Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

proquinazid (ISO); 6-iodo-2-propoxy-3-propylquinazolin-4(3*H*)-one

EC Number: -
CAS Number: 189278-12-4

CLH-O-0000007362-77-01/F

Adopted

14 September 2023

14 September 2023

CLH-O-0000007362-77-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted on **14 September 2023** by **consensus** an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **proquinazid (ISO); 6-iodo-2-propoxy-3-propylquinazolin-4(3*H*)-one**

EC Number: **-**

CAS Number: **189278-12-4**

Rapporteur, appointed by RAC: **Michal Martínek**

Co-Rapporteur, appointed by RAC: **Dania Esposito**

Administrative information on the opinion

Sweden has submitted on **16 November 2022** a CLH dossier containing a proposal together with the justification and background information documented in a CLH report.

The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **5 December 2022**.

Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **3 February 2023**.

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The following table provides a summary of the Current Annex VI entry, Dossier submitter proposal, RAC opinion and potential Annex VI entry, if agreed by the Commission.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	606-211-00-1	proquinazid (ISO); 6-iodo-2-propoxy-3-propylquinazolin-4(3*H*)-one		189278-12-4	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H400 H410	GHS08 GHS09 Wng	H351 H410		M=1 M=10	
Dossier submitters proposal	606-211-00-1	proquinazid (ISO); 6-iodo-2-propoxy-3-propylquinazolin-4(3*H*)-one		189278-12-4	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H400 H410	GHS08 GHS09 Wng	H351 H410		M=1 M=10	
RAC opinion	606-211-00-1	proquinazid (ISO); 6-iodo-2-propoxy-3-propylquinazolin-4(3*H*)-one		189278-12-4	**Retain** Carc. 2 Aquatic Acute 1 Aquatic Chronic 1 **Add** STOT RE 1	**Retain** H351 H400 H410 **Add** H372 (thyroid, liver)	GHS08 GHS09 Dgr	**Retain** H351 H410 **Add** H372 (thyroid, liver)		M=1 M=10	
Resulting Annex VI entry if agreed by COM	606-211-00-1	proquinazid (ISO); 6-iodo-2-propoxy-3-propylquinazolin-4(3*H*)-one		189278-12-4	Carc. 2 STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H351 H372 (thyroid, liver) H400 H410	GHS08 GHS09 Dgr	H351 H372 (thyroid, liver) H410		M=1 M=10	

RAC general comment

RAC opinion from 2012

The first proposal for harmonised classification and labelling of proquinazid was submitted by United Kingdom in 2011. All applicable health hazards as well as environmental hazards were evaluated by RAC at that time. In the opinion adopted in 2012, RAC proposed classification of the substance as Carc. 2; H351, Aquatic Acute 1; H400, M=1, and Aquatic Chronic 1, M=10. The classification as proposed by RAC was included in the 5th ATP to CLP (Reg. 944/2013).

Substance identity

Several batches of the test substance were used in the (eco)toxicological dataset. Batch KQ926-45 (purity ≈97%) was used for many of the key toxicology studies in the dossier, including the carcinogenicity and developmental toxicity studies. This batch was produced using an old production method and contained a higher level of impurities compared to more recent batches. Batches KQ926-75, KQ926-85 and KQ926-127 (purity ≈98%) represent the current manufacturing process.

Batches KQ926-75 and KQ926-85 were used in the acute toxicity studies. The 90-day studies in rats, 2-generation studies in rats and some genotoxicity studies were conducted with both KQ926-45 and KQ926-75/85. Comparison of the 90-day and 2-generation studies with KQ926-45 and KQ926-75/85 reveals that KQ926-45 has a stronger effect on body weight and causes a specific pattern of liver toxicity. The information on the hazard profile of individual impurities is limited, therefore it is not possible to pinpoint the impurities responsible for the generally higher toxicity of KQ926-45 compared to later batches.

As all abovementioned batches, including KQ926-45, meet the reference specification, the studies with these batches are considered relevant for classification of proquinazid.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The melting point of proquinazid (purity 99.2%) is 62 °C. A DSC (differential scanning calorimetry) test showed exothermic decomposition onset at 367 °C and a decomposition energy of 269 J/g.

Explosives

Proquinazid was not explosive in an A.14 test. The dossier submitter (DS) noted that the A.14 test method is not conclusive for classification under CLP and that the molecule contains unsaturation. The DS proposed no classification based on decomposition energy below 500 J/g.

Flammable solids

Proquinazid was not flammable in a screening test according to A.10. Accordingly, the DS proposed no classification.

Self-reactive substances

The DS proposed no classification based on exothermic decomposition energy below 300 J/g.

Pyrophoric solids

The DS proposed no classification based on experience in handling.

Self-heating substances

The DS proposed no classification based on melting point below 160 °C (in line with the Guidance on the application of the CLP criteria, hereafter CLP guidance).

Substances which in contact with water emit flammable gases

The DS proposed no classification based on structure (no metals or metalloids) and experience in handling.

Oxidising solids

The dossier contains an A.17 test, which was interpreted as negative. The DS proposed no classification based on structure (the substance contains oxygen but it is bonded only to carbon).

Organic peroxides

The hazard class is not applicable as proquinazid does not contain a peroxide group.

Corrosive to metals

The DS proposed no classification based on a melting point above 55 °C.

Comments received during consultation

A manufacturer supported the DS's proposal.

Assessment and comparison with the classification criteria

RAC concurs with the DS's assessment of the applicable physical hazards and agrees that **proquinazid warrants no classification** based on the available information.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

There is an acute oral toxicity study according to OECD TG 401 in rats reporting an LD_{50} of > 5000 mg/kg bw and 4846 mg/kg bw for males and females respectively. The DS further mentioned mortalities in *in vivo* mouse micronucleus assays and the respective range-finding experiments, which also indicated an LD_{50} above 2000 mg/kg bw. Therefore, the DS proposed no classification.

Acute dermal toxicity

The DS proposed no classification based on an acute dermal toxicity study according to OECD TG 402 (with some deviations, see CLH report) in rats reporting no mortalities at 5000 mg/kg bw.

Acute inhalation toxicity

The DS proposed no classification based on an acute inhalation toxicity study according to OECD TG 403 in rats showing no mortalities at 5,2 mg/L.

Comments received during consultation

A manufacturer supported the DS's proposal.

Assessment and comparison with the classification criteria

Acute oral toxicity

In the acute oral toxicity study in rats (1999), proquinazid was administered to 5 males at a dose level of 5000 mg/kg bw and to 5 females per group at dose levels of 2980, 5000 and 7500 mg/kg bw. The observation period was 20 days. No mortality occurred in males at 5000 mg/kg bw or females at 2980 mg/kg bw. Three and 5 females died at 5000 and 7500 mg/kg bw respectively, the last death was observed on day 12. Clinical signs included lethargy and hunched posture. The LD_{50} was >5000 mg/kg bw and 4846 mg/kg bw in males and females, respectively.

In a toxicity assay in mice preceding the micronucleus test (1999) with batch KQ926-45, mortality was observed in 1/5 and 2/5 females at 2000 mg/kg bw, 3/5 males and 2/5 females at 3000 mg/kg bw and 5/5 males and 5/5 females at 4000 mg/kg bw. The post-dose observation period was 3 days. The LD_{50} (combined sexes) was calculated to be approx. 2400 mg/kg bw. In the main test with batch KQ926-45, 1 out of 20 females died at the top dose of 1440 mg/kg bw.

In a subsequent mouse micronucleus test (1999) with batch KQ926-75, 1 out of 15 males died at 2000 mg/kg bw and 1 out of 15 females died at 1440 mg/kg bw. The third mouse micronucleus test (2019), conducted with batch KQ926-127, reported no substance-related mortality in males or females at 2000 mg/kg bw.

As the available oral LD_{50} values are greater than 2000 mg/kg bw, RAC agrees with the DS's proposal that proquinazid warrants **no classification for acute oral toxicity**.

Acute dermal toxicity

In the acute dermal toxicity study in rats (1999), proquinazid moistened with water was applied to the skin of 5 males and 5 females at a dose level of 5000 mg/kg bw for 24 hours. There was no mortality and no clinical signs except slight transient erythema in 1 animal.

As the dermal LD_{50} is >2000 mg/kg bw, RAC agrees with the DS's proposal that proquinazid warrants **no classification for acute dermal toxicity**.

Acute inhalation toxicity

In the acute inhalation toxicity study in rats (2003), 5 males and 5 females were exposed nose-only to proquinazid dust for 4 hours at a single exposure concentration of 5.2 mg/L (MMAD 3.6 µm). There were no mortalities and no gross abnormalities at necropsy at the end of the 14-day observation period. Clinical signs were limited to transient ocular and oral discharge in one animal.

As the 4-hour LC_{50} is >5 mg/L, RAC agrees with the DS's proposal that proquinazid warrants **no classification for acute inhalation toxicity**.

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

Summary of the Dossier Submitter's proposal

The DS reported that clinical signs of toxicity were seen in acute oral toxicity studies (oral, dermal, inhalation) in rats. They further discussed decreased motor activity in an acute oral neurotoxicity study in rats and ocular discharge observed in a 90-day dietary study in dogs already within the first few days. They concluded that the decreased motor activity in rats without other, more specific findings (such as gait abnormalities) appears to be secondary to general toxicity and that the ocular discharge in dogs does not represent "significant toxicity" referred to in the CLP criteria. Therefore, the DS proposed no classification.

Comments received during consultation

A manufacturer supported the DS's proposal.

Assessment and comparison with the classification criteria

RAC identified in the available studies three findings that warranted discussion in relation to a STOT SE classification: (1) reduced motor activity in the rat acute neurotoxicity study, (2) clinical signs potentially related to neurotoxicity in two of the three *in vivo* micronucleus tests in mice, and (3) ocular discharge in the 90-day study in dogs.

Acute neurotoxicity study in rats (1997) (comparable to TG OECD 424 with deviations)

Sprague-Dawley rats (12/sex/group) received a single oral dose of proquinazid, batch KQ926-45, in aqueous methyl cellulose at dose levels of 0, 100, 500 and 2000 mg/kg bw. Additional groups of females were tested at 50 mg/kg bw and below to determine the NOAEL. Functional observational battery (FOB) and motor activity tests were conducted prior to exposure, 1-3 hours post-dosing (day 1) and then again on days 8 and 15. Motor activity tests were conducted on the same days as FOB. Six animals per sex and group were used for histopathological examination of the nervous system.

There was no treatment-related mortality. Clinical signs were observed in top dose females from day 2 and included hunched posture. Decreased motor activity was observed in both sexes on day 1 from 500 mg/kg bw in males and from 100 mg/kg bw in females. In top dose females the effect persisted until day 8. Several top dose females (3/12) showed no response to tail pinch on day 8; normal response was seen in all animals on day 1. Histopathology of the nervous system did not reveal any effects.

Micronucleus tests in mice

Three oral *in vivo* micronucleus tests in mice have been conducted, each with a different batch of proquinazid. All used the same strain.

In the first test (1999; batch KQ926-45) the animals received a single dose of proquinazid in corn oil at dose levels of 0, 360, 720 and 1440 mg/kg bw. Bone marrow was sampled at 24, 48 and 72 h. A range-finding test reported 30% mortality (3/10) at 2000 mg/kg bw and an LD_{50} of 2400 mg/kg bw.

All 40 animals at 1440 mg/kg bw exhibited lethargy, 10 animals also ataxia. One animal died one day after dosing. Lethargy was also observed in all 30 animals at 720 mg/kg bw. No clinical

signs of toxicity were noted at 360 mg/kg bw. Ataxia and lethargy may be indicative of neurotoxicity.

In the second test (1999; batch KQ926-75) males received a single dose of proquinazid in corn oil at dose levels of 0, 720, 1440 and 2000 mg/kg bw, and females at 0, 360, 720 and 1440 mg/kg bw. Bone marrow was sampled at 24 and 48 h.

Clinical signs of toxicity were observed in 12/15 males and 12/15 females at the top doses (2000 and 1440 mg/kg bw, respectively), and included lethargy, salivation, hunched posture and/or abnormal gait. The incidence of individual clinical signs was not provided in the study report. One top dose male and 1 top dose female were found dead 2 days after dosing. Clinical signs were also observed in 2/5 males and 3/5 females at the mid doses; the study report does not specify whether these included abnormal gait and lethargy. Although the clinical signs are similar to those in the first micronucleus test, the level of detail is not sufficient for evaluation of target organ effects after single exposure.

In the third test (2019; batch KQ926-127) the animals received a single dose of proquinazid in an aqueous vehicle at dose levels up to 2000 mg/kg bw. No treatment-related mortality or clinical sings of toxicity were observed in this study.

90-day study in dogs (1997)

Beagle dogs (4/sex/dose) were administered proquinazid, batch KQ926-45, at dietary concentrations of 0, 500, 2000 and 4000/3000 ppm (top dose equivalent to 87/95 mg/kg bw/d in m/f). The initial top dose of 4000 ppm was reduced to 3000 ppm after 5 weeks due to decreased food consumption and body weight loss.

Increased incidence of eye discharge was observed in all treatment groups. In 1 out of 8 animals in each of the groups receiving proquinazid (1 female at 500 ppm, 1 male at 2000 ppm and 1 female at 4000 ppm) this finding was already present on the first day of exposure. However, in most of the affected animals the effect started later and is therefore considered relevant for STOT RE rather than STOT SE (see the STOT RE section for further information).

Conclusion on classification

Lethargy and ataxia or abnormal gait were observed in two *in vivo* micronucleus tests in mice via oral route. Lethargy was observed from 720 mg/kg bw and ataxia at 1440 mg/kg bw in the study with batch KQ926-45 (1999). One out of 40 animals died at 1440 mg/kg bw, a pre-test showed an LD_{50} around 2400 mg/kg bw.

According to the CLP criteria, narcotic effects in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. Such effect may warrant classification in Category 3 or higher depending on whether the effects are transient or not (CLP regulation, Annex I, 3.8.2.2.2.b). A STOT SE classification should be considered, especially when specific target organ toxicity is observed in the absence of lethality (CLP guidance, 3.8.1). In the mouse study (1999) a transient nature could not be demonstrated due to short duration (the study focused on mutagenicity, not neurotoxicity). Ataxia, which represents a relatively specific sign of neurotoxicity, occurred just below a dose causing high mortality (ataxia at 1440 mg/kg bw, 30% mortality at 2000 mg/kg bw). The most recent micronucleus test (2019) using a different batch and vehicle reported no clinical signs of toxicity.

The previous RAC assessment (2012) did not discuss clinical signs of neurotoxicity in the *in vivo* mutagenicity tests.

The acute neurotoxicity study in rats (1997; batch KQ926-45) reported decreased motor activity from 100 mg/kg bw. This effect may indicate neurotoxicity. However, when observed in isolation,

it may also represent a non-specific consequence of general toxicity. Thus, it is not considered sufficient for a STOT SE classification.

Although clinical signs in some of the mouse micronucleus tests raise a concern about neurotoxicity, the effects occurred relatively close to lethal doses and were not observed in the most recent micronucleus test in mice, nor in an acute neurotoxicity study in rats. Decreased motor activity in the rat acute neurotoxicity study is not considered to provide sufficient evidence of a specific neurotoxicity effect. Therefore, RAC agrees with the DS's proposal that proquinazid warrants **no classification for STOT SE**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

An *in vivo* skin irritation study in rabbits (TG OECD 414) reported transient erythema of low severity, not meeting the classification criteria. Accordingly, the DS proposed no classification.

Comments received during consultation

Comments were received from a manufacturer and an MSCA. The manufacturer supported no classification. The MSCA pointed out that there is evidence of phototoxicity but there is no adequate reflection of such properties in the current classification system.

Assessment and comparison with the classification criteria

In the *in vivo* skin irritation study (1999), proquinazid moistened with water was applied to the skin of 6 rabbits for 4 hours. There was no oedema. Erythema was observed in all animals at 1 h, the individual mean scores (calculated from readings at 24 h, 48 h and 72 h) ranged from 0 to 1. The effect was no longer present at 72 h. Erythema in an animal study meets the criteria for classification if the mean score is ≥2.3 in at least 2 out of 3 tested animals. As the mean scores were below 2.3 in all animals and the erythema was fully reversible, RAC agrees with the DS's proposal that proquinazid warrants **no classification for skin corrosion/irritation**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

An *in vivo* eye irritation study in rabbits (TG OECD 414) reported conjunctival redness not meeting the classification criteria. Therefore, the DS proposed no classification.

Comments received during consultation

A manufacturer supported no classification.

Assessment and comparison with the classification criteria

In the *in vivo* eye irritation study (1999), proquinazid was instilled into the eyes of 6 rabbits. No iritis or corneal opacity were observed. The maximum individual mean scores (mean of readings

at 24, 48 and 72 h) for conjunctival redness were 0.7, 0.7, 1.0, 1.7, 1.7 and 2.3. Conjunctival oedema was observed in two animals, the mean scores were 0.3 and 0.3. All effects were reversible within 7 days.

Conjunctival redness or oedema in an animal study meet the criteria for classification if the mean score is ≥ 2 in at least 2 out of 3 tested animals, or in at least 4 out of 6 tested animals (CLP guidance, v. 5.0, 3.3.2.3.2.2.). Since the mean score of ≥ 2 was reached only in 1 out of 6 tested animals, the classification criteria are not met, and RAC agrees with the DS's proposal that proquinazid **warrants no classification for eye damage/irritation**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on a negative Guinea pig maximisation test.

Comments received during consultation

A manufacturer supported no classification.

Assessment and comparison with the classification criteria

The Guinea pig maximisation test (1999),performed according to OECD 406, used 10 control and 20 test substance-treated animals. Two of the proquinazid-treated animals died during the test (days 8 and 9), the deaths were not considered substance-related. A concentration of 3% (vehicle propylene glycol) was used for intradermal induction; suspension of test substance in propylene glycol (approx. 1:1) was used for topical induction and challenge. Topical induction was preceded by sodium lauryl sulfate pre-treatment to induce irritation. A periodic positive control study with α-hexylcinnamaldehyde, performed concurrently to the proquinazid study, showed a positive response in 74% of the animals.

Positive responses were seen in 3 out of 18 (i.e., 17%) proquinazid-treated animals and in 1 out of 10 control animals. Upon rechallenge with suspension of proquinazid in propylene glycol (1:3) taking place 7 days after the first challenge, positive responses were seen in 1 out of 18 treated animals and 1 out of 10 control animals. The severity of reaction was 1 (scattered mild redness) in all cases. As the incidence of positive responses was below 30%, RAC agrees with the DS's proposal that proquinazid **warrants no classification for skin sensitisation**.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

Repeated dose toxicity of proquinazid has been investigated in rats, mice and dogs. The main target organs are the liver and the thyroid; effects below the guidance values (GVs) for classification were seen mainly in rats.

The DS concluded that the liver effects were either adaptive (hepatocellular hypertrophy) or precursor effects to liver carcinogenicity seen in females in a 2-year rat study.

Thyroid-related findings (follicular cell hyperplasia, altered hormone levels) were attributed to UDP-glucuronyltransferase (UGT) induction, in line with the previous RAC opinion (2012). However, the DS noted that according to the ED guidance (Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009), a comparative *in vitro* study of enzyme activity in the relevant test species and humans should be provided.

The DS further discussed ocular discharge in two dog studies. They concluded that the effect is related to treatment but not sufficiently adverse to meet the CLP criteria.

The DS concluded that proquinazid does not meet the criteria for classification, provided that the MoA of adverse thyroid effects in rats is considered rodent specific, and therefore not relevant to humans.

Comments received during consultation

Comments were received from an MSCA and a manufacturer. Both supported the DS's assessment and no classification.

The manufacturer informed that they had conducted an *in vitro* study on CYP and UGT induction and cell proliferation in rat and human hepatocytes. The results of this study are summarised below under 'additional key elements'.

Additional key elements

In vitro CYP and UGT induction study in rat and human hepatocytes (2023)

The objective of the study was to assess the potential of proquinazid (KQ926-127, purity 99.1%) to induce in Sprague-Dawley rat and human hepatocytes CYP1A2, CYP2B, CYP3A, CYP4A (indicative of AhR, CAR, PXR and PPARα activation respectively) and UGT (UGT1A, UGT2A, T4-UGT, Global UGT), as well as to induce cell proliferation marker MKI67.

CYP mRNA expression, UGT mRNA expression and UGT activity after 7 days of treatment was investigated in 3 rat cultures (one culture from a male animal, one culture from a female animal, one culture pooled from 18 males) and 3 human cultures (each from a single donor, 2 males and 1 female). MKI67 mRNA expression, CYP mRNA expression and CYP activity after 3 days was investigated in cultures from 3 female rats and 3 female human donors. Top concentration selection was based on cytotoxicity, top concentrations ranged between 10 and 50 µM. Positive control inducers were assessed in parallel. A ≥ 2-fold induction was considered as the threshold for a treatment-related effect by the study authors. The results are briefly summarised in the table below.

Increased AhR, CAR and PXR mRNA expression was observed in both rat and human hepatocytes, but only in rats it translated into increased enzyme activities. Increases in T4-UGT activity occurred in hepatocytes from both species.

***In vitro* CYP and UGT induction study in human and rat hepatocytes (2023)**		
Parameter	**Rat hepatocytes**	**Human hepatocytes**
CYP1A2 expression	↑ ≈ 70-fold *PC (BNF 5 µM): ↑ ≈ 100-fold*	↑ 30- to 600-fold *PC (BNF 5 µM): ↑ 70- to 400-fold*
CYP1A1/2 activity	↑ ≈ 3-fold *PC (BNF 5 µM): ↑ ≈ 30-fold*	no effect *PC (BNF 5 µM): ↑ ≈ 60-fold*

CYP2B1/6 expression	↑ ≈ 4-fold *PC (PB 1000 µM): ↑ 3- to 30-fold*	↑ ≈ 6-fold *PC (PB 1000 µM): ↑ 5- to 25-fold*
CYP2B1/2/6 activity	↑ ≈ 2-fold *PC (PB 1000 µM): ↑ ≈ 3-fold*	no effect *PC (PB 1000 µM): ↑ ≈ 3-fold*
CYP3A1/4 expression	↑ 50- to 600-fold *PC (PCN 6 µM): ↑ 60- to 1300-fold*	↑ 3- to 15-fold *PC (RIF 15 µM): ↑ 15- to 60-fold*
CYP3A1/2/4/5 activity	↑ 5- to 20-fold *PC (PCN 6 µM): ↑ 30- to 190-fold*	↑ 2-fold in 1 culture out of 3 *PC (RIF 15 µM): ↑ ≈ 8-fold*
CYP4A1/11 expression	no effect *PC (BEZA 250 µM): ↑ 45- to 670-fold*	↑ 2-fold in 2 cultures out of 5 *PC (BEZA 250 µM): ↑ ≈ 8-fold*
CYP4A1/11 activity	no effect *PC (BEZA 250 µM): ↑ ≈ 15-fold*	no effect *PC (BEZA 250 µM): no effect*
UGT1A1 expression	no effect	↑ 8- to 20-fold *PC (BNF 5 µM): ↑ ≈ 7-fold* *PC (RIF 15 µM): ↑ ≈ 18-fold* *PC (PB 1000 µM): ↑ 8- to 20-fold*
UGT1A9 expression	not measured	↑ 2- to 4-fold *PC (RIF 15 µM): ↑ ≈ 4-fold* *PC (PB 1000 µM): ↑ ≈ 4-fold*
UGT1A1 activity	no effect *NC mean: 140 pmol/min/mg*	↑ 2-fold *PC (BNF 5 µM): ↑ ≈ 3-fold* *PC (RIF 15 µM): ↑ no effect* *PC (PB 1000 µM): ↑ 2-fold in 1 cult.* *NC mean: 27 pmol/min/mg*
UGT2B1/7 expression	↑ 2- to 6-fold *PC (PCN 6 µM): ↑ 2- to 8-fold* *PC (PB 1000 µM): ↑ 4- to 15-fold*	↑ 2-fold in 1 culture out of 3
UGT2B1/7/15/17 activity	↑ 2-fold in 1 culture out of 3 *PC (PCN 6 µM): ↑ 2-fold in 2 cult.* *PC (PB 1000 µM): ↑ 2-fold in 2 cult.* *NC mean: 150 pmol/min/mg*	no effect *NC mean: 160 pmol/min/mg*
T4-UGT activity	↑ 2-fold in 2 cultures out of 3 *PC (PCN 6 µM): ↑ 2- to 3-fold* *PB (1000 µM): ↑ 2-fold in 1 cult.* *NC mean: 6.1 pmol/min/mg* *Proquinazid 10 mM mean: 10.0 pmol/min/mg*	↑ 3- to 4-fold *PC (BNF 5 µM): ↑ ≈ 3-fold* *PC (RIF 15 µM): ↑ 3-fold in 2 cult.* *PC (PB 1000 µM): ↑ 2-fold in 1 cult.* *NC mean: 0.66 pmol/min/mg* *Proquinazid 10 mM mean: 2.3 pmol/min/mg*
Global UGT activity	below limit of quantification	↑ 2- fold in 2 cultures out of 3
MKI67 expression	no effect *PC (EGF 25 ng/ml): 2- to 9-fold*	↑ 3-fold in 1 culture out of 3 *PC (EGF 25 ng/ml): 8- to 45-fold*

PC = positive control; NC = negative (vehicle) control; BNF = beta-naphthoflavone; BEZA = bezafibrate; PCN = pregnenolone carbonitrile; RIF = rifampicin; PB = phenobarbital sodium; EGF = epidermal growth factor

Assessment and comparison with the classification criteria

Effects on the liver and thyroid at doses below the (extrapolated) GVs for classification are listed in the following table. More details on the 90-day studies and the 2-year study in rats can be found under "Supplemental information" in the appendix to the opinion.

Liver and thyroid effects below the GV in the available studies			
Study type; TG; batch; year	**Dose**	**Liver effects**	**Thyroid effects**
90-day, rat, dietary; OECD 408; KQ926-45; 2003	600 ppm (50 mkd), females only	Alteration of hepatocytes, fatty change, bile duct hyperplasia	Follicular cell hypertrophy, ↑ TSH, ↓ T4, ↓ T3 MoA: ↑ UGT, ↓ DIO1
	300 ppm (19/23 mkd m/f)	↑ liver wt (m rel. 11%), alteration of hepatocytes (m), ↑ cholesterol (m)	Follicular cell hypertrophy, ↑ TSH, ↓ T3 MoA: ↑ UGT (m,f), ↓ DIO1 (f)
	100 ppm (6/8 mkd m/f)		Follicular cell hypertrophy (m), ↑ TSH (m), ↓ T3 (f) MoA: ↑ UGT (m)
	30 ppm (1.9/2.3 mkd m/f)		Follicular cell hypertrophy (m)
90-day, rat, dietary; OECD 408; KQ926-75; 2002	600 ppm (50 mkd), females only	↑ liver wt (rel. 18%)	Follicular cell hypertrophy MoA: ↑ UGT (f)
	300 ppm (19/24 mkd m/f)	↑ liver wt (m rel. 9%)	Follicular cell hypertrophy (m), ↑ TSH (m) MoA: ↑ UGT (f)
	100 ppm (6/8 mkd m/f)		Follicular cell hypertrophy (m), ↑ TSH (m)
2-year, rat, dietary; OECD 453; KQ926-45; 2002	300 ppm (12/16 mkd m/f)	1-year interim sacrifice: Fatty change (m), alteration/degeneration of hepatocytes (f)	1-year interim sacrifice: Follicular cell hypertrophy (m,f), ↑ thyroid wt (f), ↓ T4 (f), ↓ T3 (f)
		2-year group: Alteration/degeneration of hepatocytes (m,f), fatty change (m,f), hepatocellular hypertrophy (f), foci of cellular alteration (f), oval cell hyperplasia (f)	2-year group: Follicular cell hypertrophy (m,f), follicular cystic hyperplasia (m)
28-day, rat, dietary, males only; OECD 407	300 ppm (19 mkd)	↑ liver wt (rel. 21%), hepatocellular hypertrophy	Follicular cell hypertrophy, ↑ TSH, ↓ T4, ↓ T3 MoA: ↑ UGT, ↓ DIO1

KQ926-45 2002	30 ppm (1.9 mkd)		↓ T4
2-generation, rat, dietary; OECD 407; KQ926-45; 2002	600 ppm (F1 52/63 mkd m/f; P not examined)	Fatty change (m,f), alteration of hepatocytes (f), cholangiofibrosis (f)	Follicular cell hypertrophy and hyperplasia
	300 ppm (F1 24/27 mkd m/f)		Follicular cell hypertrophy
	150 ppm (F1 12/14 mkd m/f)		Follicular cell hypertrophy (m)
2-generation, rat, dietary; OECD 416; KQ926-85; 2003	600 ppm (P 35/44 mkd; F1 54/60 mkd m/f)	Hepatocellular hypertrophy	Follicular cell hypertrophy
	150 ppm (P 9/11 mkd; F1 14/16 mkd m/f)		Follicular cell hypertrophy
28-day, rat, dermal; OECD 410; KQ926-45; 2002	500 mg/kg bw/d	↑ liver wt (f rel. 15%), hepatocellular hypertrophy (f)	
90-day, dog, dietary; OECD 409; KQ926-45; 1997	2000 ppm (62/56 mkd m/f)	↑ liver wt (f rel. 45%)	
	500 ppm (17/18 mkd m/f)	↑ liver wt (f rel. 24%)	

m = males, f = females; rel. = relative; mkd = mg/kg bw/d; the mkd doses in 2-generation studies relate to the premating phase; MoA = mode of action investigations; UGT = hepatic UGT activity; DIO1 = activity of hepatic deiodinase type I

Liver

Two 90-day rat studies are available, one with batch KQ926-45 and the other one with batch KQ926-75. The study with batch KQ926-75 reported increased liver weight, and above the GV also hepatocellular hypertrophy and slightly increased cholesterol. The 90-day study with batch KQ926-45 showed alteration of hepatocytes and fatty change (midzonal) below the GV. According to the study report, alteration of periportal hepatocytes was characterised by disorganisation of hepatocytes such that hepatic cord architecture was generally lost due to individualisation of cells. This indicates significant loss of hepatocytes.

Similar changes were observed in the 1-year interim sacrifice animals of the carcinogenicity study with batch KQ926-45 already at 300 ppm (12/16 mg/kg bw/d m/f), below the extrapolated GV (25 mg/kg bw/d). Fatty change was of the micro vesicular type, which is generally considered adverse. Effects above the GV additionally included oval cell hyperplasia in both sexes and increased plasma levels of ALT, AST and SDH in females; both findings indicate liver cell damage. Histopathological examination in the main, 2-year group revealed a marked increase in hepatocellular adenomas and cholangiocarcinomas in females from 600 ppm. However, increase in hepatocellular neoplasms was seen in males.

The new *in vitro* CYP and UGT induction study (2023) showed increased expression and activity of CYP1A, CYP2B and CYP3A in rat hepatocytes, corresponding to activation of AhR, CAR and PXR respectively.

RAC agrees with the DS that increases in liver weight and hepatocellular hypertrophy observed in studies with proquinazid below the GVs may be adaptive and are not sufficient for classification. Furthermore, oval cell hyperplasia is related to liver tumours and is therefore covered by the existing classification as Carc. 2.

However, alteration/degeneration of hepatocytes and micro vesicular fatty change cannot be assigned to carcinogenicity and both lesions are considered adverse and occurred at dose levels relevant for classification. Therefore, in contrast to the previous assessment (RAC, 2012), RAC concluded that **classification in Category 2 for the liver is warranted**.

Thyroid

Effects observed below the GV for Category 2 in rats include follicular cell hypertrophy, increased TSH, reduced T3 and reduced T4. Changes below the GV for Category 1 include follicular cell hypertrophy and increased TSH in males (90-day study, 2003; 90-day study, 2002) and reduced T3 in females (90-day study, 2003; 2-year study, 2002). A 28-day mechanistic study in male rats (2002) reported reduced T4 levels already from ca. 2 mg/kg bw/d, but this finding was not reproduced in other studies. Mechanistic investigations in the 28-day and 90-day rat studies identified two modes of action related to thyroid hormones: UGT induction and deiodinase (type I) (DIO1) inhibition. In both studies the effect started at rather low doses and increased with dose. In addition, a dose dependent increase in rT3 was observed in both studies, which might be related to the inhibition of DIO1 in the liver.

No changes in thyroid weight or histopathology were observed in dogs, up to the highest dose tested of 180 mg/kg bw/d (1-year study, 2002, batch KQ926-45).

In vitro studies on TPO inhibition (2021), direct interaction with TH receptor (2021) and NIS inhibition (2021) were negative (see "supplemental information" in the appendix to the opinion). The new *in vitro* CYP and UGT induction study in human and rat hepatocytes (2023) showed increased T4-UGT activity in the hepatocytes of both species (see 'additional key elements').

In 2012 RAC considered that the effects in rats, such as follicular hypertrophy and thyroid hormone alterations, wee not relevant for humans. Therfeore, RAC concluded that a STOT RE classification for the thyroid was not warranted.

However, during the RAC discussions in 2023, the Committee considered that a classification in Category 1 could be warranted based on the following arguments:

- Effects were observed below the GV for Category 1.
- UGT induction was observed in human hepatocytes. This confirms human relevance of UGT-related thyroid findings in rats.
- Thyroid effects in rats generally started at lower doses than liver effects. Thus, liver enzyme induction is not the main MoA of thyroid effects.
- Both UGT induction and deiodinase inhibition are relevant for humans and are adverse to offspring development.

Based on these arguments, RAC agreed with the DS that **classification in Category 1 for the thyroid is warranted**.

Eyes

Increased incidence of ocular discharge was observed in dogs in the 90-day dietary study (1997) from 17 mg/kg bw/d and in the 1-year capsule study (2002) at 180 mg/kg bw/d.

Ophthalmological examination at the end of the 90-day study reported epiphora (i.e., overflow of tears) in half of the animals at the top dose (ca. 90 mg/kg bw/d). No treatment-related effects were observed on ophthalmological examination at the end of the 1-year study.

90-day study in dogs: eye effects				
Dose (ppm)	**0**	**500**	**2000**	**4000/3000**
Dose (mg/kg bw/d) m/f	0	17/18	62/56	87/95
Total no. of animals	8	8	8	8
No. of animals with ocular discharge on more than 1 day	2	3	5	6
Day first observed	8,69	1,23,32	1,3,10, 63,79	1,4,9,9, 15,20
Epiphora, week 12; (severity[a])	0	0	0	4 (1,1,2,2)
Conjunctivitis, week 12; (severity[a])	0	0	0	1 (1)

[a] severity grade: 1 = slight, 2 = moderate, 3 = severe

1-year study in dogs: eye effects				
Dose (mg/kg bw/d)	**0**	**15**	**60**	**180**
Total no. of animals	10	10	10	10
No. of animals with ocular discharge on more than 1 day	5	5	5	6
Day first observed	11,18,33, 53,232	28,50,113, 153,301	9,131,177, 195,228	3,5,9,10, 71,104

During the RAC discussion, several members expressed a view that excessive flow of tears observed in the two dog studies represents a systemic rather than local effect, and that this effect may be considered adverse. Nevertheless, it was concluded that the findings were not sufficient to warrant for a STOT RE classification.

Conclusion

RAC concluded that proquinazid **warrants classification in Category 2 for the liver and in Category 1 for the thyroid**. The resulting hazard statement is **STOT RE 1; H372 (thyroid, liver)**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Since all available *in vitro* and *in vivo* genotoxicity studies were negative, the DS proposed no classification.

Comments received during consultation

Comments were received from an MSCA and a manufacturer. Both supported no classification. The manufacturer commented on the DS's assessment of some of the studies.

Assessment and comparison with the classification criteria

The available *in vitro* mutagenicity studies are summarised in the table below.

***In vitro* mutagenicity tests**			
Study type; TG; batch; year	**Method**	**Result**	**Remarks**
Ames test; OECD 472; KQ926-45; 1997	Plate incorporation method Rat liver S9 Top concentration 5000 µg/plate Solvent DMSO	Negative ±S9 No cytotoxicity Precipitation from 1000 µg/plate	
Ames test; OECD 474 with deviations; KQ926-75; 1998	Plate incorporation method Rat liver S9 Top concentration 5000 µg/plate Solvent DMSO	Negative ±S9 No cytotoxicity Precipitation from 2500 µg/plate	
Ames test; OECD 471 with deviations; KQ926-127 2018	Plate incorporation method Rat liver S9 Top concentration 5000 µg/plate Solvent DMSO	Negative ±S9 No cytotoxicity Precipitation from 667 µg/plate	
Chromosomal aberration assay; OECD 473 with deviations; KQ926-45; 1999	Human lymphocytes Rat liver S9 Top concentration –S9 (short-term treatment) 5000 µg/ml, +S9 625 µg/ml Solvent acetone	Negative ±S9 Cytotoxicity in all experiments	Only short-term (4-h) treatment ±S9, continuous treatment –S9 not performed Top concentration selection based on cytotoxicity (adequate levels reached)
HPRT assay; OECD 476; KQ926-45; 1997	CHO cells Rat liver S9 Top concentrations 35 to 125 µg/ml Solvent acetone	Negative ±S9 Cytotoxicity in all experiments	Top concentration selection based on cytotoxicity (adequate levels reached) The study was considered unacceptable by the RMS due to considerable inter-assay variance and very low mutation frequencies in solvent controls of some assays
Mouse lymphoma assay; OECD 476; KQ926-75; 2005	Rat liver S9 Top concentrations 140 to 180 µg/ml Solvent acetone	Negative ±S9 Cytotoxicity in all experiments	Top concentration selection based on cytotoxicity (adequate levels reached)

There are three *in vivo* mouse micronucleus tests, each with a different batch of the test substance, see the table below. All three tests are negative. As for target tissue exposure, significant levels of proquinazid were found in peripheral blood in the most recent micronucleus test (2019) and clinical signs of toxicity were seen in the earlier micronucleus assays (1999). In addition, levels of radioactivity comparable to those in the blood were found in the bone marrow of rats administered radiolabelled substance in an ADME study (see the RAR). Thus, target tissue exposure is considered sufficiently demonstrated.

***In vivo* mutagenicity tests**			
Study type; batch; year	**Method**	**Result**	**Remarks**
Micronucleus, mouse, bone marrow, oral gavage; OECD 474 with deviations; KQ926-45; 1999	Crl:CD-1(ICR)BR mice 5/sex/group Doses: 0, 360, 720, 1440 mg/kg bw Vehicle corn oil Single exposure Sampling at 24 h, 48 h, 72 h (at 48 h and 72 h only control and top dose group) 2000 immature erythrocytes per animal	Negative	No stat. sign. effect on PCE/NCE ratio Clinical signs: lethargy, diarrhea, ataxia 1 female died at 1440 mg/kg bw Mortality 3/10 at 2000 mg/kg bw in a range-finding test
Micronucleus, mouse, bone marrow, oral gavage; OECD 474 with deviations; KQ926-75; 1999	Crl:CD-1(ICR)BR mice 5/sex/group Doses: males 0, 720, 1440, 2000 mg/kg bw; females 0, 360, 720, 1440 mg/kg bw Vehicle corn oil Single exposure Sampling at 24 h and 48 h (at 48 h only control and top dose group) 2000 immature erythrocytes per animal	Negative	No bone marrow toxicity Clinical signs: lethargy, salivation, hunched posture, abnormal gait 1 male died at 2000 mg/kg bw, 1 female died at 1440 mg/kg bw
Micronucleus, mouse, peripheral blood, oral gavage; OECD 474; KQ926-127; 2019	Crl:CD-1(ICR)BR mice 5/sex/group Doses: 0, 500, 1000, 2000 mg/kg bw Vehicle water with 0.1% Tween 80 and 0.5% CMC Single exposure Sampling at 48 h and 72 h 2000 immature erythrocytes per animal Additional 4 animals/sex at 500 mg/kg bw for measurement of proquinazid levels in blood	Negative	No treatment-related mortality No clinical signs of toxicity No decrease in the proportion of reticulocytes in blood Concentration of proquinazid in pooled peripheral blood from the 500 mg/kg bw group, 4 hours post-dosing: males 560 ng/ml, females 896 ng/ml

As all available *in vitro* and *in vivo* genotoxicity tests are negative, RAC agrees with the DS's proposal that proquinazid **warrants no classification for germ cell mutagenicity**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

There are two carcinogenicity studies, one in rats and one in mice. The current classification as Carc. 2 is based on liver and thyroid tumours in rats and mice (RAC, 2012).

In the current CLH proposal, the DS proposed to retain the current classification as Carc. 2. They noted that given the occurrence of tumours in two tissues (liver, thyroid) and two species (rat, mouse), Cat. 1B could be considered. However, the concern is reduced by the following factors:

- The thyroid tumours seem to be caused by a rodent-specific mechanism.
- The tumours were observed at doses close to or above the Maximum Tolerated Dose (MTD) (based on reduced body weight and/or hepatotoxicity).
- Some of the tumours were benign.
- There is no indication of a genotoxic potential.

Comments received during consultation

Comments were received from an MSCA and a manufacturer. Both supported the DS's proposal of Carc. 2.

The manufacturer referred to results of the new *in vitro* study on CYP and UGT induction and cell proliferation in rat and human hepatocytes (summarised in the STOT RE section under 'Additional key elements'), which in their view questions human relevance of the liver and thyroid tumours. Further, they stated that the slight increase in hepatocellular carcinomas in male mice was unrelated to treatment.

Assessment and comparison with the classification criteria

2-year chronic toxicity and carcinogenicity study in rats (2002) (TG OECD 453);

Sprague-Dawley rats (80/sex/group) were administered proquinazid, batch KQ926-45, at dietary concentrations up to 2000 ppm (92 mg/kg bw/d) for males and 1200 ppm (76 mg/kg bw/d) for females. Ten animals/sex/group were sacrificed after 1 year for histopathological examination. Further 10 animals/sex/group were used for mechanistic endpoints related to the liver (cytochrome P-450 content, cell proliferation). The remaining 60 animals/sex/group were used for the 2-year study.

Survival of top dose males and females was higher than in control, probably as a secondary effect to decreased body weights. Body weights of top dose males were ca. 20% below control throughout the study (from week 4 until termination). The effect was more profound in females, the decrease was around 40% in the second year of the study at 1200 ppm and ca. 25% at the next lower dose of 600 ppm.

Examinations at 1-year interim sacrifice revealed considerable hepatic injury, particularly in females. Histopathological findings included alteration/degeneration of hepatocytes, micro vesicular fatty change and oval cell hyperplasia in both sexes. Clinical pathology showed increased ALT, AST and SDH in females. Thyroid-related findings included follicular cell hypertrophy and reduced T4 in both sexes. Details can be found under "supplemental information" in the appendix to the opinion.

Histopathological examination of the 2-year group showed three neoplastic findings: thyroid follicular adenoma in males (statistically significant from 1000 ppm), hepatocellular adenoma in females (from 600 ppm) and cholangiocarcinoma in females (from 600 ppm).

2-year study in rats (2002): neoplastic and selected non-neoplastic findings						
Males						
Dose (ppm)	**0**	**10**	**30**	**300**	**1000**	**2000**
Dose (mg/kg bw/d)	0	0.42	1.2	12	43	92
Survival (%)	32	45	40	45	53	68
No. of animals for histopathological examination	60	60	61	60	60	60
Thyroid: follicular cell adenoma	1	0	1	3	6#	8#
Thyroid: follicular cell carcinoma	0	0	0	0	1	1
Thyroid: follicular hypertrophy	0	1	0	14#	23#	30#
Thyroid: follicular cyst/cystic hyperplasia	2	1	5	7#	9#	16#
Liver: alteration/degeneration of hepatocytes	0	0	0	16#	22#	34#
Liver: cholangiofibrosis	0	0	0	0	5#	0
Liver: fatty change, centrilobular	4	2	7	7	24#	24#
Liver: fatty change, midzonal	1	0	0	5#	7#	24#
Liver: focus of cellular alteration, eosinophilic	10	7	10	15	14	18#
Liver: oval cell hyperplasia	0	0	0	2	9#	7#
Liver: hepatocyte hypertrophy, centrilobular	0	0	2	1	1	5#
Females						
Dose (ppm)	**0**	**10**	**30**	**300**	**600**	**1200**
Dose (mg/kg bw/d)	0	0.49	1.4	16	35	76
Survival (%)	33	38	34	37	63	59
No. of animals for histopathological examination	60	61	60	60	60	61
Liver: animals with at least one hepatocellular neoplasm	1	0	0	2	17#	35#
Liver: hepatocellular adenoma	1	0	0	2	11#	29#
Liver: hepatocellular carcinoma	0	0	0	0	2	1
Liver: cholangiocarcinoma, intestinal type	0	0	0	0	8#	12#
Liver: alteration/degeneration of hepatocytes	0	0	0	13#	46#	59#
Liver: cholangiofibrosis	0	0	0	1	4#	10#
Liver: biliary cyst	0	0	0	0	3#	8#
Liver: fatty change, midzonal	0	0	0	5#	22#	37#

Liver: focus of cellular alteration, eosinophilic	10	5	7	20[#]	48[#]	36[#]
Liver: oval cell hyperplasia	0	0	0	4[#]	42[#]	52[#]
Liver: bile duct hyperplasia	18	22	17	13	26	29[#]
Liver: hepatocyte hypertrophy, panlobular	0	0	0	20[#]	4[#]	0
Thyroid: follicular hypertrophy	1	1	0	10[#]	36[#]	45[#]
No. of animals on study	61	61	60	60	60	61
Found dead or sacrificed in extremis	40	38	39	37	22	25
Probable cause of death/moribundity: liver tumour	0	0	0	0	4	4
Probably cause of death/moribundity: liver toxicity	0	0	0	0	2	7

[#] Statistically significant difference from control, $p \leq 0.05$, by Cochran-Armitage trend test

Cholangiocarcinomas in this study were morphologically similar to cholangiofibrosis and did not metastasise. Cholangiofibrosis generally originates from oval cell hyperplasia, which was present at a high incidence in females. Foci of cellular alteration might represent precursor lesions to hepatocellular adenomas.

Although an increase in hepatocellular neoplasms was only observed in females, the precursor lesions such as oval cell hyperplasia, cholangiofibrosis and foci of cellular alteration were also seen in males at a lower incidence. The difference in neoplastic response appears to be a consequence of higher susceptibility of females to proquinazid-induced hepatic injury.

As to the thyroid tumours, both sexes showed precursor lesions (follicular hypertrophy) but adenomas developed only in males, the more susceptible sex with regard to thyroid effects of proquinazid (see results of the 90-day study with batch KQ926-45 under "Supplemental information" in the STOT RE section in the appendix to the opinion).

18-month carcinogenicity study in mice (2002) (TG OECD 451)

CD-1 mice (70/sex/group) were administered proquinazid, batch KQ926-45, at dietary concentrations up to 2000 ppm (282/415 mg/kg bw/d in m/f). Ten animals/sex/group were sacrificed after 6 months for histopathological examination. Further 5 animals/sex/group were used for mechanistic endpoints related to the liver (cytochrome P-450 content, peroxisomal β-oxidation) at 1 week. The remaining 55 animals/sex/group were used in the 18-month study. The top dose selection was based on a 90-day study where 3000 ppm induced liver toxicity (hypertrophy, fatty change, necrosis).

Survival and body weights were not significantly affected. Examinations at the 6-month interim sacrifice revealed markedly increased liver weight (absolute by 34%/58% in m/f), hepatocellular hypertrophy, and in females also individual hepatocyte necrosis. Mechanistic investigations showed increased cytochrome P-450 content in the liver and increased peroxisomal β-oxidation activity in both sexes at 2000 ppm.

There was no statistically significant increase in neoplastic findings on pairwise comparison. RAC in 2012 as well as the DS in the current CLH proposal discussed the slight increases in hepatocellular carcinomas in males, hepatocellular adenomas in females and thyroid follicular adenomas in females. The importance attached to these findings was apparently derived from

exceedance of historical control ranges (hepatocellular carcinoma males: 0-6.3%; hepatocellular adenoma females: 0-2.6%; thyroid follicular adenoma females: 0-1.3%).

Non-neoplastic findings in the 18-month group included hepatocellular hypertrophy, hepatocyte alteration, fatty change, necrosis of individual hepatocytes, thyroid inflammation and in females also thyroid follicular cell hypertrophy and hyperplasia. The overall pattern of non-neoplastic liver effects in mice is similar to that observed in rats.

18-month study in mice (2002): incidence of liver and thyroid tumours and selected non-neoplastic findings					
Dose (ppm)	**0**	**5**	**30**	**200**	**2000**
Males					
Dose (mg/kg bw/d)	0	0.7	3.9	27	282
Liver: no. of animals for histopathological examination	55	55	55	56	55
Liver: animals with at least one hepatocellular neoplasm	12	4	8	8	12
Liver: hepatocellular adenoma	12	4	6	8	10
Liver: hepatocellular carcinoma	1	0	2	0	4
Liver: hepatocyte hypertrophy	2	3	3	11#	42#
Liver: hepatocyte alteration, multifocal/diffuse	0	0	0	4#	10#
Liver: necrosis	5	3	7	6	12#
Liver: necrosis, individual hepatocyte	0	1	0	2	15#
Liver: fatty change, single cell	0	0	0	1	13#
Thyroid: no. of animals for histopathological examination	51	54	55	53	54
Thyroid: follicular cell adenoma	0	0	0	0	1
Thyroid: follicular cell hyperplasia	0	0	0	1	0
Thyroid: inflammation, subacute/chronic	2	1	1	0	15#
Females					
Dose (mg/kg bw/d)	0	1.0	5.6	38	415
Liver: no. of animals for histopathological examination	55	55	55	55	55
Liver: animals with at least one hepatocellular neoplasm	1	1	1	0	3
Liver: hepatocellular adenoma	1	1	0	0	3
Liver: hepatocellular carcinoma	0	0	1	0	0
Liver: hepatocyte hypertrophy	1	2	4	11#	44#
Liver: hepatocyte alteration, multifocal/diffuse	0	0	0	5#	40#
Liver: necrosis	7	7	7	5	7

Liver: necrosis, individual hepatocyte	2	2	2	6	27[#]
Liver: fatty change, single cell	0	0	0	2	24[#]
Liver: oval cell hyperplasia	0	0	0	1	7[#]
Thyroid: no. of animals for histopathological examination	55	52	51	53	55
Thyroid: follicular cell adenoma	0	0	0	0	2[#]
Thyroid: follicular cyst	0	0	0	0	2[#]
Thyroid: follicular cell hypertrophy	0	0	0	0	6[#]
Thyroid: follicular cell hyperplasia	0	0	0	0	7[#]
Thyroid: inflammation, subacute/chronic	4	2	5	7	22[#]

[#] Statistically significant difference from control, $p \leq 0.05$, by Cochran-Armitage trend test

Mode of action of liver and thyroid tumours

Proquinazid is not considered genotoxic since all available genotoxicity tests were negative. A plausible MoA has been proposed for cholangiocarcinomas in female rats according to the CLH report: severe liver toxicity leads to oval cell proliferation (a regenerative response to liver cell damage), then metaplasia of pluripotent oval cells results in cholangiofibrosis and eventually cholangiocarcinoma. In the absence of data to the contrary, these tumours are considered relevant for humans.

No MoA has been proposed for hepatocellular adenomas in female rats. The new *in vitro* study (2023) showed induction of CYP1A, CYP2B and CYP3A in rat hepatocytes. Thus, it is plausible that activation of nuclear receptors (AhR, CAR, PXR) was involved in tumorigenesis.

Two possible MoAs of thyroid tumours have been identified for proquinazid: UGT induction and deiodinase inhibition.

Conclusion on classification

According to the CLP criteria, sufficient evidence of carcinogenicity in experimental animals, corresponding to Category 1B, is available when a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species.

An increased incidence of tumours in both sexes of a single species in a well-conducted study can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.

Category 2 classification is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (CLP Regulation, Annex I, 3.6.2.2.6). Such evidence may be derived either from limited(1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

In the case of proquinazid, there are distinct and statistically significant increases in three neoplastic findings:

- Cholangiocarcinomas in female rats;
- Hepatocellular adenomas in female rats;
- Thyroid follicular cell adenomas in male rats.

The increases in liver and thyroid tumours in the mouse carcinogenicity study are rather weak and therefore are considered equivocal. On the other hand, RAC notes that the incidence of precursor effects to liver tumours was observed in both sexes of mice, and thyroid follicular cell hyperplasia was increased in top dose females. Further, precursor lesions to thyroid and liver tumours were observed in both sexes of rat. Thus, none of the three neoplastic findings is a purely one-species, one-sex phenomenon.

Out of the three tumour types, only cholangiocarcinomas are malignant. However, the concern about cholangiocarcinomas is reduced by the following factors:

- The cholangiocarcinomas in the study with proquinazid were not metastatic.
- The tumours were only observed at doses associated with marked general toxicity (body weight decrease > 20%, liver toxicity).
- The carcinogenic effect has a threshold. Proquinazid is not genotoxic, the presumed MoA involves sustained hepatotoxicity leading to oval cell hyperplasia, cholangiofibrosis and eventually cholangiocarcinoma. No increase in precursor lesions was observed at 30 and 100 ppm in the 2-year and 90-day studies respectively.

The thyroid tumours in male rats can be attributed to UGT induction and deiodinase inhibition. Although human relevance cannot be excluded, humans may be less sensitive to the development of malignant thyroid tumours from sustained stimulation by TSH compared to rats.

To aid in the weight of evidence assessment, the CLP regulation provides a list of factors increasing or decreasing the level of concern for human carcinogenicity (CLP Regulation, Annex I, 3.6.2.2.6). An overview of these factors together with relevant information on proquinazid is provided in the following table.

Factors increasing or decreasing the level of concern for human carcinogenicity	
Factor	**Evidence for proquinazid**
Tumour type and background incidence	All three tumour types occur in humans. Hepatocellular adenoma and thyroid follicular adenoma have a low background incidence in rats. Cholangiocarcinoma is rarely a spontaneous lesion in rats.
Multi-site responses	No
Progression of lesions to malignancy	Yes (cholangiocarcinoma)
Reduced tumour latency	Comparison with control is not possible due to low control incidence.
Whether responses are in single or both sexes	Significant increases in liver tumours in female rats and thyroid tumours in male rats. Precursor lesions to liver tumours observed in both sexes of both species.
Whether responses are in a single species or several species	A clear neoplastic response only in a single species (rat)
Structural similarity to a substance(s) for which there is good evidence of carcinogenicity	No

Routes of exposure	Oral route is relevant for humans.
Comparison of ADME between test animals and humans	Human information is limited to an *in vitro* study, no indication of interspecies differences in metabolic profile.
The possibility of a confounding effect of excessive toxicity at test doses	Liver tumours in rats occurred only in the presence of excessive toxicity (marked body weight reduction, hepatotoxicity). Thyroid tumours occurred at a dose without excessive toxicity.
Mode of action and its relevance for humans	Cholangiocarcinoma: Sustained hepatotoxicity leading to oval cell hyperplasia, cholangiofibrosis and cholangiocarcinoma. Non-genotoxic, threshold MoA. Relevant for humans. Hepatocellular adenomas: MoA uncertain, possible involvement of AhR, CAR and PXR activation. MoA assumed to be non-genotoxic, with a threshold. Relevant for humans. Thyroid follicular adenomas: UGT induction leads to sustained stimulation of the thyroid by TSH, resulting in hypertrophy, hyperplasia and neoplasia. Deiodinase inhibition might be involved as a second MoA. Non-genotoxic, threshold MoAs. Human relevance cannot be excluded, but humans may be less sensitive than rats to development of malignant thyroid tumours from sustained stimulation by TSH.

In a weight of evidence assessment, considering all the factors increasing and decreasing the concern as reported above , RAC is of the view that the current **classification as Carc. 2; H351 is still warranted** (as agreed by RAC in 2012 and proposed by the DS in the current CLH process).

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Reproductive toxicity of proquinazid was investigated in two 2-generation studies in rats, a prenatal developmental toxicity (PNDT) study in rats and a PNDT study in rabbits. The DS discussed decreased number of implantation sites, reduced litter size, increased pup mortality and decreased pup weight in one of the 2-generation studies. However, they did not consider these effects sufficient for classification as they occurred only in the presence of maternal toxicity (reduced maternal body weight) and were not observed in the other 2-generation study.

Further, the DS discussed increased incidences of several developmental variations seen in the PNDT studies (persistent ductus arteriosus in rats, delayed ossification in rats and rabbits) but did not consider them sufficient for classification.

In addition, a 90-day mouse study (1997) and a 1-year oral (capsule) study in dogs (2002; batch KQ926-45) were also assessed.

Therefore, the DS proposed no classification for fertility or for developmental toxicity. Effects on or via lactation were not assessed.

Comments received during consultation

A manufacturer supported no classification. They stated that the increases in variations in the PNDT studies were within the HCD and/or occurred in the presence of maternal toxicity.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

Two-generation study in rats (2002; batch KQ926-45; TG OECD 407)

Sprague-Dawley rats (30/sex/group) were fed diets containing proquinazid, batch KQ926-45, at concentrations of 0, 150, 300 and 600 ppm (top concentration equivalent to 35/44 mg/kg bw/d in m/f during P premating (NB. P is the first parental generation, can also be identified as F0)). Top dose selection was based on range-finding studies, where 3000 ppm and 1000 ppm had a marked effect on body weights. Histopathological examination in the F1 generation included, besides reproductive organs, also liver, kidneys and thyroid (these three additional organs were not examined in the P generation). Puberty onset was not investigated.

A significant effect on body weight was observed in top dose animals, particularly females, of in the main study. Body weight of females was 13% and 29% below control at the end of premating in the P and F1 generation respectively. Parental body weight was only slightly affected at 300 ppm. Histopathological examination of F1 animals revealed changes in the liver (fatty change, alteration of hepatocytes, cholangiofibrosis) and thyroid (follicular cell hypertrophy and hyperplasia).

Reproductive and offspring parameters are summarised in the table below and discussed below the table. To avoid repetition, all effects are described in the fertility section, including those related to development and lactation.

2-generation study (2002; batch KQ926-45)				
Dose (ppm)	**0**	**150**	**300**	**600**
Dose (mg/kg bw/d) – P m/f premating	0	9/11	17/21	35/44
Dose (mg/kg bw/d) – F1 m/f premating	0	12/14	24/27	52/63
P/F1				
Female body weight at the end of premating (g)	272	276	271	236* (-13%)
Female body weight LD 0 (g)	304	302	296	253* (-17%)
Female body weight LD 7 (g)	324	326	310	259* (-20%)
Female body weight LD 14 (g)	329	329	307*	257* (-22%)
Mating index (%)	100	97	93	97
Fertility index (%)	86	96	86	93
No. of implantation sites[a]	13.1	13.8	14.3	13.3
Mean litter size	12.7	13.1	13.8	12.0
Post-implantation loss (%)[a]	9.6	8.8	6.2	9.6
Viability PND 0-4 (%)	99.5	98.5	98.7	89.3*

No. of pups born alive (no. of litters)	303 (24)	338 (26)	332 (24)	316 (27)
No. of pups that died between PND 0 and 4 (no. of litters affected)	1 (1)	6 (4)	5 (4)	36 (8)
Pup weight PND 0 (g)	6.5	6.6	6.2	5.4* (-17%)
Pup weight PND 7 (g)	16.5	16.7	14.7*	11.6* (-30%)
Pup weight PND 14 (g)	33.7	34.3	29.8*	22.2* (-34%)
Pup weight PND 21 (g)	55.7	56.7	49.4*	36.7* (-34%)
F1/F2				
Female body weight at the end of premating (g)	281	288	262*	200* (-29%)
Female body weight LD 0 (g)	310	313	288*	228* (-26%)
Female body weight LD 7 (g)	333	341	309*	238* (-29%)
Female body weight LD 14 (g)	346	360	322*	244* (-30%)
Mating index (%)	100	100	100	100
Fertility index (%)	80	80	77	83
No. of implantation sites[a]	13.2	13.1	13.5	11.3*
Mean litter size	13.2	13.4	13.8	10.3*
Post-implantation loss (%)[a]	6.1	2.9	7.9	10.6
Viability PND 0-4 (%)	95.6	97.4	99.1	94.6
Pup weight PND 0 (g)	6.3	6.5	6.3	5.7* (-10%)
Pup weight PND 7 (g)	15.7	15.7	14.7	11.7* (-25%)
Pup weight PND 14 (g)	32.9	33.9	30.1*	23.2* (-29%)
Pup weight PND 21 (g)	53.6	55.6	48.3*	37.4* (-30%)

* Statistically significant difference, $p \leq 0.05$

[a] Instead of post-implantation loss the study reported 'implantation efficiency', calculated as pups born / implantation sites × 100%. Post-implantation loss = 100% − implantation efficiency. Data on the no. of implantation sites in this study are considered unreliable (see the text below), this applies also to post-implantation loss

Early postnatal mortality (PND 0-4) was increased in top dose pups of the first generation. This effect pertains to developmental toxicity. Six of the dead pups belonged to a dam (no. 597805) with an unusually high litter size (19 liveborn pups). The other 22 out of the 36 pups that died belonged to two dams suffering total litter loss (animals no. 597809 and 597825). Mean birth weights of pups in these two litters were the lowest in the group (4.3 and 3.6 g, respectively, compared to a group mean of 5.4 g), as were the maternal body weights at the beginning of lactation (211 and 205 g, respectively, compared to a group mean of 253 g). Increased postnatal mortality can thus be partly explained by maternal toxicity. No increase in postnatal mortality was observed in the second generation.

Significantly reduced litter size was observed at the top dose in the second generation (10.3 vs 13.2). Relevant historical control data (within 5 years before the current study) is limited to two studies, where litter sizes in F1 were 12.8 and 12.7. It is not clear whether this effect is related to fertility or development. The information on implantation sites, and consequently also post-implantation loss, is considered unreliable since the number of identified implantation sites was lower than the number of pups born in 31% of F1 dams. The concern about the reduced litter

size is somewhat decreased by the concurrent maternal toxicity (maternal body weight at the top dose was > 25% below control).

The third effect, reduced pup weight, may be relevant for developmental toxicity or lactation. Marked reductions were seen in the top dose group, where maternal weights were also affected. A less pronounced effect on pup weight was observed at the mid-dose. In the P/F1 litters the pup weights at 300 ppm were ca. 11% below control throughout lactation; maternal toxicity was only slight. In the F1/F2 litters the decrease in pup weight was around 8% in the presence of a significant maternal body weight reduction.

Two-generation study in rats (2003; batch KQ926-85)

The second two-generation study was conducted with a different batch of proquinazid, containing impurities generally at a lower level than the batch used in the first study (see RAC general comment). The study with batch KQ926-85 was conducted according to the current version of OECD TG 416 and investigated endocrine-sensitive parameters such as puberty onset. The top dose was the same as in the first study, 600 ppm. This dose was expected to produce systemic and reproductive toxicity without excessive mortality.

Parental body weights were unaffected or only slightly decreased. Histopathological examination revealed hepatocellular hypertrophy and thyroid follicular cell hypertrophy in both sexes of both generations.

No significant changes were observed in any of the reproductive and offspring parameters investigated, including the number of implantation sites, post-implantation loss, pup survival and pup weight. RAC notes that a higher dose of proquinazid batch KQ926-85 than 600 ppm would probably be tolerated without excessive general toxicity.

Repeated dose toxicity studies

An overview of effects on reproductive organs in repeated dose toxicity studies with proquinazid can be found in Table 2.10.2.2-1 of the CLH report (p. 223-228).

A 90-day mouse study (1997) reported ovarian atrophy from 7000 ppm, equivalent to 2270 mg/kg bw/d. Findings at such a high dose, far above the limit dose of 1000 mg/kg bw/d, are considered of low relevance for classification.

The 2-generation study with batch KQ926-45 reported decreased absolute weight of testes and ovaries in the F1 generation. Given that (1) the decreases occurred in the presence of marked effects on body weight, (2) relative weights were unaffected and (3) there were no corresponding histopathological changes, the study is not considered to provide sufficient evidence of a specific effect on reproductive organs.

A 1-year oral (capsule) study in dogs (2002; batch KQ926-45) reported increased severity but not incidence of testicular degeneration at the top dose of 180 mg/kg bw/d. Testicular weight was not affected. According to the DS, atrophy of seminiferous tubules, which commonly occurs in beagle dogs of all ages, may have been aggravated by the bodyweight depression (terminal body weight 11% below control, not statistically significant).

1-year study in dogs (2002): testicular findings				
Dose (mg/kg bw/d)	**0**	**15**	**60**	**180**
No. of animals	5	5	5	5
Terminal body weight (kg)	11.1	11.7	10.1	9.9
Body weight gain week 0-54 (kg)	2.7	3.3	1.9	1.5
Testes with epididymides weight, absolute (g)	17.6	18.7	17.5	16.9
Testes with epididymides weight, relative (%)	0.159	0.159	0.174	0.173
Testes: degeneration/atrophy of seminiferous tubules	2 (+, +)	0	1 (++)	2 (+++, ++++)
Epididymides: oligospermia/germ cell debris	2 (+, +)	0	1 (++)	2 (++++, ++++)

Severity: +, minimal; ++, mild; +++, moderate; ++++, severe

Conclusion on classification

No relevant effects related to fertility or sexual function were observed in the available 2-generation studies in rats or in the repeated dose toxicity studies in rats and mice.

Increased severity but not incidence of testicular degeneration was observed in the 1-year dog study in the presence of some but not excessive general toxicity. The evidence **is not considered sufficiently strong to warrant classification for adverse effects on sexual function and fertility**.

Adverse effects on development

PNDT study in rats (1997; batch KQ926-45; TG OECD 414)

Mated female Sprague-Dawley rats (25/group) were administered proquinazid in aqueous methyl cellulose via gavage from GD 7 to 21 at dose levels of 0, 5, 10, 30 and 60 mg/kg bw/d. The study was terminated on GD 22. All foetuses were subject to skeletal examination, approximately half of the foetuses were examined for visceral abnormalities. Top dose selection was based on a pilot study in pregnant rats, where 3 out of 8 animals had to be sacrificed *in extremis* at 200 mg/kg bw/d. Effects at 100 mg/kg bw/d included decreased food consumption, decreased body weight gain and clinical signs of toxicity (not further specified in the study report).

Maternal toxicity in the main study was mild. Developmental effects at the top dose included decreased foetal weight (by 10%), retarded ossification of sternebrae and increased incidence of patent ductus arteriosus, classified in the study report as a variation due to retarded development. The increase was statistically significant and above the HCD range (7 studies, maximum 2 foetuses in 2 litters). Ductus arteriosus is a small vessel connecting the foetal pulmonary artery to the aorta. It allows part of the oxygenated blood to bypass the developing lungs *in utero*. Failure of its postnatal closure has adverse consequences. However, toxicological significance of increased incidence of patent ductus arteriosus *in utero* is unclear. RAC agrees with the DS that this finding does not warrant classification.

PNDT study in rats (1997)					
Dose (mg/kg bw/d)	**0**	**5**	**10**	**30**	**60**
Mated females	25	25	25	25	25
Pregnant females	23	24	21	24	25
No. of litters	23	24	21	24	25
Food consumption GD 7-22 (g/day)	24.4	24.0	24.5	24.4	22.8*
Body weight GD 22 minus uterus weight (g)	322	321	322	325	304
Clinical signs: piloerection	0	0	0	0	2*
Mean litter size	14.7	14.8	14.5	15.0	15.4
Resorptions	0.4	0.7	0.6	0.9	0.4
Foetal weight (g)	5.18	5.03	5.05	5.10	4.65*
Visceral examination: no. of foetuses	176	184	157	184	196
Patent ductus arteriosus: foetuses (litters)	0	1 (1)	1 (1)	1 (1)	13 (5)*
Skeletal examination: no. of foetuses	339	356	304	359	385
Sternebra – retarded ossification: foetuses (litters)	2 (2)	11 (8)	25 (4)	4 (4)	28 (13)*

* Statistically significant difference from control, $p \leq 0.05$

PNDT study in rabbits (1998; batch KQ926-45; TG OECD 414)

Mated female NZW rabbits (22/group) were administered proquinazid in aqueous methyl cellulose via gavage from GD 7 to 28 at dose levels of 0, 1, 2.5, 5 and 10 mg/kg bw/d. The study was terminated on GD 29. All foetuses were examined for external, visceral and skeletal anomalies. Top dose selection was based on a range-finding study, showing significantly reduced maternal body weight and food consumption at 10 and 20 mg/kg bw/d (no further details provided).

Final body weight corrected for uterus weight was not affected. Transient marked decreases in food consumption were observed in several females, particularly at the top dose. Two top dose females aborted after prolonged periods of markedly reduced food intake. Female no. 32531 aborted on day 26, average food intake over GD 11-25 was 36 g/day compared to a group mean of 137 g/day. Female no. 32536 aborted on day 20, average food intake over GD 13-19 was only 5 g/day.

Mean foetal body weight was decreased by 11% and 9% at 10 and 5 mg/kg bw/d respectively. Skeletal examination revealed a few foetuses with delayed pelvic ossification. The incidence at the top dose (6 foetuses, 2 litters) was still well within the HCD range (9 studies, maximum foetal incidence 8, maximum litter incidence 4).

Two-generation studies in rats

The first two-generation study (batch KQ926-45) showed increased early postnatal mortality in the first generation, partly attributed to maternal toxicity. No effect on postnatal survival was observed in the second generation, nor in the subsequent two-generation study with a different batch (KQ926-85).

Further, the two-generation study with batch KQ926-45 reported reduced litter size in the second generation. This effect was associated with significant maternal toxicity (maternal body weight 26-29% below control). No such effect was seen in the other study, where the substance (batch KQ926-85) did not induce any significant maternal or developmental toxicity.

Similarly, reduced pup weight in both generations of the 2-generation study with KQ926-45 can be partly attributed to concurrent maternal toxicity.

Conclusion on classification

The rat PNDT study reported increased incidence of patent ductus arteriosus. As this finding was observed *in utero* and in connection with reduced foetal weight, it is considered to represent a developmental delay rather than a malformation.

The two-generation study with batch KQ926-45 (representing the old manufacturing process) reported increased early postnatal mortality in the first generation, reduced litter size in the second generation, and markedly reduced pup weight in both generations. Although involvement of a specific reprotoxic mechanism cannot be excluded, the significant concurrent maternal toxicity decreases the concern. None of these effects was observed in the two-generation study with batch KQ926-85 (representing the current manufacturing process).

RAC agrees with the DS that **classification for developmental toxicity is not warranted**.

Effects on or via lactation

Classification for effects on or via lactation was not assessed by the DS due to lack of data. According to the CLP criteria, classification can be assigned based on results of one- or two-generation studies in animals providing clear evidence of adverse effects in the offspring due to transfer in the milk or adverse effect on the quality of the milk.

As described above, proquinazid batch KQ926-45 caused a marked retardation in pup body weight development in the 2-generation study. Body weights of top dose (600 ppm) first generation pups were 17%, 30% and 34% below control on PND 0, PND 7 and PND 14 respectively. Maternal body weights at these time points were 17%, 20% and 23% below control respectively. Even stronger effects on body weight were observed in the second generation (PND 7: pup weight 25% below control, maternal body weight 29% below control). Batch KQ926-85 did not induce any marked toxicity in the dams nor in the offspring at the same dose level. Thus, the effects on pup weight in the former study appear to be at least partly related to maternal toxicity.

RAC **proposes no classification for effects on or via lactation**.

Overall conclusion on reproductive toxicity

RAC agrees with the DS's proposal that proquinazid warrants **no classification for reproductive toxicity,** for effects on sexual function and fertility or for developmental toxicity. RAC further concludes that **no classification for effects on or via lactation** is warranted.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

Proquinazid is a solid with a melting point of 62 °C. Therefore, it does not meet the classification criteria in terms of viscosity. Accordingly, the DS proposed no classification.

Comments received during consultation

A manufacturer supported no classification.

Assessment and comparison with the classification criteria

The substance is a solid. RAC agrees with the DS's proposal that proquinazid warrants **no classification for aspiration toxicity**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) reported evidence that proquinazid is not rapidly degradable and has a high potential to bioaccumulate in aquatic species. Based on the available information, the DS proposed to retain the current Annex VI entry for aquatic hazard as Aquatic Acute 1 (H400), with an M factor of 1, and Aquatic Chronic 1 (H410) with an M factor of 10.

Environmental degradation

Hydrolysis

The hydrolysis of [phenyl-^{14}C(U)]proquinazid was investigated according to OECD TG 111 at pH 4, 7 and 9 and 20 ± 1 °C. The substance, which was tested at nominal 0.20 mg/L, remained stable at all pH levels (89-97% AR at 30 days); no known metabolite was detected.

Four supportive OECD TG 111 studies assessing the hydrolysis of the metabolites IN-MM671, IN-MM986, IN-MM991 and IN-MT884 were presented. All tested substances were hydrolytically stable at pH 4, 7 and 9 after 30 days.

Although the required tier I assessment at 50 °C was not performed in all presented hydrolysis studies, the DS considered these acceptable as (almost) no hydrolysis was shown at 30 days.

Photolysis

The photochemical degradation of [phenyl-^{14}C(U)]proquinazid was assessed at nominal 0.20 mg/L (pH 7; 20 ± 1 °C) under continuous irradiation with artificial sunlight (filtered xenon lamp). The substance showed a photochemical DT_{50} < 1h, with mineralisation reaching 21% AR after 15 days. Four metabolites were detected and assessed for photodegradation during the study: IN-MM671 (DT_{50} = 5 days), IN-MM986 (DT_{50} = 11 days), IN-MT884 (DT_{50} = 39 days) and IN-MM986 (DT_{50} not calculated due to unacceptable kinetic fitting).

Theoretical lifetimes of proquinazid and its metabolites were also calculated using the software GCSolar (summer conditions; 40° latitude). Results showed a half-life of 0.03 days for proquinazid, 16.1 days for IN-MM671, 32.8 days for IN-MM986, 132 days for IN-MT884 and 12.7 days for IN-MM991, the latter being very uncertain due to unacceptable photolysis rate.

The DS stated that based on the available information, proquinazid is highly susceptible to photodegradation; however, due to its relatively quick adsorption to organic matter, it may not be present in the upper water column for a sufficient time for photolysis to play a role in its degradation in surface water.

Ready Biodegradability

A study carried according to OECD TG 301B (CO_2 evolution; modified Sturm test) was performed by incubating 22 mg/L proquinazid (10 mg [carbon]C/L) with activated sludge (30 mg solids/L) for 29 days. No significant CO_2 evolution was observed in the Proquinazid-treated vessels (cumulative CO_2 production = 1% TCO_2). The degradation of the reference substance sodium benzoate was rapid, with 68% TCO_2 after 7 days and 91% after 29 days. No inhibitory effect of proquinazid was noted in the inhibition controls (containing a 1:1 mixture of sodium benzoate and proquinazid). According to the results, proquinazid is considered as not readily biodegradable.

Aerobic degradation

The mineralisation of [phenyl-^{14}C(U)]proquinazid was investigated in an OECD TG 309 simulation study using surface water from Tuckahoe Lake (Maryland, US). The substance was tested in bioreactors (250 mL Erlenmeyer flasks) at nominal 26 and 256 µg/L in the dark at 20 ± 2 °C day 14. The mineralisation of the test substance was very low, with max 0.07% AR detected as $^{14}CO_2$ after 60 days at the highest concentration. A maximum of 5.2 % of unidentified radioactivity was recorded throughout the test, most of which (4.9%) was confirmed to be present as IN-MM671 via HPLC-UV.

In a second study, the metabolism and fate of [phenyl-^{14}C(U)]proquinazid in water/sediment systems was assessed according to US-EPA 162-4 using two different matrices: one from a stream (Red oak stream, Middletown, US) and one from a pond (Town Park pond, Middletown, US). Experimental vessels containing sediment and water at a 1 to 4 ratio (w/v) were dosed with 0.1 mg/L test substance and sampled for analyses at 0, 3, 7, 15, 30, 60, and 100 days. A rapid migration of proquinazid into sediments occurred in both stream and pond system, with single first order (SFO) $DisTs_{50}$ in the water phase being 0.6 and 0.5 days, respectively. Conversely, the substance dissipated slowly in sediments, where $DisT_{50}$ values were 97.1 days for pond and 185 days for stream. Overall, a faster degradation from the total system occurred in pond (SFO $Degt_{50}$ = 35.5 days) compared to stream (SFO $Degt_{50}$ = 131 days). The major metabolite IN-MM671 reached a maximum of 28% AR (at day 60) and 71% AR (at day 100) for the stream and pond systems, respectively.

The mineralisation to $^{14}CO_2$ was low, with maximum levels of 1.4% measured at day 100.

Conclusion on the rapidly degradable of proquinazid

The DS concluded that proquinazid **is not rapidly degradable** based on the available information, notably:

- proquinazid was not readily biodegradable in a 28-day ready biodegradability test according to OECD TG 301B;
- half-lives measured in surface water simulation test were longer than 16 days and ultimate degradation did not reach levels > 70% in 28 days;
- ultimate (aerobic) degradation in water/sediments test did not reach levels > 70% in 28 days;
- proquinazid was hydrolytically stable at environmentally realistic temperatures and pH values;
- under favourable photolytic conditions, half-lives for primary degradation were shorter than 16 days, but ultimate degradation did not reach levels > 70% in 28 days.

Bioaccumulation

The DS presented data from a suite of experimental studies addressing the measurement of the partition coefficient n-octanol/water (Log P_{ow}) and bioconcentration factor (BCF) of proquinazid and its metabolites.

Data from an OECD TG 107 shake-flask test showed that Log P_{ow} of proquinazid at 25 °C is 5.5, (i.e., greater than the cut-off level of 4), indicating a high potential for bioaccumulation. Proquinazid was tested for bioaccumulation in the bluegill *Lepomis macrochirus* according to OECD TG 305, resulting in a steady state, whole fish wet weight, lipid normalised and growth corrected bioconcentration factor (BCF) of 813 and a clearance time of 5.8 days. IN-MM671 was the only metabolite showing a Log P_{ow} > 4 (4.05 at 20 °C according to OECD TG 107), and a moderate bioaccumulation in fish (BCF = 439 observed in both *in vitro* and *in vivo* systems).

The DS concluded that since the measured BCF for proquinazid (813) exceeds the trigger of 500 for bioaccumulation, **proquinazid is expected to bioaccumulate in fish**.

Aquatic toxicity

Acute aquatic toxicity

The relevant acute aquatic toxicity data presented by the DS are displayed in the table below.

Method/Species	**Test material**	**Results**	**Remarks**
Fish (for reference: Vol. 3CA, section B.9.2.1)			
OECD TG 203 *Oncorhynchus mykiss*	Proquinazid	96 h LC_{50} = 0.349 mg/L	Flow through; mean measured
OECD TG 203 *Lepomis macrochirus*	Proquinazid	96 h LC_{50} = 0.454 mg/L	Flow through; mean measured
OECD TG 203 *Cyprinodon variegatus*	Proquinazid	96 h LC_{50} > 0.58 mg/L	Flow through; mean measured
OECD TG 203 *Oncorhynchus mykiss*	IN-MM671	96 h LC_{50} = 2.2 mg/L	Static; mean measured
OECD TG 203 *Lepomis macrochirus*	IN-MM671	96 h LC_{50} = 4.2 mg/L	Static; mean measured
OECD TG 203 *Oncorhynchus mykiss*	IN-MM986	96 h LC_{50} ≥ 1.03 mg/L	Static; mean measured
OECD TG 203 *Oncorhynchus mykiss*	IN-MM991	96 h LC_{50} = 28.4 mg/L	Static; mean measured
OECD TG 203 *Oncorhynchus mykiss*	Proquinazid 200 g/L EC	96 h LC_{50} = 2.3 mg f.p./L (0.446 mg a.s./L)	Supportive study; static; nominal
Aquatic invertebrates (for reference: Vol. 3CA, section B.9.2.4)			
OECD TG 202 *Daphnia magna*	Proquinazid	48 h EC_{50} = 0.287 mg/L	Flow through; mean measured
US EPA 72-3 *Crassostrea Virginica*	Proquinazid	96 h EC_{50} = 0.219 mg/L	Flow through; mean measured
US EPA 72-4; OCSPP 850.1035 *Mysidopsis bahia*	Proquinazid	96 h EC_{50} = 0.110 mg/L	Flow through; mean measured
OECD TG 202 *Daphnia magna*	IN-MM671	48 h EC_{50} = 5.4 mg/L	Static; mean measured

OECD TG 202 *Daphnia magna*	IN-MM986	48 h EC_{50} ≥ 0.791 mg/L	Static; mean measured
OECD TG 203 *Oncorhynchus mykiss*	IN-MM991	48 h EC_{50} ≥ 45.5 mg/L	Static; mean measured
OECD TG 202 *Daphnia magna*	IN-MT884	48 h EC_{50} ≥ 114 mg/L	Static; mean measured
OECD TG 202 *Daphnia magna*	Proquinazid 200 g/L EC	48 h EC_{50} = 1.8 mg f.p./L (0.349 mg a.s./L)	Supportive study; static; nominal
Algae and aquatic plants (for reference: Vol. 3CA, sections B.9.2.6 and B.9.2.7)			
OECD TG 201 *Pseudokirchneriella subcapitata*	Proquinazid	72 h Er/bC_{50} > 0.12 mg a.s./L	Static; mean measured
OECD TG 201 *Selenastrum capricornutum*	IN-MM671	72 h Er/bC_{50} > 0.5 mg/L	Static; mean measured
OECD TG 201 *Pseudokirchneriella subcapitata*	Proquinazid 200 g/L EC	72 h EbC_{50} = 1.4 mg f.p./L (0.28 mg a.s./L); EC_{50} = 1.3 mg f.p./L (0.28 mg a.s./L); ErC_{50} = 2.5 mg f.p./L (0.5 mg a.s./L)	Supportive study; static; nominal

Short-term toxicity data on proquinazid are available for the three trophic levels (fish, invertebrates and algae). Effects of metabolites and formulated products were also presented, indicating similar or lower toxicity compared to the active ingredient. The acute toxicity of proquinazid to fish (*O. mykiss*; 96 h LC_{50} = 0.349 mg/L), invertebrates (*M. bahia*; 96 h EC_{50} = 0.110 mg/L) and algae (*P. subcapitata*; 72 h Er/bC_{50} > 0.12 mg/L) fulfils the criteria for classification as **Aquatic Acute 1 (H400) with an M-factor of 1** (L/EC_{50} between 0.1 and 1 mg/L).

Chronic aquatic toxicity

The relevant chronic aquatic toxicity data presented by the DS are displayed in the table below.

Method/Species	**Test material**	**Results**	**Remarks**
Fish (for reference: Vol. 3CA, section B.9.2.2)			
OECD TG 210 *Oncorhynchus mykiss*	Proquinazid	90 d NOEC = 0.0030 mg/L EC_{10} = 0.0074 mg/L	Flow through; mean measured
US EPA 72- *Cyprinodon variegatus*	Proquinazid	36 d NOEC = 0.00872 mg/L EC_{10} = 0.0093 mg/L	Flow through; mean measured
Aquatic invertebrates (for reference: Vol. 3CA, section B.9.2.5)			
OECD TG 211 *Daphnia magna*	Proquinazid	21 d NOEC = 0.0018 mg/L	Semi-static; mean measured
US EPA 72-4 *Mysidopsis bahia*	Proquinazid	28 d NOEC = 0.0105 mg/L	Flow through; mean measured

OECD TG 219 *Chironomus riparius*	Proquinazid	28 d NOEC = 0.456 mg/L	Semi-static; mean measured
OECD TG 211 *Daphnia magna*	IN-MM671	21 d NOEC = 0.519 mg/L EC_{10} = 0.939 mg/L	Semi-static; mean measured
Algae and aquatic plants (for reference: Vol. 3CA, section B.9.2.6 and B.9.2.7)			
OECD TG 201 *Pseudokirchneriella subcapitata*	Proquinazid	72 h EC_{10} > 0.12 mg/L	Static; mean measured

Long-term toxicity data on proquinazid are available for the three trophic levels (fish, invertebrates and algae). Considering that the lowest long-term toxicity value in aquatic species was a 21-day NOEC of 0.0018 mg/L derived in an aquatic invertebrate (*D. magna*), the DS concluded that proquinazid fulfils the criteria for classification as **Aquatic Chronic 1 (H410), with an M-factor of 10** (NOEC/EC_{10} values between 0.01 and 0.001 mg/L for a not rapidly degradable substance).

Comments received during consultation

One MSCA and one stakeholder commented the (ENV) classification proposal for proquinazid during public consultation.

The MS expressed general agreement with the proposed aquatic hazard classification and the calculated M-factors.

The stakeholder agreed with the proposed acute aquatic hazard classification but reported that according to a new irradiated water-sediment study on proquinazid (ID: 220042) the rate of degradation in water and sediment is significantly reduced under sunlight (DT_{50} in sediment approximate of 5 days and total system of < 1 day) and this may have an impact on BCF values, as overall exposure may be significantly reduced under realistic conditions. The stakeholder also generated two new aquatic organisms' studies (ID: 211160 and 211087) which provide additional information (confirms and/or improves the current end points) for the classification of aquatic hazard.

Detailed reports of the three studies are provided for the consideration of RAC and RMS/EFSA upon request.

The three studies have been requested and assessed by RAC, which considers that the newly submitted information does not affect the current classification proposal. A detailed RAC response to the stakeholder's comment is reported in RCOM (comment nr. 29).

Additional key elements

A summary of the aerobic degradation and aquatic toxicity studies provided during public consultation is reported below.

Aerobic degradation in irradiated water/sediment systems

In an OECD TG 308 study (ID 220042, 2023), the aerobic (bio)transformation of [^{14}C]Proquinazid was assessed in two irradiated water/sediment systems from USA (Taunton and Wewantic river systems). The experiment was conducted for 16 days at a sediment/water ratio of 1/3 (w/v),

under artificial sunlight (Atlas Suntest Unit, 290 to 800 nm, 700 W/m^2) and at a temperature of 20 ºC; [^{14}C]Proquinazid was initially spiked at 0.20 mg a.i./L and samples analyzed at 0, 1, 3, 5, 7, 12, and 16 days of incubation. Actual test concentration was analytically verified in pre- and post-dose solutions by LSC with measured values being 0.203 and 0.201 mg/L, respectively, thus confirming nominal levels.

The concentration of parent [^{14}C]Proquinazid in water rapidly decreased from 71.0% (Taunton) and 69.1% (Wewantic) at day 0, to 3.9% (Taunton) and < 1 % (Wewantic) at day 1. The concentration of parent [^{14}C]Proquinazid in the sediment started at 19.9% (Taunton) and 28.7 (Wewantic) at day 0 and decreased to 7.3% (Taunton) and 6.1% (Wewantic) at the end of the study period. At day 16, the percentages of applied radioactivity (AR) remained in the water layer were 5.9 % and 26.0 for Taunton and Wewantic systems, while partitioning from the water layer to the sediment accounted for 79.9% and 43.3% AR, respectively.

Proquinazid showed a total system DT_{50} ranging from 0.458 to 0.533 days in the two aerobic water-sediment test systems. Due to the rapid partitioning to the sediments, it was not possible to calculate DT_{50} in water from the two systems, while DT_{50} values of 7.89 and 1.43 days were measured for Taunton and Weweantic sediments, respectively.

The major transformation products IN-MM671 was detected in sediments, with maximum concentrations of 16.6% in Taunton (day 16) and 9.9% in Weweantic system (day 1). The minor transformation products IN-MT884 and IN-MM991 and the unidentified ^{14}C were individually detected below 5% in both systems.

At day 16, the amount of mineralisation to $^{14}CO_2$ in Taunton and Wewantic systems was 23.1% and 29.9% AR, respectively.

Aquatic Chronic toxicity studies

A 21-day *Daphnia magna* life cycle toxicity test was performed according to OECD TG 211 (ID 211087, 2021). Organisms were exposed to nominal 1.8, 3.6, 7.3, 15 and 29 µg Proquinazid per litre in flow-through conditions at a target T° of 20 ± 1 °C, pH range of 7.9-8.2 and mean oxygen concentration ≥ 6.3 mg/L. Test concentrations were analytically verified prior the test (days -1 and -2), at test initiation (day 0) and at regular intervals (weekly) thereafter. Since test concentrations were not maintained within ± 20% of nominal concentrations, the results of the study were based on the time weighted mean measured concentrations, namely 1.5, 3.1, 6.1, 13 and 25 µg a.s./L. A NOEC of 13 µg/L and an IC_{10} of 19 µg/L was determined for the survival of first generation daphnids. For both reproduction and growth, a NOEC of 25 µg/L was reported, while IC_{10} values were determined to be greater than the highest tested concentration.

A Prolonged Sediment Toxicity Test was performed according to OECD TG 225 to investigate the long-term effects of proquinazid in the oligochaete *Lumbriculus variegatus*. Adult organisms were exposed for 28 days to spiked sediments at mean measured concentrations of 0.41 to 106 mg a.s./kg at a target T° of 20 ± 2 °C, pH 8.4-8.8 and dissolved oxygen concentrations ≥ 83% (7.6 mg/L) of air saturation. A 28-day EC_{10} of 44 mg a.s./kg was derived for survival, while NOEC value for both survival and total dry weight was 35 mg a.s./kg. All validity criteria according to OECD TG 225 were fulfilled.

RAC highlights that data obtained using spiked sediments for *Lumbriculus variegatus* are not acceptable for classification purpose as exposure via the water column is required.

Assessment and comparison with the classification criteria

Degradation

Relevant information regarding the environmental degradation of proquinazid and its metabolites has been presented by the DS through an array of data on hydrolysis, photolysis, ready biodegradability and aerobic degradation.

Considering all the available evidence, RAC agrees with the DS's proposal to consider proquinazid as **not rapidly degradable:**

- Proquinazid was stable to hydrolysis at pH 4, 7 and 9, and did not ultimately degrade in photodegradation studies at levels > 70% in 28 days.
- Proquinazid was not readily biodegradable according to test guideline OECD TG 301B.
- Ultimate degradation did not reach levels > 70% in 28 days (nor led to half-lives < 16 days) in simulated surface water (OECD TG 309) and water/sediments (OECD TG 308) systems.

Bioaccumulation

RAC agrees with the DS that proquinazid has a **high potential for bioaccumulation** in aquatic organisms:

- The measured BCF of 813 in fish (*L. macrochirus*) exceeds the CLP criterion of 500.
- The measured Log K_{ow} of 5.5 is greater than the cut-off value of 4.

Aquatic toxicity

Acute toxicity

Reliable acute toxicity data on proquinazid are available for the three trophic levels (fish, invertebrates and algae). The most stringent short-term toxicity outcome was obtained in the Mysid shrimp *Mysidopsis bahia* (EPA guideline OCSPP 850.1035*),* with a 96 h EC_{50} = 0.110 mg/L (95% C.I. = 0.088-0.17 mg/L). This study is compliant with GLP and shows no deviations from test guideline OCSPP 850.1035 validity criteria. Based on the key (EC_{50}) value of 0.110 mg/L, and in line with the DS proposal, RAC is of the opinion that proquinazid **warrants classification as Aquatic Acute 1 (H400) with an M-factor of 1** (0.1 < EC_{50} ≤ 1 mg/L).

Chronic toxicity

Reliable chronic toxicity data were available for the three trophic levels (fish, invertebrates and algae). The lowest long-term effect value for proquinazid was a 21-day NOEC of 0.0018 mg/L derived in the cladoceran *D. magna*. This value was obtained in an OECD TG 211 test performed in compliance with GLP; all validity criteria according to test guideline were fulfilled.

Overall, RAC agrees with the DS that proquinazid **warrants classification for Aquatic Chronic 1 (H410), with an M-factor of 10** (0.001 < NOEC/EC_{10} ≤ 0.01 mg/L) based on the key (NOEC) value of 0.0018 mg/L.

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

There were no specific data available on the potential hazard to the ozone layer. However, proquinazid is non-volatile (vapour pressure: 9×10^{-5} mm Pa at 25 °C) and it is not included in Annex I or Annex II to Regulation (EC) 1005/2009.

Based on the above considerations, the DS concludes that Proquinazid does not fulfil classification criteria for 'hazardous to the ozone layer'.

Comments received during consultation

One MSCA and one stakeholder commented the CLH proposal for this hazard class

The MS generically commented that this hazard class "Was not reviewed". The stakeholder expressed general agreement with the DS proposal of no classification.

Assessment and comparison with the classification criteria

RAC agrees with the DS that since proquinazid is non-volatile (vapour pressure: 9×10^{-5} mm Pa at 25 °C) and is unlikely to imply a hazard to the stratospheric ozone. RAC notes that it is not included in Annex I or Annex II to Regulation (EC) 1005/2009, although this is not itself a classification criterion.

RAC concludes that proquinazid does not warrant classification as 'hazardous to the ozone layer'.

Additional references

EU Specialised Experts (1999) Summary record. Commission Group of Specialised Experts in the fields of Carcinogenicity, Mutagenicity and Reprotoxicity. Meeting at Arona, 1-2 September 1999. ECBI/49/99 – Add. 1 Rev. 2. 17.12.1999

IARC (1999) Species differences in thyroid, kidney and urinary bladder carcinogenesis. IARC Scientific publications no. 147

RAC (2012) Opinion proposing harmonised classification and labelling at EU level of Proquinazid. ECHA/RAC/CLH O-0000002607-72-01/F. Adopted 9 March 2012

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter and additional information (if applicable).

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).

Appendix

Supplemental information – in depth analysis by RAC on the following endpoints

RAC evaluation of skin corrosion/irritation

The dataset also contains a 28-day dermal study in rats (2002; batch KQ926-45; TG OECD 410). In this study, proquinazid in water was applied to the skin of 10 animals per sex and group at dose levels of 0, 100, 500, 1000 and 2000 mg/kg bw/d. The incidence of mild erythema and desquamation at the test site was significantly increased from 1000 mg/kg bw/d, see the table below. Thus, the supplemental hazard statement EUH066 ('Repeated exposure may cause sky dryness or cracking') could be considered. However, RAC concludes that the severity of skin irritation is not sufficient to warrant EUH066.

28-day dermal study in rats: irritation at the test site					
Dose (mg/kg bw/d)	**0**	**100**	**500**	**1000**	**2000**
Males					
No. of animals	10	10	10	10	10
Desquamation	2	3	6	8*	8*
Mild erythema	2	2	3	7*	7*
Females					
No. of animals	10	10	10	10	10
Desquamation	1	0	1	5*	5*
Mild erythema	5	5	6	6	5

* Statistically significant difference from control, $p \leq 0.05$

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

In vitro studies on thyroid-related MoAs

In vitro thyroid receptor activity (2021)

The study used a commercial test kit from INDIGO Bioscience for detection of agonists of human thyroid hormone receptor alpha (TRα) or beta (TRβ). T3 was used as positive control. The substance was considered positive if the maximum response was ≥ 10% of the response of the positive control at non-cytotoxic concentrations (cell viability > 80%).

The maximum concentration of proquinazid of 1×10^{-5} M was selected on the basis of solubility. However, in some experiments the top concentrations evaluated were lower due to cytotoxicity. The maximum response of proquinazid was 0.3% of positive control. Thus, the study was considered negative.

In vitro TPO inhibition (2021)

The study was conducted with F344 rat thyroid microsomes using the Amplex UtraRed assay. Propylthiouracil (PTU) was used as positive control.

The top concentration of 2.5×10^{-6} M was chosen based on solubility. Proquinazid did not inhibit TPO at any concentration. IC50 for PTU was between 1.8×10^{-6} and 6.1×10^{-6} M.

In vitro NIS inhibition (2021)

The assay was based on Sandell-Kolthoff reaction and used FRTL5 cells (a rat cell line). Potassium perchlorate was used as positive control.

The top concentration of proquinazid was 10^{-6} M. No cytotoxicity was observed. Proquinazid did not inhibit NIS at any concentration. The positive control gave the expected response.

Liver- and thyroid-related findings in rat studies

90-day subchronic toxicity and neurotoxicity study in rats (2003; batch KQ926-45)

Sprague-Dawley rats (22/sex/group) were administered proquinazid, batch KQ926-45, at dietary concentrations up to 2000 ppm in males and 600 ppm in females. Within each group, 10 rats were designated as the subchronic toxicity subgroup, and 12 rats were designated as the neurotoxicity subgroup. Information on liver- and thyroid-related parameters in animals from the subchronic toxicity subgroup (10/sex) is provided in the table below.

Hepatic UGT activity was measured spectrophotometrically using *p*-nitrophenol as the substrate. 5'-deiodinase activity was measured using $^{[125]}$I-rT_3 as the substrate. The release of $^{[125]}$I, following the conversion of $^{[125]}$I-rT_3 to T_2 by 5'-deiodinase type I, was measured by a gamma counter.

90-day study in rats (2003; batch KQ926-45): liver- and thyroid-related parameters					
Males					
Dose (ppm)	**0**	**30**	**100**	**300**	**2000**
Dose (mg/kg bw/d)	0	1.9	6.2	19	135
Terminal body weight (g)	500	494	490	500	315*
Liver weight, absolute (g)	13.4	14.0	14.3	14.8	10.4*

Liver weight, relative (%)	2.7	2.8	2.9*	3.0*	3.3*
Thyroid weight, absolute (mg)	30	32	35	38	28
No. of animals for histopathological examination	10	10	10	10	10
Liver: alteration of periportal hepatocytes	0	0	0	3 (3 +)	10 (9 +, 1 ++)
Liver: fatty change, midzonal	0	0	0	1 (1 +)	7 (4 +, 2 ++, 1 +++)
Liver: oval cell hyperplasia	0	0	0	0	2
Liver: increased pigment in Kupffer cells	0	0	0	0	2
Thyroid: follicular cell hypertrophy	0	5	8	7	7
Cholesterol (mg/dl), day 90	68	82	68	89*	111*
T4 (µg/dl), 2 weeks	4.3	4.3	4.2	4.6	3.2
T3 (ng/dl), 2 weeks	71	79	73	77	73
rT3 (ng/ml), 2 weeks	0.13	0.13	0.15*	0.17*	0.20*
TSH (ng/ml), 2 weeks	8.2	10.8*	10.9*	11.9*	14.2*
T4 (µg/dl), day 90	4.3	4.6	4.7	4.7	2.3*
T3 (ng/dl), day 90	62	62	58	52*	56*
rT3 (ng/ml), day 90	0.073	0.059	0.083	0.076	0.089*
TSH (ng/ml), day 90	8.7	10.0	11.5*	12.0*	15.2*
Hepatic 5'-deiodinase activity (nmol/hr/mg protein)[a]	5.4	5.2	5.8	5.2	3.2*
Hepatic glucuronyltransferase activity (nmol/min/mg protein)[a]	34	33	46*	55*	134*
Females					
Dose (ppm)	**0**	**30**	**100**	**300**	**600**
Dose (mg/kg bw/d)	0	2.3	7.8	23	50
Terminal body weight (g)	271	267	251	251	206*
Liver weight, absolute (g)	7.7	8.0	8.0	8.0	7.0
Liver weight, relative (%)	2.8	3.0	3.2*	3.2	3.4*
Thyroid weight, absolute (mg)	24	26	24	27	28
No. of animals for histopathological examination	10	10	10	10	10
Liver: alteration of periportal hepatocytes	0	0	0	0	6 (2 +, 3 ++, 1 +++)
Liver: fatty change, midzonal	0	0	0	0	5 (3 +, 2 ++)
Liver: oval cell hyperplasia	0	0	0	0	2

Liver: bile duct hyperplasia	0	0	0	1	7
Thyroid: follicular cell hypertrophy	0	0	0	4	2
T4 (µg/dl), 2 weeks	2.4	3.3	2.4	2.6	1.5*
T3 (ng/dl), 2 weeks	81	72	62*	62*	66*
rT3 (ng/ml), 2 weeks	0.10	0.12	0.11	0.15*	0.13*
TSH (ng/ml), 2 weeks	5.8	6.0	6.0	7.0*	6.6*
T4 (µg/dl), day 90	2.4	2.9	2.6	2.6	1.3*
T3 (ng/dl), day 90	90	78	69*	74*	56*
rT3 (ng/ml), day 90	0.088	0.085	0.092	0.108	0.083
TSH (ng/ml), day 90	6.4	6.6	7.0	7.6	9.1*
Hepatic 5'-deiodinase activity (nmol/hr/mg protein)[a]	5.5	4.5	5.0	4.6*	3.2*
Hepatic glucuronyltransferase activity (nmol/min/mg protein)[a]	29	25	36	57*	76*

* Statistically significant difference from control, $p \leq 0.05$. Histopathological findings were not subject to statistical analysis

[a] n = 5/group

Lesion grades: +, minimal; ++, mild; +++, moderate; ++++, severe

Alteration of periportal hepatocytes is described in the study report as follows: "Alteration of periportal hepatocytes was characterised by disorganisation of hepatocytes such that hepatic cord architecture was generally lost due to individualisation of cells. Affected hepatocytes had abundant eosinophilic cytoplasm and moderately enlarged nuclei. This lesion was considered test substance-related and also adverse."

<u>90-day study in rats (2002; batch KQ926-75)</u>

Sprague-Dawley rats (10/sex/group) were administered proquinazid, batch KQ926-75, at dietary concentrations up to 2000 ppm in males and 600 ppm in females. The study was performed in the same laboratory as the 90-day study (2003) with batch KQ926-45. Information on liver- and thyroid-related parameters is provided in the following table.

90-day study in rats (2002; batch KQ926-75): liver- and thyroid-related parameters					
Males					
Dose (ppm)	**0**	**30**	**100**	**300**	**2000**
Dose (mg/kg bw/d)	0	2.0	6.3	19	127
Terminal body weight (g)	525	520	558	520	500
Liver weight, absolute (g)	18.0	17.6	20.2*	19.3	21.9*
Liver weight, relative (%)	3.4	3.4	3.6	3.7*	4.4*
Thyroid weight, absolute (mg)	25	26	28	27	33*
No. of animals for histopathological examination	10	10	10	10	10
Liver: hepatocyte hypertrophy, centrilobular	0	0	0	0	6

Thyroid: follicular cell hypertrophy	0	0	7	9	10
Cholesterol (mg/dl), day 92	66	75	77	76	95*
T4 (µg/dl), 2 weeks	4.9	4.8	4.8	4.7	4.2*
T3 (ng/dl), 2 weeks	82	82	72	72	55*
rT3 (ng/ml), 2 weeks	0.12	0.12	0.16	0.12	0.16*
TSH (ng/ml), 2 weeks	8.1	9.1	11.2*	13.8*	18.6*
T4 (µg/dl), day 90	4.6	5.1	5.3	5.2	5.8*
T3 (ng/dl), day 90	80	81	78	72	62*
rT3 (ng/ml), day 90	0.13	0.16	0.18	0.16	0.19*
TSH (ng/ml), day 90	7.7	9.0	8.6	14.0*	11.1*
Hepatic 5'-deiodinase activity (nmol/hr/mg protein)[a]	7.0	5.7	6.8	5.6	3.3*
Hepatic glucuronyltransferase activity (nmol/min/mg protein)[a]	52	62	46	64	126*
Females					
Dose (ppm)	**0**	**30**	**100**	**300**	**600**
Dose (mg/kg bw/d)	0	2.4	8.0	24	50
Terminal body weight (g)	292	307	296	307	267
Liver weight, absolute (g)	9.9	10.5	10.0	11.1	10.6
Liver weight, relative (%)	3.4	3.4	3.4	3.6	4.0*
Thyroid weight, absolute (mg)	23	21	21	23	24
No. of animals for histopathological examination	10	10	9	10	10
Thyroid: follicular cell hypertrophy	0	0	0	0	7
T4 (µg/dl), 2 weeks	3.9	3.7	3.8	4.6	4.5
T3 (ng/dl), 2 weeks	82	82	82	80	86
rT3 (ng/ml), 2 weeks	0.094	0.094	0.090	0.104	0.110
TSH (ng/ml), 2 weeks	5.3	5.8	5.7	5.5	6.6
T4 (µg/dl), day 90	3.1	2.9	3.1	3.4	3.2
T3 (ng/dl), day 90	88	90	87	86	97
rT3 (ng/ml), day 90	0.066	0.084	0.072	0.076	0.052
TSH (ng/ml), day 90	5.2	5.7	5.8	5.8	6.5
Hepatic 5'-deiodinase activity (nmol/hr/mg protein)[a]	5.5	5.4	6.5	5.0	4.1
Hepatic glucuronyltransferase activity (nmol/min/mg protein)[a]	26	32	33	47*	64*

* Statistically significant difference from control, $p \leq 0.05$. Histopathological findings were not subject to statistical analysis

[a] n = 5/group

2-year chronic toxicity and carcinogenicity study in rats (2002; batch KQ926-45)

Sprague-Dawley rats (80/sex/group) were administered proquinazid at dietary concentrations up to 2000 ppm for males and 1200 ppm for females. 10 animals per group were sacrificed after 1 year for histopathological examination. Further 5 animals per sex, group and time point were sacrificed after 1 week and after 1 year for hepatocellular proliferation and cytochrome P-450 concentration. The remaining 60 animals/sex/group were used for the 2-year study. Thyroid hormones were measured in 15 animals/sex/group at 1 week and 1 year.

2-year study in rats (2002): liver- and thyroid-related parameters						
Males						
Dose (ppm)	**0**	**10**	**30**	**300**	**1000**	**2000**
Dose (mg/kg bw/d)	0	0.42	1.2	12	43	92
1 week						
Body weight	340	329	330	340	302	261*
Liver weight, absolute (g)	15.0	15.2	15.1	16.0	14.7	12.6
Liver weight, relative (%)	4.4	4.6	4.6	4.7	4.8	4.8
Liver cytochrome P-450 content (nmol/mg protein)	0.83	0.86	0.83	1.00	1.19*	1.30*
Hepatocyte BrdU labelling index (per 1000 cells)	3.1					2.2
T4 (µg/dl)	5.2	4.8	4.5	5.1	4.2*	2.8*
T3 (ng/dl)	94	87	81*	78*	75*	69*
rT3 (ng/ml)	0.13	0.13	0.13	0.16*	0.16*	0.15*
TSH (ng/ml)	14	12	11	14	20*	23*
3 months (clinical chemistry)						
Cholesterol (mg/dl)	74	75	71	86	100*	112*
1 year						
Body weight	703	721	696	686	637	513*
Liver weight, absolute (g)	19.0	18.7	19.7	19.7	21.5	18.6
Liver weight, relative (%)	2.7	2.6	2.8	2.9	3.4*	3.6*
Thyroid weight, absolute (mg)	45	42	46	44	49	44
No. of animals for histopathological examination	10	10	10	10	10	10
Liver: alteration/degeneration of hepatocytes	0	0	0	0	1	10[#]
Liver: cholangiofibrosis	0	0	0	0	1	1
Liver: fatty change, midzonal	0	1 (1 +)	0	6[#] (3 +, 3 ++)	10[#] (7 ++, 2 +++, 1 ++++)	10[#] (2 +, 4 ++, 2 +++, 2 ++++)
Liver: focus of cellular alteration, basophilic	0	0	0	0	1	3[#]

Liver: oval cell hyperplasia	0	0	0	0	0	2[#]
Thyroid: follicular hypertrophy	0	0	0	3[#]	7[#]	7[#]
Cholesterol (mg/dl)	113	106	112	119	121	123
T4 (µg/dl)	2.5	2.6	2.3	2.8	2.2	1.8*
T3 (ng/dl)	68	76	64	63*	61*	61*
rT3 (ng/ml)	0.040	0.046	0.042	0.068*	0.064*	0.063*
TSH (ng/ml)	15	14	13	17	18	18
Liver cytochrome P-450 content (nmol/mg protein)	0.70	0.69	0.73	0.78	0.82	0.85
2-year study						
No. of animals alive on day 693	19	27	23	27	32	40
Percent survival (%)	32	45	40	45	53	68
Body weight	644	682	714	634	600	545*
Liver weight, absolute (g)	17.8	18.1	18.3	18.7	19.2	18.4
Liver weight, relative (%)	2.8	2.9	2.6	2.9	3.4*	3.7*
Thyroid weight, absolute (mg)	54	53	51	61	62	63
No. of animals for histopathological examination	60	60	61	60	60	60
Liver: alteration/degeneration of hepatocytes	0	0	0	16[#] (13 +, 3 ++)	22[#] (10 +, 8 ++, 2 +++, 2 ++++)	34[#] (15 +, 13 ++, 6 +++)
Liver: cholangiofibrosis	0	0	0	0	5[#]	0
Liver: fatty change, centrilobular	4	2	7	7	24[#]	24[#]
Liver: fatty change, focal	3	5	3	6	1	13[#]
Liver: fatty change, midzonal	1 (1 +)	0	0	5[#] (2 +, 1 ++, 2 +++)	7[#] (2 +, 5 ++)	24[#] (6 +, 9 ++, 9 +++)
Liver: focus of cellular alteration, eosinophilic	10	7	10	15	14	18[#]
Liver: focus of cellular alteration, mixed	0	1	0	1	3	4[#]
Liver: oval cell hyperplasia	0	0	0	2	9[#]	7[#]
Liver: hepatocyte hypertrophy, centrilobular	0	0	2	1	1	5[#]
Thyroid: follicular cell adenoma	1	0	1	3	6[#]	8[#]
Thyroid: follicular cell carcinoma	0	0	0	0	1	1
Thyroid: follicular hypertrophy	0	1	0	14[#]	23[#]	30[#]

Thyroid: follicular cyst/cystic hyperplasia	2	1	5	7#	9#	16#
Females						
Dose (ppm)	**0**	**10**	**30**	**300**	**600**	**1200**
Dose (mg/kg bw/d)	0	0.49	1.4	16	35	76
1 week						
Body weight	228	221	215	224	184*	166*
Liver weight, absolute (g)	10.1	9.5	9.5	10.0	7.6*	6.8*
Liver weight, relative (%)	4.4	4.3	4.4	4.5	4.1	4.1
Liver cytochrome P-450 content (nmol/mg protein)	0.53	0.57	0.55	0.64	0.72*	0.82*
Hepatocyte BrdU labelling index (per 1000 cells)	3.2					2.1
T4 (µg/dl)	3.0	2.9	2.6	2.7	2.6	1.6*
T3 (ng/dl)	86	88	77	68*	73*	70*
rT3 (ng/ml)	0.11	0.12	0.11	0.11	0.15*	0.14*
TSH (ng/ml)	7.1	7.3	6.6	7.7	7.2	6.8
3 months (clinical chemistry)						
ALT (U/L)	37	30	29	29	30	34
AST (U/L)	79	72	72	74	79	93
SDH (U/L)	20	19	20	21	21	25
Cholesterol (mg/dl)	94	82	77	93	107	132*
1 year						
Body weight	345	435*	417*	380	304	237*
Liver weight, absolute (g)	9.9	11.8	10.6	10.9	10.2	10.9
Liver weight, relative (%)	2.9	2.7	2.6	2.9	3.4*	4.6*
Thyroid weight, absolute (mg)	24	30	28	34*	32*	22
No. of animals for histopathological examination	9	10	10	10	10	10
Liver: alteration/degeneration of hepatocytes	0	0	0	3# (2 +, 1 ++)	9# (4 +, 5 ++)	10# (2 +, 8 ++)
Liver: cholangiofibrosis	0	0	0	0	1	1
Liver: fatty change, midzonal	0	0	0	0	3#	6#
Liver: focus of cellular alteration, eosinophilic	0	0	0	2	1	2#
Liver: bile duct hyperplasia	1	0	1	1	2	4#
Liver: oval cell hyperplasia	0	0	0	2	5#	8#
Liver: increased pigment in Kupffer cells	0	0	0	0	5#	5#

Thyroid: follicular hypertrophy	0	0	0	6#	9#	9#
ALT (U/L)	37	52	63	37	111*	89*
AST (U/L)	87	118	102	86	196*	179*
SDH (U/L)	23	27	30	33	92*	66*
Cholesterol (mg/dl)	127	124	125	144	166	188*
T4 (µg/dl)	1.85	1.96	1.62	1.43*	0.77*	0.42*
T3 (ng/dl)	100	107	91*	84*	73*	75*
rT3 (ng/ml)	0.036	0.035	0.036	0.037	0.031	0.019*
TSH (ng/ml)	8.5	10.3	9.3	10.3	10.6	8.8
Cytochrome P-450 content (nmol/mg protein)	0.47	0.45	0.56	0.54	0.45	0.56
2-year study						
No. of animals alive on day 693	20	23	20	22	38	36
Percent survival (%)	33	38	34	37	63	59
Body weight	424	457	449	411	345*	281*
Liver weight, absolute (g)	11.8	12.2	12.5	12.4	14.2*	15.7*
Liver weight, relative (%)	2.9	2.8	2.9	3.1	4.4*	5.7*
Thyroid weight, absolute (mg)	41	42	41	39	41	35
No. of animals for histopathological examination	60	61	60	60	60	61
Liver: animals with at least one hepatocellular neoplasm	1	0	0	2	17#	35#
Liver: hepatocellular adenoma	1	0	0	2	11#	29#
Liver: hepatocellular carcinoma	0	0	0	0	2	1
Liver: cholangiocarcinoma, intestinal type	0	0	0	0	8#	12#
Liver: alteration/degeneration of hepatocytes	0	0	0	13#	46#	59#
Liver: cholangiofibrosis	0	0	0	1	4#	10#
Liver: biliary cyst	0	0	0	0	3#	8#
Liver: fatty change, individual cell	0	0	0	6#	17#	4#
Liver: fatty change, midzonal	0	0	0	5#	22#	37#
Liver: focus of cellular alteration, basophilic	13	11	10	17	25*	7
Liver: focus of cellular alteration, eosinophilic	10	5	7	20#	48#	36#
Liver: focus of cellular alteration, mixed	1	0	2	3	3	6#
Liver: bile duct hyperplasia	18	22	17	13	26	29#

Liver: oval cell hyperplasia	0	0	0	4#	42#	52#
Liver: hepatocyte hypertrophy, panlobular	0	0	0	20#	4#	0
Thyroid: follicular hypertrophy	1	1	0	10#	36#	45#

* Statistically significant difference from control, $p \leq 0.05$, by Dunnett's test (body and organ weights, hormones) or Dunn's test (clinical chemistry)

Statistically significant difference from control, $p \leq 0.05$, by Cochran-Armitage trend test

Lesion grades: +, minimal; ++, mild; +++, moderate; ++++, severe

Survival to the final sacrifice was significantly increased in 2000 ppm males and in 600 ppm and 1200 ppm females. The histopathological findings in the liver are further described in the study report as follows (verbatim citation, only selected diagnoses presented here):

- The diagnosis of "alteration/degeneration, hepatocytes" was made when large areas of the liver contained hepatocytes having the appearance of those found in foci of cellular alteration. These areas did not have distinct boundaries, hence were not focal in nature. The affected hepatocytes most often resembled those in eosinophilic foci, being large with dense eosinophilic cytoplasm and enlarged nuclei, but also could present as smaller basophilic cells, vacuolated or clear cells, or any mixture of these types. Hepatic cords were typically disorganised with individualisation of hepatocytes and there was increased single cell necrosis, pigment in Kupffer cells and to a lesser extent in hepatocytes, and an increase in scattered individual cells with very large and/or multiple nuclei. Alteration/degeneration of hepatocytes was interpreted to be a direct test substance-related adverse effect, and one that was not expected to occur spontaneously in this strain of rat.
- The term "cholangiofibrosis" in the liver was used to diagnose focal to multifocal proliferations of large hyperchromatic epithelial cells, which had a striking resemblance to intestinal epithelium. These cells were arranged in acini and ducts which were partly or completely surrounded by dense fibrous tissue. This lesion was generally not compressive, often displayed some degree of retraction as evidenced by depression of the hepatic capsule over the lesion, and its central portions were mostly composed of fibrotic tissue.
- The diagnosis of "cholangiocarcinoma, intestinal type" was applied to the cholangiofibrotic lesion when involvement of the liver was extensive, with compression of the adjacent parenchyma, the presence of many glandular structures at the periphery of the lesions, and outward bulging of the overlying hepatic capsule. However, there were no instances of extrahepatic involvement of cholangiofibrotic lesions in the current study. Determination of the exact biological nature of the lesions would require additional information, such as their ability to be successfully transplanted into another host.
- Oval cell hyperplasia depicted the proliferation of small cells with scant cytoplasm and oval nuclei with marginated chromatin. These cells were often observed in single or double width chains of varying lengths extending along hepatic sinusoids from portal areas. They are reported to be primitive progenitor cells that proliferate in response to hepatic injury and can differentiate into hepatic or biliary cells.
- Fatty change of individual cells depicted scattered individual hepatocytes containing prominent cytoplasmic micro vesicles and was considered adverse.