss.* DuPont Haskell Laboratory. DuPont-1994

Kreamer, G-L.C. 1999b IN-MM671 (metabolite of DPX-KQ926): Static, acute, 48-hour EC_{50} to *Daphnia magna* DuPont Haskell Laboratory DuPont-1993

Samel, A. 2001 IN-MM671: Static, acute, 96-hour, toxicity test to bluegill sunfish, *Lepomis macrochirus* DuPont Haskell Laboratory. DuPont-6353. GLP: Yes

Samel, A. 2003a IN-MM986: Static, acute, 96-hour LC_{50} to rainbow trout, *Oncorhyncus mykiss* DuPont Haskell Laboratory. DuPont-12075

Samel, A. 2003b IN-MM991: Static, acute, 96-hour LC_{50} to rainbow trout, *Oncorhynchus mykiss* DuPont Haskell Laboratory. DuPont-12080

Samel, A. 2003c IN-MT884: Static, acute, 48-hour EC_{50} to *Daphnia magna* DuPont Haskell Laboratory DuPont-12074

Samel, A. 2003d IN-MM986: Static, acute, 48-hour EC_{50} to *Daphnia magna* DuPont Haskell Laboratory DuPont-12077

Samel, A. 2003e IN-MM991: Static, acute, 48-hour EC_{50} to *Daphnia magna* DuPont Haskell Laboratory. DuPont-12079

Sloman, T.L. 1999 IN-MM671 (metabolite of DPX-KQ926): Influence on growth and growth rate of the green alga *Selenastrum capricornutum* DuPont Haskell Laboratory DuPont-2757

Sloman, T.L. 2003a IN-MM986: Influence on growth and growth rate of the green alga *Selenastrum capricornutum* DuPont Haskell Laboratory DuPont-12078

Sloman, T.L. 2003b IN-MM991: Influence on growth and growth rate of the green alga *Selenastrum capricornutum* DuPont Haskell Laboratory DuPont-12081

Sloman, T.L., Leva, S.E. 1997 DPX-KQ926 technical: Influence on growth and reproduction of *Lemma gibba* G3 DuPont Haskell Laboratory AMR 4580-97

8 ANNEXES

Postulated mode of action for rat thyroid tumours

It was postulated that the thyroid follicular tumours observed in male rats were the result of a perturbation of hypothalamus, pituitary and thyroid (HPT) axis caused by an increase in UDP-glucuronyltransferase (UGT) activity. Increased UGT activity results in increased excretion of T4, lowering serum T4 levels (and sometimes T3 levels). To counter this decrease, the pituitary releases more thyroid-stimulating hormone (TSH). Chronic TSH stimulation of the thyroid gland leads to thyroid hypertrophy, hyperplasia and adenoma of the thyroid gland.

If this mode of action is correct, several key events should be observed. These are increased UGT activity, changes in thyroid levels, increased thyroid growth and thyroid lesions. To investigate this, an analysis of the available data has been performed according to the IPCS framework, using the example for thyroid tumours outlined in Dellarco, (2006). The analysis draws on the findings from the two rat 90-day studies, the 2-year rat chronic/carcinogenicity study, and a mechanistic 28-day study conducted with proquinazid, the results of which are presented below.

Table 19b: Summary table of mechanistic data relevant for carcinogenicity

<table>
<tr><th>Method</th><th>Results</th><th>Reference</th></tr>
<tr><td>28-day feeding study with 14, 28 and 42 days recovery

Male Sprague Dawley rat

100 male rats/dose

0, 10, 30 and 300 ppm

Corresponding to 0, 0.62, 2, 19 mg/kg bw/day

A 3000 ppm dose group was included but discontinued on day 18 due to excessive weight loss</td><td><u>3000 ppm</u>
Discontinued on day 18

<u>300 ppm</u>
↑absolute and relative liver weight (wk 1 and 4) and ↑relative liver weight (wk 2)
Liver cell and thyroid follicular cell hypertrophy observed from day 28 (reversible by day 56)
↑ hepatic UDP-glucuronyltransferase activity (week 1 – 66 %, week 2 – 100 % and week 4 – 70 %)
↓5'-deiodinace activity (29 %)

<table><tr><td>Week</td><td>TSH</td><td>T4</td><td>T3</td><td>rT3</td></tr><tr><td>1</td><td>68 %</td><td>-18 %</td><td>-20 %</td><td>-</td></tr><tr><td>2</td><td>34 %</td><td>- 16 %</td><td></td><td>63 %</td></tr><tr><td>4</td><td>60 %</td><td>-18 %</td><td>-17 %</td><td>78 %</td></tr></table>
Only TSH levels remained higher at the end of the recovery period (6 weeks – 120 %)

<u>30 ppm</u>
↑ Hepatic UDP-glucuronyltransferase activity (week 2 – 36 %)
<table><tr><td>Week</td><td>TSH</td><td>T4</td><td>T3</td><td>rT3</td></tr><tr><td>1</td><td>32 %</td><td>-15 %</td><td></td><td></td></tr><tr><td>2</td><td>9 %</td><td>-18 %</td><td></td><td></td></tr><tr><td>4</td><td></td><td>-16 %</td><td></td><td>25 %</td></tr></table>
<u>10 ppm</u>
<table><tr><td>Week</td><td>TSH</td><td>T4</td><td>T3</td><td>rT3</td></tr><tr><td>1</td><td></td><td>-16 %</td><td></td><td></td></tr><tr><td>2</td><td></td><td></td><td></td><td></td></tr><tr><td>4</td><td></td><td></td><td></td><td></td></tr></table></td><td>O'Connor (2002)</td></tr>
</table>

Dose response relationship and concordance

A summary of the LOAELs for the key events observed in the two 90-day studies and the 2-year chronic carcinogenicity study in male rats are presented in the table below.

Effect	LOAEL
	Liver
Induction of UDP-transferase	6 mg/kg bw/day - 90-day (Malley, 2003b)
	127 mg/kg bw/day - 90-day (Malley, 2002b)
Increase of T4 biliary elimination	Not measured
Increase in liver weight	19 mg kg bw/day - 90-day (Malley, 2003b)
	127 mg/kg bw/day - 90-day (Malley, 2002b)
	43 mg/kg bw/day - 2-year study
Hepatocellular hypertrophy	No effects noted
	127 mg/kg bw/day - 90-day (Malley, 2002b)
	95 mg/kg bw day - 2-year study

	Hormones
Decrease in serum T4	135 mg/kg bw/day - 90-day (Malley, 2003b)
	127 mg/kg bw/day - 90-day (Malley, 2002b)
	43 mg/kg bw /day (2-year study)
Increase in serum TSH	19 mg/kg bw/day - 90-day (Malley, 2003b)
	19 mg/kg bw/day - 90-day (Malley, 2002b)
	43 mg/kg bw/day (2-year study)
	Thyroid
Increase in thyroid weight	135 mg/kg bw/day - 90-day (Malley, 2003b)
	127 mg/kg bw/day - 90-day (Malley, 2002b)
	92 mg/kg bw/day (2 year study)
Increase in thyroid hyperplasia	12 mg/kg bw/day (2-year study)
Increase in thyroid tumours	43 mg/kg bw/day (2-year study)

In the 28-day mechanistic study conducted with proquinazid (O'Connor, 2002), a statistically significant increase in UGT activity was observed in 30 and 300 ppm (2 and 19 mg/kg bw/day) animals and, consistent with the proposed mode of action, was accompanied by a decrease in T4 and an increase in TSH at the same dose levels. Liver weight was increased at the top dose level due to hepatocellular hypertrophy. Follicular cell hypertrophy was also observed in the top dose by day 28.

In one 90-day study (Malley, 2003a), male SD rats (22/dose) were fed diets containing 0, 30, 100, 300 or 2000 ppm (estimated to be 0, 2, 6, 19 and 135 mg/kg bw/day) for 90-days. In this study, effects on the liver (weights, heptic UGT activity, hepatic 5'-deiodinase), thyroid (weights, hypertrophy/hyperplasia), and hormones (serum levels of T4, T3, rT3 and TSH) were investigated.

Statistically significant increases in UGT activity were observed from 6 mg/kg bw/day, with activity increasing almost 300 % in the high dose groups compared to the control. Statistically significant increases in relative liver weight were observed from 19 mg/kg bw/day. Consistent with enhanced hepatic excretion of T4, decreases in serum T4 levels (46 %) were observed at the top dose and TSH levels were increased from 19 mg/kg bw/day (38 %) and were 75 % higher at the top dose. An increase in relative thyroid weight was observed at the top dose. In the other 90-day study (Malley 2002b), similar effects were observed, but, in general, did not tend to occur until the top dose (apart from TSH, which was elevated from 19 mg/kg bw/day in males).

In the 2-year study, UGT activity was not measured (Malley, 2002a). A statistically significant increase in relative liver weight was observed from 43 mg/kg bw/day. Statistically significant decreases in serum T4 were observed after one week at 43 mg/kg bw/day (but not one year) and at one week and one year in the top dose (29 % decrease after one year). TSH levels were significantly increased after one week, but not one year, in the top two doses (by 41 ad 61 %, respectively). The lowest dose of proquinazid producing a statistically significant increase in thyroid follicular cell tumours in male SD rats was 43 mg/kg bw/day in the 2-year study.

The data shows concordance between dose levels causing effects on the liver and those that cause thyroid changes, supporting the mode of action.

Temporal relationship

To support the postulated mode of action there must be a temporal relationship between the key events and the emergence of thyroid follicular tumours. The effect of proquinazid at different timepoints (7, 14 and 28 days) in male rats is available from the 28-day mechanistic study, conducted in Sprague-Dawley rats, dosed up to 19 mg/kg bw/day. Hepatic UGT activity was increased at 2 mg/kg bw/day (week 2 only) and 19 mg/kg bw/day, resulting in an increased liver weight at the top dose on day 28. Serum T4 was reduced in both 19 mg/kg bw/day and 2 mg/kg bw/day dose levels from week 1 and was accompanied by increased TSH levels. Thyroid hypertrophy was observed in the top dose on day 28. In the 2-year rat study, follicular hypertrophy, but no tumours were observed at the interim sacrifice. Overall, key events appear to precede tumour cell formation and thus support the proposed mode of action.

Strength, consistency and specificity of association of the tumour response with key events

The results of the repeat dose and 28-day mechanistic studies are largely consistent with the proposed mode of action. Hepatic UGT activity was generally increased at the same dose level as effects on T4 and TSH levels were observed and occurred before thyroid follicular-cell hypertrophy/hyperplasia was observed. There was a consistent decrease in T4 levels and increase in TSH levels across studies. Furthermore, in subchronic studies, the increases in thyroid weight and the development of hypertrophy/hyperplasia mainly occurred at dose levels that also resulted in hormonal changes. The recovery period in the 28-day mechanistic study showed that cessation of proquinazid dosing was followed by a return of hormone levels to control values, (apart from TSH which remained slightly elevated), as well as a reduction in liver weight and reversal of hypertrophy of the hepatocytes and thyroid follicular cells.

Biological plausibility and coherence

Evidence from laboratory studies have demonstrated that sustained perturbation of the hypothalamic-pituitary-thyroid axis, resulting in prolonged stimulation of the thyroid gland by TSH, can lead to hypertrophy, hyperplasia and eventually neoplasia of the follicular cells of the rat thyroid.

Other modes of action

In addition to the increased activity of UGT, either the decrease in 5'-deiodinase activity or the increase in rT_3 levels, observed in the 28-day mechanistic study and the 90-day repeat dose study (Mallet, 2002a), could also explain the changes observed. However, these proposed mechanisms are likely to contribute to the effects seen rather than present an alternative mechanism for thyroid tumour generation.

Proquniazid has shown to be non-genotoxic in a suite of *in vitro* and *in vivo* assays investigating gene mutation and chromosomal aberrations. Therefore, a genotoxic mechanism is unlikely.

Uncertainties, inconsistencies and data gaps

In the 2-year study, hypertrophy and hyperplasia were observed at doses lower than effects on hormones etc. This finding is not felt to significantly undermine the hypothesised mode of action as it is possible that the effects may be due to small, but prolonged, changes in hormone level caused by elevated UGT activity (which was not measured).

Assessment of postulated mode of Action

The data presented are judged to be adequate to explain the development of follicular-cell tumours in males rats following chronic exposure to proquinazid.

Human applicability of the proposed MOA

The human relevance of this mode of action has already been published (Dellarco, 2006). The main arguments presented in this paper regarding human relevance are summarised below.

1. *Is the weight of evidence sufficient to establish a mode of action in animals*

The evidence suggests that proquinazid alters thyroid homeostasis by increasing hepatic UGT activity (and inhibiting 5'-deionidase activity), reducing serum T4 levels and consequently elevating serum TSH levels.

2. *Can human relevance of the mode of action be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?*

In humans, the regulation of the HPT axis is essentially similar to rats; however, unlike in rats, no increase in TSH is observed in humans following exposure to substances that cause a decrease in serum T4 levels as a result of increased hepatic enzyme activity. Therefore, a key event of the proposed mode of action in rats is missing in humans.

3. *Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?*

Although no information is available for proquinazid in humans, there is information available for other substances, which also indirectly affect the thyroid via the liver. These substances produce hypothyroidism by decreasing T4 levels, but do not result in elevated TSH levels in humans. Furthermore, epidemiological studies with such substances, e.g. phenobarbitone, do not show any increased risk of thyroid cancer.

There are two main quantitative differences between the rat and human thyroid. Firstly, the half-life of T4 in the serum of humans is longer than in rats (5-9 days compared to 12 hours, respectively). This is probably due to the presence of a high affinity globulin for T4 in humans, which results in a lower rate of T4 degradation in humans than in rats. Secondly, TSH levels are also approximately 25-times higher in rats than humans reflecting the higher activity of the HPT axis in rats. These differences suggest that humans are quantitatively less sensitive than rats to substances that lower T4 levels and elevate TSH.

In addition, there are also histopathological differences, which are consistent with higher metabolic activity in rats. In rats, more of the follicular cells are tall cuboidal and appear to be active, whereas in humans the cells tend to be short or almost squamous in appearance suggesting they are inactive. Since more cells are inactive in humans, stimulation with TSH is likely to stimulate these inactive cells to produce hormone, whereas in rats, where cells are already active, TSH is more likely to result in hyperplasia. Therefore, the primary response in humans (hypertrophy) is likely to differ from that in rats (hyperplasia).

Overall, these differences indicate that rats and humans are differently susceptible to hypothyroidism. In contrast to humans, modest changes in thyroid homeostasis will promote tumour formation in rats. Therefore, thyroid tumours induced by proquinazid involving increased hepatic

clearance of hormone and altered homeostasis of the pituitary-thyroid axis in rodents are considered not relevant to humans.

The Specialist Advisory Panel (Committee of Carcinogenicity) also reached the same conclusion on mode of action and human relevance.

References

O'Connor (2002) DPX-KQ926 Technical: Mechanistic Studies for Thyroid Effects 28-Day Feeding Study with 14, 28 or 42 days of Recovery in Rats. Unpublished.

Dellarco, V, Mc Cregor, D, Berry, C, Cohen, S, Boobis, A (2006) Thiazopyr and Thyroid Disruption: Case Study Withinthe Context of the 2006 IPCS Human Relevance Framework for Analysis of a Cancer Mode of Action. Critical Reviews in Toxicology. 36: 763-801.

Hurley, P.M., Hill, R.N., and Whiting, R.J. (1998). Mode of carcinogenic action of pesticides includeing thyroid follicular-cell tumors in rodents. Environ. Health Perspect. 106(8): 437-445.