Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

fenpyrazamine

EC number: not assigned
CAS number: 473798-59-3

ECHA/RAC/CLH-O-0000003187-73-01/A2

Adopted

30 November 2012

Annex 2.1: Comments and response to comments on CLH Proposal and Justification

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

ECHA has compiled the comments received via internet that refer to several hazard classes and entered them under each of the relevant categories/headings as comprehensive as possible. Please note that some of the comments might occur under several headings when splitting the given information is not reasonable.

Substance name: fenpyrazamine
EC number: not assigned
CAS number: 473798-59-3

General comments

Date	Country / Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
07/02/ 2012	France/	I fully agree with the ECHA CLH report as published for public consultation and with the proposed C&L for EU harmonised classification and labelling of the compound	Noted	N/A
08/02/ 2012	Japan / Individual	I fully agree with the content and proposed classfication and labelling indicated in CLH report that is scientifically evaluated. I think that the CLH report shows a good toxicology evaluation.	Noted	N/A
10/02/ 2012	Japan / Individual	I agree with the ECHA CLH report and the C&L proposals for a EU harmonised classification and labelling.	Noted	N/A
10/02/ 2012	Japan / Individual	Fully agree with the ECHA CLH report as published for comments and with the C&L proposals for a EU harmonised classification and labelling of the conpound.	Noted	N/A
10/02/ 2012	Japan / Individual	I fully agree with the ECHA CLH report and the proposed C&L.	Noted	N/A
16/02/ 2012	Japan / Individual	Fully agree.	Noted	N/A
17/02/ 2012	France / Individual	Fully agree with the ECHA CLH report proposal for EU classification of the active substance Fenpyrazamine.	Noted	N/A
17/02/ 2012	United States / Individual	I fully agree with the ECHA CLH report as published for comments and with the C&L proposals for a EU harmonised classification and labelling of the conpound	Noted	N/A

Date	Country / Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
17/02/ 2012	United States / Individual	Fully agree with the ECHA CLH report as published for comments and with the C&L proposals for a EU harmonised classification and labelling of the conpound	Noted	N/A
18/02/ 2012	United States / Individual	I agree that based on scientific facts, the ECHA CLH report on fenpyrazamine is correct, and the C&L proposals for an EU harmonized classification and labelling of the compound are correct.	Noted	N/A
18/02/ 2012	Japan / Individual	Completely agrew with the EGHA CLH report as published for comments and with the C&L proposals for the harmonized classification and labelling of compounds	Noted	N/A
20/02/ 2012	Japan / Individual	Fully agree with ECHA CLH report and with the C&L proposals for a EU harmonised classification and labelling of Fenpyrazamine.	Noted	N/A
21/02/ 2012	France / Individual	Fully agree with the ECHA CLH report proposal for EU classification of the active substance Fenpyrazamine.	Noted	N/A
21/02/ 2012	United Kingdom / Individual	Fully agree with the ECHA CLH report proposal for EU classification of the active substance Fenpyrazamine	Noted	N/A
21/02/ 2012	United Kingdom / Company-Downstream user	I fully agree with the ECHA CLH report proposal for EU classification of the active substance fenpyrazamine	Noted	N/A
25/02/ 2012	Japan/ Individual	I fully support the ECHA CLH report.	Noted	N/A
25/02/ 2012	Japan/ Individual	The ECHA CHL report as published for comments is well written, and I fully agree with the report and with the C&L proposals for a EU harmonised classification and labelling of the conpound.a EU harmonized classification and labeling of the compound.	Noted	N/A
27/02/ 2012	France / Company-Downstream user	I agree with the ECHA CLH report proposal for EU classification of the substance fenpyrazamine	Noted	N/A
08/03/ 2012	Netherlands / RIVM Bureau REACH / MSCA	Editorial comment: at page 97 part of the first sentence below Table 3 is missing.	The missing sentence is added in the revised version.	Noted
09/03/ 2012	France / MSCA	FR agrees with the classification proposal for Fenpyrazamine by Austria.	Noted	N/A

Date	Country / Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
10/03/ 2012	United States / Individual	I fully agree with the ECHA CLH report as published. I also agree with the C&L proposals for a EU harmonised classification and labelling of the compound.	Noted	N/A
12/03/ 2012	France / Company-Manufacturer	I fully agree with ECHA's proposal which is consistent with the results of our studies and study evaluations	Noted	N/A

Carcinogenicity

Date	Country / Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
07/02/2012	France / Individual	I fully agree with the evaluation in the CLH report and with the argumentation to justify that this compound does not need C&L proposals for the carcinogenic effcets observed in the rat carcinogenic study. The argumentation is based on the latest available science demonstrating clearly that the effects observed are not relevant and linked to a human mode of action.	Noted	N/A
08/02/2012	Japan / Individual	I fully agree with the proposed classification in CLH report, that is based on the scientific evidence for which non-human relevance was clearly demonstrated.	Noted	N/A
10/02/2012	Japan / Individual	I agree that the effects in the carcinogenicity rat study do not require C&L proposals. Because the CLH report justifies that the effects are not related to a human mode of action.	Noted	N/A
10/02/2012	Japan / Individual	Fully agree with the evaluation.	Noted	N/A
10/02/2012	Japan / Individual	I fully agree with that no classification for carcinogenity. CLH report fully justifies that the effects observed are not related to a human mode of action.	Noted	N/A
16/02/2012	Japan / Individual	No specific comment. Evaluation seems to be fair.	Noted	N/A
17/02/2012	France / Individual	I fully agree with the carcinogenic evaluation and explanation that the effects observed are not related to human mode of action.	Noted	N/A

Date	Country / Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
17/02/2012	United States / Individual	I fully agree that the effects observed in the carcinogenic rat study do not require C&L proposals because the CLH report justifies fully that the effects observed are not related to a human mode of action. These evaluations were made following the latest available science.	Noted	N/A
17/02/2012	United States / Individual	I fully agree that the effects observed in the carcinogenic rat study do not require C&L proposals because the CLH report justifies fully that the effects observed are not related to a human mode of action. These evaluations were made following the latest available science.	Noted	N/A
18/02/2012	United States / Individual	I agree that the effects seen in the rat carcinogenicity study do not require C&L proposals because the effects are not relevant to humans, based on the scientific facts considered in the CLH report.	Noted	N/A
18/02/2012	Japan / Individual	Fully agree with the evaluation conducted by the EU authorities and also agree with the conclusion that this compound does not need classification and labelling for carcinogenicity	Noted	N/A
20/02/2012	Japan / Individual	no comments	Noted	N/A
21/02/2012	France / Individual	Fully agree with the carcinogenic evaluation and explanation that the effects observed are not related to human mode of action.	Noted	N/A
21/02/2012	United Kingdom / Individual	Fully agree with the carcinogenic evaluation and explanation that the effects observed are not related to a human mode of action.	Noted	N/A
21/02/2012	United Kingdom / Company-Downstream user	Fully agree with the carcinogenicity evaluation and explanation that the effects observed are not related to a human mode of action	Noted	N/A
25/02/2012	Japan / Individual	I fully support that the effects observed in the carcinogenic rat study do not require C&L proposals.	Noted	N/A
25/02/2012	Japan / Individual	Human relevance of the effects observed in the rat carcinogenic bioassay is well discussed. I fully agree that the effects do not require C&L proposals because the CLH report justifies fully that the effects observed are not related to a human mode of action. These evaluations were based	Noted	N/A

Date	Country / Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		on the latest available science.		
27/02/2012	France / Company-Downstream user	I agree with the carcinogenic evaluation and explanation that the effects observed are not related to human mode of action	Noted	N/A
08/03/2012	Netherlands / RIVM Bureau REACH / MSCA	Malignant tumors were observed in male rats in liver and thyroid. Essential criteria in favor of no classification are the absence of an induced tumor incidence in female rats and male and female mice and the MoA that is considered not relevant for humans (based on the comparability to phenobarbital). In our opinion, there is sufficient evidence showing that this MoA responsible for thyroid tumor induction is not relevant for humans. Fenpyrazamine induces biotransformation enzymes that enhance metabolism and excretion of thyroid hormones, thereby disturbing the hypothalamus-pituitary-thyroid axis. Numerous studies have shown that especially the rat thyroid gland axis is much more susceptible to physiological disturbances by xenobiotics than humans. The rat thyroid gland shows a high incidence of proliferative lesions such as hyperplasia and adenomas of follicular cells upon long term exposure to various xenobiotics, due to the 'hyperactive state' of the rat thyroid (increased turnover thyroid hormones, high circulating TSH levels). Additionally, adult male rats have higher circulating TSH levels than females, explaining why the increased incidence of thyroid tumors was only observed in male rats. Based on these data, the increased incidence of thyroid tumors observed in rat is considered not relevant for humans. The relevance of the fenpyrazamine-induced hepatic tumors for humans is less clear. The dossier submitter states that the MoA of fenpyrazamine resembles the MoA of phenobarbital and that the described MoA of phenobarbital that leads to an enhanced incidence of liver tumors is not relevant for humans. First, the resemblance between fenpyrazamine and phenobarbital should be sufficiently proven. We suggest making a more extensive comparison of the two substances, including a comparison of the tumors observed.	Noted	In general RAC supports the dossier submitter's proposal that there is no justification to classify fenpyrazamine for <u>thyroid gland carcinogenicity</u> (corresponding specialised expert recommendation on the non-relevance of specific MOA's for thyroid gland tumour). With the following set of information and evaluations • borderline incidence of liver carcinomas in male rats for fenpyrazamine • experimental evidence for activation of CAR receptor • no indication for

Date	Country / Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		For example, fenpyrazamine induced hepatic tumors in male rats only, while phenobarbital induced tumors in rats and (more pronounced) in mice. In addition, the tumors induced by fenpyrazamine were carcinomas, while phenobarbital-induced hepatic tumors were mainly adenomas. Next to this, also the role of pregnane X receptor, which is known to be activated by phenobarbital, should be evaluated. Second, the human relevance of the MoA of phenobarbital leading to liver tumors should be more extensively evaluated and described. The dossier submitter considers this MoA not relevant for humans, based on epidemiological studies and on cell proliferation studies. In our opinion, the absence of an increased liver tumor risk in humans at exposure levels similar to those in rodents does not provide sufficient evidence for the absence of a carcinogenic effect in humans. It cannot be excluded that hepatic tumors will form in humans at a higher exposure level, especially since phenobarbital is not genotoxic, the effect is threshold-based and classification of a substance is independent of dose. Proliferation studies were performed in vitro or with mice with humanized PXR and CAR and were only very briefly described. Further, the essential role of phenobarbital-induced proliferation in the development of liver tumors might be questioned, since there is no justification provided and increased DNA synthesis is only observed after a short treatment period (3 days) and is reduced after 7 and 14 days treatment (which was also observed after fenpyrazamine treatment). This is not what would be expected from a tumor promoter. Finally, it is remarked that musk xylene has a MoA that resembles the MoA of phenobarbital. Musk xylene induces an increased incidence of hepatocellular adenomas en carcinomas in mice and the current harmonized classification is Carc. 2 (CLP). We suggest re-evaluating the proposal for no classification regarding carcinogenicity, taking into account the above mentioned comments.	All available information on liver tumours is compiled in the CLH report. Based on very low liver tumours incidences and plausible explanation for their non-relevance, the dossier submitter did not propose C&L for carcinogenicity. However, it should be discussed by RAC members if the proposed justification is acceptable and if (proposed) phenobarbital MoA is relevant or not for humans.	genotoxicity or cytotoxicity RAC comes to the conclusion that there is no sufficient human potential for the fenpyrazamine-related liver carcinogenicity observed. RAC proposes to follow the recommendation of the dossier submitter, not to classify fenpyrazamine for liver carcinogenicity. Thus, overall, with respect to both target organs (thyroid gland and liver), RAC concludes that there is no sufficient human relevance of tumour data. RAC proposes not to classify fenpyrazamine for carcinogenicity.
10/03/2012	United States / Individual	I fully agree that the effects observed in the carcinogenic rat study do not require C&L proposals. The report justifies fully that the effects observed are not related to a human mode of action.	Noted	N/A

Date	Country / Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
12/03/2012	France / Company-Manufacturer	I fully agree with ECHA's proposal	Noted	N/A
12/03/2012	Germany / MSCA	Page 70 A phenobarbital-like mechanism behind liver tumour formation in high-dose male rats is not a convincing argument not to classify the compound, at least if CLP criteria are applied. For Metazachlor, RAC dismissed the argumentation that a phenobarbital-like mechanism is a priori not relevant for humans. Instead, the rather low incidence in spite of a very high dose level might be considered to lower the concern for fenpyrazamin. For thyroid tumours, non-relevance to man seems to be shown. However, the whole issue of carcinogenicity and possible classification might need further discussion by RAC.	All available information on liver tumours is compiled in the CLH report. Based on very low liver tumours incidences and plausible explanation for their non-relevance, the dossier submitter did not propose C&L for carcinogenicity. However, it should be discussed by RAC members if the proposed justification is acceptable and if (proposed) phenobarbital MoA is relevant or not for humans.	See response to the Dutch comment
12/03/2012	United Kingdom / UK CA / MSCA	Rat Liver tumours There is no agreed EU position on the human relevance of substances inducing rodent liver tumours by activation of the constitutive androstane receptor (CAR), such as phenobarbitone, Therefore it is difficult to dismiss rodent liver tumours arising via CAR activation at present. However, there were 2/50 high dose male rats with hepatocellular carcinoma, compared to 0/50 in controls and other dose levels. The historical control incidence is 2.8%, The observed increase is just outside the expected background incidence for this tumour type. We note that there was very little evidence of hepatocyte hypertrophy and hepatocellular adenoma observed in these animals. Given the low level of	Noted	See response to the Dutch comment

Date	Country / Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		general hepatocellular proliferation and very slight increase in hepatocellular carcinoma, we consider these tumours to be a chance finding and of little toxicological significance. Thyroid follicular tumours Taking account of the interpretational framework developed by the EU Specialised Experts, it is possible to dismiss these tumours as not being relevant for human health. Conclusion Overall, we can support the proposal of no classification for carcinogenicity.		

Mutagenicity

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
07/02/2012	France / Individual	no comment, I fully agree with the evaluatuion available in the CLH report	Noted	N/A
08/02/2012	Japan / Individual	I fully agree with the proposed classification.	Noted	N/A
10/02/2012	Japan / Individual	I agree with the evaluation in the CLH report.	Noted	N/A
10/02/2012	Japan / Individual	Fully agree with the evaluation.	Noted	N/A
10/02/2012	Japan / Individual	No comment.	Noted	N/A
16/02/2012	Japan / Individual	No comments.	Noted	N/A
17/02/2012	France / Individual	Fully Agree with the evaluation of the CLH Report.	Noted	N/A
17/02/2012	United States / Individual	I fully agree with the evaluation in the CLH report	Noted	N/A

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
17/02/2012	United States / Individual	No comment, fully agree with the evaluation in the CLH report	Noted	N/A
18/02/2012	United States / Individual	I agree with the evaluation in the CLH report.	Noted	N/A
18/02/2012	Japan / Individual	Have no comment on the conclusion described in the CLH report, fully agree with the evaluation	Noted	N/A
20/02/2012	Japan / Individual	no comments	Noted	N/A
21/02/2012	France / Individual	Fully agree with the evaluation of the CLH report.	Noted	N/A
21/02/2012	United Kingdom / Individual	Fully agree with the evaluation of the CLH report.	Noted	N/A
21/02/2012	United Kingdom / Company-Downstream user	Fully agree with the evaluation of the CLH report.	Noted	N/A
25/02/2012	Japan / Individual	I have no comment and fully support the evaluation in the CLH report.	Noted	N/A
25/02/2012	Japan Individual	I have no comments, fully agree with the evaluation in the CLH report.	Noted	N/A
27/02/2012	France / Company-Downstream user	I agree with the evaluation of the CLH Report	Noted	N/A
12/03/2012	France / Company-Manufacturer	I fully agree with ECHA's proposal	Noted	N/A

Toxicity to reproduction

Date	Country / Organisation /MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
07/02/2012	France / Individual	no comment, I fully agree with the evaluation in the CLH report	Noted	N/A

Date	Country / Organisation /MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
08/02/2012	Japan / Individual	I fully agree with the proposed classification.	Noted	N/A
10/02/2012	Japan / Individual	I agree with the evaluation in the CLH report.	Noted	N/A
10/02/2012	Japan / Individual	Fully agree with the evaluation.	Noted	N/A
10/02/2012	Japan / Individual	No comment.	Noted	N/A
16/02/2012	Japan / Individual	No comments.	Noted	N/A
17/02/2012	France / Individual	Fully Agree with the evaluation of the CLH Report	Noted	N/A
17/02/2012	United States / Individual	I fully agree with the evaluation in the CLH report	Noted	N/A
17/02/2012	United States / Individual	No comment, fully agree with the evaluation in the CLH report	Noted	N/A
18/02/2012	United States / Individual	I agree with the evaluation in the CLH report.	Noted	N/A
18/02/2012	Japan / Individual	Have no comment on the conclusion described in the CLH report, fully agree with the evaluation	Noted	N/A
20/02/2012	Japan / Individual	no comments	Noted	N/A
21/02/2012	France / Individual	Fully agree with the evaluation of the CLH report.	Noted	N/A
21/02/2012	United Kingdom / Individual	Fully agree with the evaluation of the CLH report	Noted	N/A
21/02/2012	United Kingdom / Company-Downstream user	Fully agree with the evaluation of the CLH report	Noted	N/A
25/02/2012	Japan / Individual	I have no comment and fully support the evaluation in the CLH report.	Noted	N/A
25/02/2012	Japan / Individual	I have no comments, fully agree with the evaluation in the CLH report.	Noted	N/A
27/02/2012	France / Company-Downstream user	I agree with the evaluation of the CLH Report	Noted	N/A

Date	Country / Organisation /MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
12/03/2012	France / Company-Manufacturer	I fully agree with ECHA's proposal	Noted	N/A

Respiratory sensitisation

Date	Country / Organisation /MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
07/02/2012	France / / Individual	no comment, no effects observed, so I fully agree with the evaluation in the CLH report	Noted	N/A
08/02/2012	Japan / Individual	I fully agree with the proposed classification.	Noted	N/A
10/02/2012	Japan / Individual	I agree with the evaluation in the CLH report.	Noted	N/A
10/02/2012	Japan / Individual	Fully agree with the evaluation.	Noted	N/A
10/02/2012	Japan / Individual	No comment.	Noted	N/A
16/02/2012	Japan / Individual	Fully agree.	Noted	N/A
17/02/2012	France / Individual	Fully Agree with the evaluation of the CLH Report	Noted	N/A
17/02/2012	United States / Individual	I fully agree with the evaluation in the CLH report	Noted	N/A
17/02/2012	United States / Individual	No comment, fully agree with the evaluation in the CLH report	Noted	N/A
18/02/2012	United States / Individual	I agree with the evaluation in the CLH report.	Noted	N/A
18/02/2012	Japan / Individual	Have no comment on the conclusion described in the CLH report, fully agree with the evaluation	Noted	N/A
20/02/2012	Japan / Individual	no comments	Noted	N/A
21/02/2012	France /	Fully agree with the evaluation of the CLH report.	Noted	N/A

Date	Country / Organisation /MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
	Individual			
21/02/2012	United Kingdom / Individual	Fully agree with the evaluation of the CLH report	Noted	N/A
21/02/2012	United Kingdom / Company-Downstream user	Fully agree with the evaluation of the CLH report.	Noted	N/A
25/02/2012	Japan / Individual	I have no comment and fully support the evaluation in the CLH report.	Noted	N/A
25/02/2012	/ Individual	I have no comments, fully agree with the evaluation in the CLH report.	Noted	N/A
27/02/2012	France / Company-Downstream user	I agree with the evaluation of the CLH Report	Noted	N/A
12/03/2012	France / Company-Manufacturer	I fully agree with ECHA's proposal	Noted	N/A

Other hazards and endpoints

Date	Country /Organisa-tion /MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
07/02/ 2012	France / Individual	no comment, I fully agree with the proposed C&L for environmental effcets and with the C&L proposals for phys chem properties	Noted	N/A
08/02/ 2012	Japan / Individual	I fully agree with the proposed classification.	Noted	N/A
10/02/ 2012	Japan / Individual	I agree with the evaluation in the CLH report about environmental classification and proposals for classification and labelling.	Noted	N/A
10/02/ 2012	Japan / Individual	Fully agree with the evaluation.	Noted	N/A

Date	Country /Organisation /MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
10/02/ 2012	Japan Individual	I fully agree with the C&L proposals.	Noted	N/A
16/02/ 2012	Japan / Individual	No comments.	Noted	N/A
17/02/ 2012	France / Individual	Fully agree with the evaluation of the CLH Report, and the proposed classification in the environment.	Noted	N/A
17/02/ 2012	United States / Individual	I fully agree with the evaluation in the CLH report about environmental classification and about proposals for classification and labelling based on the aquatic toxicity.	Noted	N/A
17/02/ 2012	United States / Individual	Fully agree with the evaluation in the CLH report about environmental classification and about proposals for classification and labelling based on the aquatic toxicity.	Noted	N/A
18/02/ 2012	United States Individual	I agree with the evaluation in the CLH report regarding the environmental classification, and with the C&L proposals made on the scientific basis of the aquatic toxicity.	Noted	N/A
18/02/ 2012	Japan / Individual	Completely agree with the environmental classification and labelling and also the evaluation in the CLH report	Noted	N/A
20/02/ 2012	Japan / Individual	no comments	Noted	N/A
21/02/ 2012	France / Individual	Fully agree with the evaluation of the CLH Report and the proposed classification in the environment.	Noted	N/A
21/02/ 2012	United Kingdom / Individual	Fully agree with the evaluation of the CLH report, and the proposed classification according to the environment evaluation.	Noted	N/A
21/02/ 2012	United Kingdom / Company-Downstream user	Fully agree with the evaluation of the CLH report, and the proposed classification according to the environment evaluation.	Noted	N/A
25/02/ 2012	Japan / Individual	I have no comment and fully support the evaluation in the CLH report.	Noted	N/A
25/02/ 2012	Japan Individual	I have no comments, fully agree with the evaluation in the CLH report.	Noted	N/A
27/02/ 2012	France / Company-Downstream	I agree with the evaluation of the CLH Report, and the proposed classification in the environment	Noted	N/A

Date	Country /Organisation /MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
	user			
05/03/2012	Belgium / Els Boel / MSCA	Environment : Based on the results of the aquatic toxicity tests (LC50>1mg/l, lowest NOEC between 0.1 and 1mg/l) the fact that the substance is considered as not rapidly degradable it is justified to classify, following the classification criteria of the 2nd ATP, as Aquatic chronic2, H411. The substance shows low potential to bioaccumulate (Log Pow=3.52 but BCFwhole fish =9, which is <500). Based on the classification and labelling criteria in accordance with dir. 67/548/EEC, Fenpyrazamine should be classified as N, R51/53 (LC50>1mg/l, not rapidly degradable and BCF<100) In conclusion : we agree with the proposed environmental classification (based on DSD and CLP criteria) by the Austrian MSCA. Some editorial or/and minor comments: • General remark :reference for tables in the text are often those from the DAR, furthermore table numbers in the CLH report do not succeed. Please let table numbers succeed correctly and use correct references in the text of the CLH report. • 5.1.2.3 simulation tests : p.159, first sentence should read "S-2188 degrades in both water/sediment systems …" instead of "S-2188 degrades in both sediment/sediment systems …" p.160 : the first order multi compartment kinetics are missing in the table 75 p.160 : last paragraph : please delete the sentence about the KinGui graph reports as they are not copied from the DAR into the CLH report. P.161 : conclusion : first sentence : " … maximum in whole system 20.5% of AR and 15.9% of AR respectively)." Based on the results of table 73, the maximum in the whole system of metabolite S-2188-OH is 54.5% of AR and not 15.9%. P162 : last sentence : "major metabolites" is listed twice.	Noted	N/A

Date	Country /Organisa-tion /MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		• 5.5. comparison with criteria for environmental hazards P.186 Please give a clear comparison with the criteria for DSD and CLP, CLP justification : the explanation given in 5.6 is part of the comparison with the criteria (5.5), also for the CLP-proposal a justification should be given why a classification for acute toxicity is not warranted (LC50>1mg/l) Please delete the precautionary statements in the proposal as they need not to be specified in a CLH report. They will not be included in annex VI and the final responsibility for the allocation of P statements lies with the supplier. DSD-justification : the log Pow >3, but BCF <100 which is the trigger for bioaccumulation in DSD. • 5.6 Conclusion on classification and labelling for environmental hazards Please give both proposals following DSD and CLP		
05/03/ 2012	Spain / MSCA	We are in agreement with the classification for environmental hazards proposed by Austria	Noted	N/A
09/03/ 2012	France / MSCA	We agree on the classification proposal on the environmental hazards. It could be interesting to add the toxicity data on sediment dwelling organisms in the CLH report.	Noted	Toxicity data for aquatic organisms exposed via sediment are not used for classification purposes. It is therefore not relevant to consider them.
10/03/ 2012	United States / Individual	I fully agree with the report concerning environmental classification and the proposals for classification and labelling based on the aquatic toxicity.	Noted	N/A
12/03/ 2012	France / Company-Manufacturer	I fully agree with ECHA's proposal	Noted	N/A
12/03/	Sweden / MSCA	Environmental classification:	According to the	The slope of the dose

Date	Country /Organisa-tion /MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
2012		The current proposal for harmonized classification of Fenpyrazamine (CAS No 473798-59-3) is: Aquatic Chronic 2, H411. We reason, however, that also category Acute 1 is justified because, based on the reasons specified below, toxicity of fenpyrazamine to algae Pseudokirchineriella subcapitata would probably be higher and the effect level would be lower than 1 mg a.s /l (Pseudokirchineriella subcapitata EC50/LC50 >0.9 mg a.s. /L). There are two reasons for this: One is that the biomass endpoint of the algae instead of growth rate would be more reliable in this case since we do not know exactly the toxicity endpoint (EC50/LC50>0.9 mg a.s. /L). The EC50/LC50 for biomass for this algae is 0.42 mg a.s./L which is < 1 mg a.s. /L and will consequently fall under the category Acute 1. The second is that the photolysis of fenpyrazamine is rapid with an experimental half-life of 1.7 days at summer sunlight in UK/US. This indicates that the concentration of fenpyrazamine would decrease over time in the toxicity test of Pseudokirchineriella subcapitata and the EC50/LC50 value would actually be lower than 1 mg a.s. /L. Based on the arguments above the proposal for fenpyrazamine would be: Acute 1, H400 (very toxic to aquatic life with long lasting effects), and Aquatic Chronic 2, H411 (Toxic to aquatic life with long lasting effects).	regulation 1272/2008 classification and labelling should be based on the algae growth rate endpoint. We agree that classification and labelling on the basis of a "greater than" endpoint is problematic. However, a valid E_rC_{50} is available and at the concentration of 0.9 mg a.s./L no adverse effects > 50% on algae were observed. However, a discussion about how to use an E_bC_{50} and under which conditions might be preferable. About the second point made regarding the rapid photolysis of fenpyrazamine. The study was conducted under conditions of continuous illumination. Hence, the rapid photolysis is already considered.	response curve suggests that 50% inhibition of growth rate would have been achieved at a concentration slightly above 1 mg/L in this case. The CLP Guidance indicates that growth rate is preferred to biomass as an end point. This is because the direct use of the biomass concentration without logarithmic transformation cannot be applied to an analysis of results from a system in exponential growth. Since the results are expressed in terms of mean measured concentrations (which were in the range of 83 – 90% of nominal concentrations over the whole test duration), photolysis is not an issue. The suggested addition of Aquatic acute 1 is therefore

Date	Country /Organisa-tion /MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
				not appropriate.

ATTACHMENTS RECEIVED: None

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: Fenpyrazamine

EC Number: ***Not allocated***

CAS Number: ***473798-59-3***

Index Number: **-**

Contact details for dossier submitter:

Austrian Agency for Health and Food Safety

Institute for Plant Protection Products Evaluation and Authorisation

Spargelfeldstraße 191, 1220 Vienna

Austria

Version number: 4 **Date: March 2012**

CONTENTS

Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Fenpyrazamine***
EC number:	***Not allocated***
CAS number:	***473798-59-3***
Annex VI Index number:	**-**
Degree of purity:	***Minimum purity 94.0 % w/w (based on a pilot plant)***
Impurities:	***No relevant impurities***

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	-	-
Current proposal for consideration by RAC	Aquatic Chronic 2, H411	N, R51/53
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Aquatic Chronic 2, H411	N, R51/53

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

Table 3: Proposed classification according to the CLP Regulation

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

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.2.	Skin corrosion / irritation	-		-	Conclusive, but not sufficient for classification
3.3.	Serious eye damage / eye irritation	-		-	Conclusive, but not sufficient for classification
3.4.	Respiratory sensitisation	-		-	Conclusive, but not sufficient for classification
3.4.	Skin sensitisation	-		-	Conclusive, but not sufficient for classification
3.5.	Germ cell mutagenicity	-		-	Conclusive, but not sufficient for classification
3.6.	Carcinogenicity	-		-	Conclusive, but not sufficient for classification
3.7.	Reproductive toxicity	-		-	Conclusive, but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	-		-	Conclusive, but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	-		-	Conclusive, but not sufficient for classification
3.10.	Aspiration hazard	-		-	Conclusive, but not sufficient for classification
4.1.	Hazardous to the aquatic environment	H411, P273, P391, P501		-	-
5.1.	Hazardous to the ozone layer	-		-	Conclusive, but not sufficient for classification

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

Labelling: Signal word: -
Pictogram: GHS9
Hazard statements: H411
Precautionary statements: P273, P391, P501

Proposed notes assigned to an entry:

Table 4: Proposed classification according to DSD

Hazardous property	**Proposed classification**	**Proposed SCLs**	**Current classification [1)]**	**Reason for no classification [2)]**
Explosiveness	-		-	Conclusive, but not sufficient for classification
Oxidising properties	-		-	Conclusive, but not sufficient for classification
Flammability	-		-	Conclusive, but not sufficient for classification
Other physico-chemical properties *[Add rows when relevant]*	-		-	Conclusive, but not sufficient for classification
Thermal stability	-		-	Conclusive, but not sufficient for classification
Acute toxicity	-		-	Conclusive, but not sufficient for classification
Acute toxicity – irreversible damage after single exposure	-		-	Conclusive, but not sufficient for classification
Repeated dose toxicity	-		-	Conclusive, but not sufficient for classification
Irritation / Corrosion	-		-	Conclusive, but not sufficient for classification
Sensitisation	-		-	Conclusive, but not sufficient for classification
Carcinogenicity	-		-	Conclusive, but not sufficient for classification
Mutagenicity – Genetic toxicity	-		-	Conclusive, but not sufficient for classification
Toxicity to reproduction – fertility	-		-	Conclusive, but not sufficient for classification
Toxicity to reproduction – development	-		-	Conclusive, but not sufficient for classification
Toxicity to reproduction – breastfed babies. Effects on or via lactation	-		-	Conclusive, but not sufficient for classification
Environment	N, R51/53, S61		-	-

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

Labelling: Indication of danger: N, Dangerous for the environment
R-phrases: R51/53
S-phrases: S61

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Fenpyrazamine has not yet been approved for Annex I under Council Directive 91/414/EEC (new active substance), with Austria as Rapporteur Member State. The open issues in the European peer-review of fenpyrazamine (not relevant for classification and labelling) considering mammalian toxicology were discussed in an expert meeting (PRAPeR) end of September 2011; the EFSA (European Food Safety Authority) conclusion on the substance is expected in 2012. The experts did not discuss C&L of fenpyrazamine.

In accordance with Article 36(2) of the CLP Regulation, fenpyrazamine should be considered for harmonised classification and labelling (including the criteria of the 2nd ATP). Therefore, this proposal considers all physico-chemical, human health and environmental end points. This Annex VI dossier presents a classification and labelling proposal based mainly on the information presented in the assessment of fenpyrazamine under Directive 91/414/EEC. The assessment made under that Directive is attached to the IUCLID 5 dossier. No other registration dossiers are available for fenpyrazamine at time of the submission of the revised CLH report.

Fenpyrazamine is not listed on Annex VI of the CLP Regulation This proposal seeks for classification for environment. No classification is required for human health and physico-chemical properties.

2.2 Short summary of the scientific justification for the CLH proposal

For Fenpyrazamine, no classification and labelling has been proposed regarding physical and chemical properties and human health.

Justification for the proposal with respect to environmental effects:
The classification and labelling of the active substance Fenpyrazamine is based on the toxicity to fish (*Oncorhynchus mykiss*, LC_{50} = 5.2 mg/L, Cafarella, 2006a) and the fact that the active substance is not rapidly biodegradable (Lewis, C.J. & Troth, K., 2007). The log P_{ow} of fenpyrazamine is 3.52 (Lentz, N.R., 2005b).

Combing all these criteria for classification with respect to environmental effects, according to Directive 67/548/EEC, *R51/53, Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment* and according to Regulation 1272/2008, *H411, Toxic to aquatic life with long lasting effects*, is proposed for Fenpyrazamine.

2.3 Current harmonised classification and labelling

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

No current entry in Annex VI, Table 3.1 in the CLP Regulation.

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

No current entry in Annex VI, Table 3.2 in the CLP Regulation.

2.4 Current self-classification and labelling

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

No current self-classification based on the CLP Regulation criteria.

2.4.2 Current self-classification and labelling based on DSD criteria

The notifier provided self-classification and labelling as follows:

Classification and labelling of active substance according to EU directive 2001/59/EC: S-2188 TG

Hazard Symbol:		Dangerous for the environment (N)
Risk Phrases:	R50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
Safety Phrases:	S60	This material and its container must be disposed of as hazardous waste
	S61	Avoid release to the environment. Refer to special instructions/Safety data sheets.

The notifier provided no information about the scientific basis for this conclusion.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

No need for justification for pesticides.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

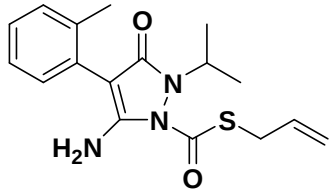
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	-
EC name:	-
CAS number (EC inventory):	-
CAS number:	473798-59-3
CAS name:	1H-pyrazole-1-carbothioic-acid, 5-amino-2,3-dihydro-2-(1-methylethyl)-4-(2-methylphenyl)-3-oxo, *S*-2-propen-1-yl ester
IUPAC name:	S-allyl 5-amino-2-isopropyl-4-(2-methylphenyl)-3-oxo-2,3-dihydro-1H-pyrazole-1-carbothioate
CLP Annex VI Index number:	-
Molecular formula:	$C_{17}H_{21}N_3O_2S$
Molecular weight range:	331.43 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Fenpyrazamine	940 g/kg	Minimum purity, no range	The minimum purity based on a pilot plant and should be considered provisionally. If commercial production launches (2012) a different minimum purity might be specified.

Current Annex VI entry: no entry

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
No relevant impurities	-	-	-

Current Annex VI entry: -

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
No additives	-	-	-	-

Current Annex VI entry: -

1.2.1 Composition of test material

Physico-chemical properties: see table 9 (purity of tested technical material in the range from 94.7% to 99.3%)

Human health hazard assessment: purity of tested technical material either 94.7% (all toxicological studies performed with the same batch) or 99.4% (ADME studies only).

Environmental hazard assessment: purity of tested technical material in the range from 94.7 % to 99.3 %.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties:

Property (Annex point as reference to the DAR)	Method	Material / Batch	Results	Conclusion/Comment	Reference (Study)
B.2.1.1 Melting point, freezing point or solidification point (IIA 2.1.1)	OECD 102 Equivalent to EEC Method A.1 DSC GLP	PGAI R-4CM03G 99.3%	Melting point: 116.4 °C (389.6 K)	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
B.2.1.2 Boiling point (IIA 2.1.2)	US EPA OPPTS 830.7220 – Boiling point Equivalent to EEC Method A.2 DSC and capillary GLP	PGAI R-4CM03G 99.3%	Boiling point: 239.8 °C (513.0 K) at a nominal pressure of 745 mm/Hg	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
B.2.1.3 Temperature of decomposition or sublimation (IIA 2.1.3)	US EPA OPPTS 830.7220 – Boiling point Equivalent to EEC Method A.2 GLP	PGAI R-4CM03G 99.3%	No decomposition was observed. Individual melting and boiling points determined, therefore no sublimation occurred.	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
B.2.1.4 Relative density (IIA 2.2)	US EPA OPPTS 830.7300 – Density Equivalent to EEC Method A.3 Pycnometer method GLP	PGAI R-4CM03G 99.3%	Relative density at 20 °C: 1.262	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
		TGAI 030-050914-1G 94.7%	Relative density at 20 °C: 1.250	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006b (QNP-0007)
B.2.1.5 Vapour pressure (IIA 2.3.1)	OECD 104 EEC Method A.4 – gas saturation method and calculation (MPBPWin)	PGAI R-4CM03G 99.3% *)	Vapour pressure by gas saturation method: $<10^{-5}$ Pa at 25 °C (too low to be determined experimentally). Vapour pressure by MPBPWin calculation: 2.89×10^{-8} Pa at 25 °C.	Acceptable According to EEC A.4 calculated values can be used if the vapour pressure is likely $<10^{-5}$ Pa at ambient	DiFrancesco, D., 2006 (QNP-0004)

Property (Annex point as reference to the DAR)	Method	Material / Batch	Results	Conclusion/Comment	Reference (Study)
	GLP			temperature. The calculation confirms the low value. *) The original study reveals a purity of > 98%. The certificate of analysis confirms the purity of 99.3%.	
B.2.1.6 Volatility, Henry's law constant (IIA 2.3.2)	calculation	--	The Henry's Law Constant at 20 ˚C is calculated to be 1.62 x 10^{-4} Pa.m^3/mole. (Calculated from vapour pressure of 10^{-5} Pa and water solubility of 20.4 mg/L at 20 °C.)	Acceptable	Document M II Section 1
B.2.1.7 Appearance: physical state and colour (IIA 2.4.1)	US EPA OPPTS 830.6302 - Color / ASTM D-1535 US EPA OPPTS 830.6303 - Physical State GLP	PGAI R-4CM03G 99.3%	White, Munsell reference: N9.5/90% at 21.7 °C Solid at 25 °C	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
	US EPA OPPTS 830.6302 - Color / ASTM D-1535 US EPA OPPTS 830.6303 - Physical State GLP	TGAI 030-050914-1G 94.7%	Very pale yellow, Munsell reference: 10Y 9/2 at 20.7 °C Solid at 25 °C	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006b (QNP-0007)
B.2.1.8 Appearance: odour (IIA 2.4.2)	US EPA OPPTS 830.6304 - Odor GLP	PGAI R-4CM03G 99.3%	Slight odour at 25 °C	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
	US EPA OPPTS 830.6304 - Odor GLP	TGAI 030-050914-1G 94.7%	Odour characteristic of garlic at 25 °C	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006b (QNP-0007)

<table>
<tr><th>Property (Annex point as reference to the DAR)</th><th>Method</th><th>Material / Batch</th><th>Results</th><th>Conclusion/Comment</th><th>Reference (Study)</th></tr>
<tr><td>B.2.1.9.1
Spectra of the active substance [UV/VIS]
(IIA 2.5.1.1)</td><td>US EPA OPPTS 830.7050
OECD 101
GLP</td><td>PGAI
R-4CM03G
99.3%</td><td>UV/Vis: Spectra in 90/10 v/v/ water/methanol measured in acidic (addition of aqueous HCl), unadjusted and basic solutions (addition of aqueous NaOH).

<table><tr><th>Solution</th><th>λ_{max} (nm)</th><th>ε [L x · cm^{-1} x mol^{-1}]</th></tr><tr><td rowspan="2">Acidic
pH 1.4-1.5</td><td>243</td><td>16600</td></tr><tr><td>274</td><td>13800</td></tr><tr><td rowspan="2">Unadjusted
pH 7.8-8.1</td><td>243</td><td>16700</td></tr><tr><td>274</td><td>13900</td></tr><tr><td>Basic
pH 12.7</td><td colspan="2">No maxima due to decomposition in basic medium.</td></tr></table></td><td>Acceptable</td><td>Sweetapple, G.G. & Lentz, N.R., 2006a
(QNP-0006)</td></tr>
<tr><td>B.2.1.9.2
Spectra of the active substance [IR]
(IIA 2.5.1.2)</td><td>US EPA OPPTS 830.7050
GLP</td><td>PGAI
R-4CM03G
99.3%</td><td>IR spectrum provided and consistent with the structure of S-2188.

<table><tr><th>cm^{-1}</th><th>Assignment</th></tr><tr><td>3423</td><td>N-H stretch</td></tr><tr><td>2970, 2932</td><td>C-H stretch</td></tr><tr><td>1668</td><td>C=O stretch</td></tr></table></td><td>Acceptable</td><td>Sweetapple, G.G. & Lentz, N.R., 2006a
(QNP-0006)</td></tr>
<tr><td>B.2.1.9.3
Spectra of the active substance [NMR]
(IIA 2.5.1.3)</td><td>US EPA OPPTS 830.7050
GLP</td><td>PGAI
R-4CM03G
99.3%</td><td>^{1}H and ^{13}C NMR spectra provided and consistent with the structure of S-2188.
This is demonstrated by the chemical shift peak assignments.</td><td>Acceptable</td><td>Sweetapple, G.G. & Lentz, N.R., 2006a
(QNP-0006)</td></tr>
<tr><td>B.2.1.9.4
Spectra of the active substance [MS]
(IIA 2.5.1.4)</td><td>US EPA OPPTS 830.7050
GLP</td><td>PGAI
R-4CM03G
99.3%</td><td>Electron Impact Mass Spectrum (EI/MS) provided and consistent with structure of S-2188.</td><td>Acceptable</td><td>Sweetapple, G.G. & Lentz, N.R., 2006a
(QNP-0006)</td></tr>
<tr><td>B.2.1.9.5
Wavelengths at which UV/VIS molecular extinction occurs, where appropriate, to</td><td></td><td>PGAI
R-4CM03G
99.3%</td><td>Measurements up to 750 nm show no more absorptions as reported in B.2.1.9.1.</td><td>Acceptable
No absorption above 290 nm.</td><td>Sweetapple, G.G. & Lentz, N.R., 2006a
(QNP-0006)</td></tr>
</table>

Property (Annex point as reference to the DAR)	Method	Material / Batch	Results	Conclusion/Comment	Reference (Study)
include a wavelength at the highest absorption above 290 nm (IIA 2.5.1.5)					
B.2.1.9.6 Optical purity (IIA 2.5.1.6)			Not relevant since active substance is no resolved isomer		
B.2.1.10 Spectra of relevant impurities (IIA 2.5.2)			Not relevant since no impurities of toxicological or environmental concern are stated.		
B.2.1.11 Solubility in water (IIA 2.6)	Japanese MAFF (12-Nousan-No. 8147, Part 2-9-8, 2000) OECD 105, US EPA OPPTS 830.7840, and EEC Method A6 shake flask method; determination HPLC GLP	PGAI R-4CM03G 99.3%	Water solubility at neutral pH at 20 °C: 20.4 mg/L The effect of pH on water solubility was not determined as S-2188 does not dissociate under acidic or basic conditions.	Acceptable	Lentz, N.R., 2005a (QNP-0003)
B.2.1.12 Solubility in organic solvents (IIA 2.7)	US EPA OPPTS 830.7840 OECD 105 determination HPLC GLP	PGAI R-4CM03G 99.3%	n-hexane: 902 mg/L n-octanol: 84403 mg/L (99174 mg/kg) toluene: 112978 mg/L (126297 mg/kg) acetone: > 250 g/L (> 250 g/kg) methanol: > 250 g/L (> 250 g/kg) dichloromethane: > 250 g/L (>250 g/kg) ethyl acetate: > 250 g/L (> 250 g/kg)	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
	US EPA OPPTS 830.7840 OECD 105 determination HPLC GLP	TGAI 030-050914-1G 94.7%	n-hexane: 811 mg/L n-octanol: 99223 mg/L (105230 mg/kg) toluene: 129308 mg/L (132262 mg/kg) acetone: > 250 g/L (> 250 g/kg)	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006b (QNP-0007)

<table>
<tr><th>Property (Annex point as reference to the DAR)</th><th>Method</th><th>Material / Batch</th><th>Results</th><th>Conclusion/Comment</th><th>Reference (Study)</th></tr>
<tr><td></td><td></td><td></td><td>methanol: > 250 g/L (> 250 g/kg)
dichloromethane: > 250 g/L (>250 g/kg)
ethyl acetate: > 250 g/L (> 250 g/kg)</td><td></td><td></td></tr>
<tr><td>B.2.1.13
Partition coefficient n-octanol/water
(IIA 2.8.1)</td><td rowspan="2">Japanese MAFF (12-Nousan-No. 8147, Part 2-9-11, 2000)
OECD 107
US EPA OPPTS 830.7550
EEC Method A8
shake flask method
GLP</td><td rowspan="2">PGAI
R-4CM03G
99.3%</td><td>n-octanol/water partition coefficient: 3307.32
log Pow = 3.52 at 25 ± 1 ºC (pH: 7.2)</td><td rowspan="2">Acceptable</td><td rowspan="2">Lentz, N.R., 2005b
(QNP-0002)</td></tr>
<tr><td>Effect of pH (4-10) on the n-octanol/water partition co-efficient
(IIA 2.8.2)</td><td>The effect of pH on partition coefficient was not determined as S-2188 does not dissociate under acidic or basic conditions.</td></tr>
<tr><td>B.2.1.16
Quantum yield
(IIA 2.9.3)</td><td>Japanese MAFF (12-Nousan-No. 8147, Part 2-6-2, 2000)
EPA Pesticide Assessment Guidelines, Sub-division N, Section 161-2
SETAC Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, Section 10
GLP</td><td>[Phenyl-^{14}C] S-2188 batch number CFQ 14367
Radio-chemical purity: 99.4%

[Pyrazolyl-5-^{14}C] S-2188 batch number CFQ 14368
Radio-chemical purity: 99.4%</td><td>Quantum yield in pH 7 buffer: 0.021</td><td>Acceptable</td><td>Lewis, C.J. & Troth, K., 2007d
(QNM-0029)</td></tr>
<tr><td>B.2.1.17
Lifetime in the top layer of aqueous systems (calculated and real)
(IIA 2.9.4)</td><td>Japanese MAFF (12-Nousan-No. 8147, Part 2-6-2, 2000)
EPA Pesticide Assessment Guidelines, Sub-division N, Section</td><td>[Phenyl-^{14}C] S-2188 batch number CFQ 14367
Radio-chemical</td><td>Photodegradation in sterile water at pH 7 and 25 ºC in summer sunlight at UK/US conditions(ca 25 Watt/m2 at 300-400 nm):

<table><tr><th>Label</th><th>DT_{50} (days)</th><th>DT_{90} (days)</th></tr><tr><td>Pyrazolyl</td><td>1.7</td><td>5.5</td></tr></table></td><td>Acceptable</td><td>Lewis, C.J. & Troth, K., 2007d
(QNM-0029)</td></tr>
</table>

Property (Annex point as reference to the DAR)	Method	Material / Batch	Results	Conclusion/Comment	Reference (Study)
	161-2 SETAC Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, Section 10 GLP	purity: 99.4% [Pyrazolyl-5-^{14}C] S-2188 batch number CFQ 14368 Radio-chemical purity: 99.4%	Phenyl 1.6 5.4		
B.2.1.18 Dissociation constant (pKa) (IIA 2.9.5)	Japanese MAFF (12-Nousan-No. 8147, Part 2-9-14, 2000) US EPA OPPTS 830.7370 OECD 112 spectrophotometric method GLP	PGAI R-4CM03G 99.3%	In a pH screening experiment on S-2188, absorbance bands at 243 and 273 nm were observed in acidic and unadjusted 90/10 v/v water/methanol solutions (~ pH 1 and 7). A shift to lower wavelengths was observed in basic solution (~ pH 13). This shift was not reversible, indicating that the shift was due to basic decomposition rather than dissociation. No dissociation activity was observed in the approximate pH range 1 – 13.	Acceptable	Beckwith, R.C. & DiFrancesco, D., 2005 (QNP-0001)
B.2.1.19 Stability in air, photochemical oxidative degradation (IIA 2.10)	Atkinson calculation, performed using Atmospheric Oxidation programme EPIWIN (AOPWIN v 1.9) GLP	--	Photochemical reaction with OH radicals. Assuming a 12 hr day and a hydroxyl radical concentration of 1.5 x 10^6 OH/cm³ (EPA), decomposition half life was calculated to be 1.221 hrs. i.e. <2 days.	Acceptable	Liney, P. & Jarvis, T., 2009 (QNM-0032)
B.2.1.20 Flammability (IIA 2.11.1)	EEC Method A.10 GLP	TGAI 030-050914-1G 94.7%	In the preliminary screen S-2188 did not ignite. Not classified as flammable.	Acceptable according to Directive 67/548/EEC. The result is acceptable according to Regulation 1272/2008 as well. No classification.	Sweetapple, G.G. & Lentz, N.R., 2006b (QNP-0007)
B.2.1.21 Auto-flammability	EEC Method A.16 - Relative self-ignition temperature	TGAI 030-050914-1G	S-2188 showed no exothermic reaction. Not auto-flammable. (Measurement up to 400 °C).	Acceptable according to Directive 67/548/EEC.	Weissenfeld,M., 2009

Property (Annex point as reference to the DAR)	Method	Material / Batch	Results	Conclusion/Comment	Reference (Study)
(IIA 2.11.2)	GLP	94.7%		According to the Regulation 1272/2008 no test procedure for self heating (N.4) is required if the substance is completely molten at 160 °C. This is demonstrated by DSC plots for determination of melting point and boiling point [Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)] No classification.	(QNP-0014)
B.2.1.22 Flash point (IIA 2.12)			Not required, as S-2188 does not melt below 40 ºC.		
B.2.1.23 Explosive properties (IIA 2.13)	US EPA OPPTS 830.6316 GLP	TGAI 030-050914-1G 94.7%	Preliminary thermal explodability screen: No evidence of explodability observed up to 200ºC. Impact explodability: No evidence of explodability at the maximum impact drop height.	Test is not according to EEC A14.	Sweetapple, G.G. & Lentz, N.R., 2006b (QNP-0007)
	Statement		Evaluation based on oxygen balance and structural consideration: The calculated oxygen balance is -205.2%. This value is considered to be outside of the potential for explosivity. The chemical structure does not indicate any potential for explosivity.	Statement is acceptable S-2188 (Fenpyrazamine) is considered having no explosive properties according to Directive 67/548/EEC. The statement is acceptable according to Regulation 1272/2008 as well. No classification.	Asada, Y., 2010 (QNP-0019)
B.2.1.24 Surface tension	OECD 115	TGAI 030-050914-1G	Surface tension: 66.9 mN/m at a concentration of 90% of the saturation solubility and 20 ºC.	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006b

Property (Annex point as reference to the DAR)	Method	Material / Batch	Results	Conclusion/Comment	Reference (Study)
(IIA 2.14)	EEC Method A.5 GLP	94.7%			(QNP-0007)
B.2.1.25 Oxidizing properties (IIA 2.15)	Statement according to EEC Method A17		An examination of the structure of S-2188 reveals that it contains none of the reactive groups or oxidizing compounds known to increase oxidizing power. It does contain some electronegative atoms (N, S, O), but these are bonded only to carbon and/or hydrogen, and therefore, are unlikely to add to the oxidizing power. The structural examination of S-2188 suggests it is not likely to possess oxidizing properties.	Acceptable The statement is acceptable according to Regulation 1272/2008 as well. No classification.	Liney, P. & Jarvis, T., 2009 (QNP-0008)
B.2.1.2.26 pH (IIA 2.16)			Not required for EU		
B.2.1.2.27 Storage stability (IIA 2.17.1)			Not required for EU		
B.2.1.2.28 Stability (temperature, metals) (IIA 2.17.2)			Not required for EU		
B.2.1.2.29 Other/special studies (IIA 2.18)			None		

According to Directive 91/414/EEC, granulometry is not required for active substances. Thus, no study considering this end-point has been provided

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for Classification and Labelling.

2.2 Identified uses

Fenpyrazamine is a fungicide to be used for control of grey mould (*Botrytis)*. It is not systemic but there is some translocation in plants. Fenpyrazamine shows its fungicidal activity through inhibition on germ tube elongation and mycelium elongation. However, the biochemical mechanism of fungicidal activity is not clarified to date.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

No classification required.

4 HUMAN HEALTH HAZARD ASSESSMENT

Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Absorption, distribution, excretion and metabolism (toxicokinetics):

Absorption and Excretion

Fenpyrazamine was rapidly absorbed and than rapidly eliminated after single oral administration of 3 mg/kg body weight and 300 mg/kg body weight whereby the absorption was more rapid at low than at high dose. Based on the toxicokinetic parameters investigated (AUC), the systemic availability increased with dose level applied. Longer $T_{½}$ and over proportionally higher AUC at high dose might indicate that in the high dose animals the saturation of elimination could be reached.

Absorption of fenpyrazamine was extensive, and fenpyrazamine was almost totally metabolised after administration of single oral low and high dose. Excretion of the low dose was almost complete within 24 hours and of the high dose within 48 hours. Urine was the most important route of elimination (> 80% for males and females, at low or high doses), and elimination as CO_2 almost insignificant. In the repeated dose study recovery of eliminated radioactivity in excreta after 24 days was 96.5% of administered dose in males and 97.2% in females. The majority of radioactivity was recovered in urine (up to 78.7%) and faeces (up to 13.6%). While the excretion up to last dose administration was high and constant, it declined rapidly after the dosing was finished. After ceasing of administration, only very low amounts of radioactivity were found in urine, cage wash and faeces. The results of excretion (route of excretion and metabolite pattern) obtained in repeated dose study were in line with those from single dose study. The amount of radioactivity found in urine after single and repeated dose administration indicates a high resorption from GI tract.

Distribution

While after the administration of single oral low dose the highest radioactivity concentration in most tissues was reached 1 hour post-dosing, in high dose animals the highest concentration was reached 6 hours post-dosing. This is supported by the pharmacokinetic study showing higher T_{max} in blood/plasma of high dose animals compared to low dose. 7 days after administration of single oral low and high dose, the proportion of dose remaining in tissues was very low. Distribution of radioactivity into tissues was very even. There was no evidence of accumulation, with the exception of stomach and caecum (including contents) which was considered unusual but was not further explained in the study report. There were no significant dose or sex differences in tissue distribution. During repeated dosing the concentration of radioactivity in most tissues maximised approximately up to day 7 and then remained relatively constant for the rest of the dosing period. Tissue residues declined after finishing of dosing period. As noticed in the single dose study, no evidence of unusual accumulation into tissue and no sex differences were observed in the

repeated dose study.

Metabolism

Regarding the metabolite pattern in plasma of low and high dose animals, there were some differences in sex and doses after administration of single low and high dose. While S-2188-DC was the major metabolite in both sexes (but much higher amount were measured in females) and for both doses applied, the unknown metabolite "RT 31 min" was present at high amounts in low dose males but was not quantified in low dose females, which may indicate slightly different metabolite pattern in low dose males and females. For both sexes and doses, the parent fenpyrazamine was minor in plasma at each time point. In high dose animals the difference in metabolites occurrence and amount measured in males and females was less pronounced. In repeated dose study, a mixture of S-2188-CH_2OH-DC and MPPZ was the major identified metabolite fraction in plasma of both males and females.

In liver of low and high dose animals after single exposure, additional unknown metabolites occurred compared to plasma samples, but no identification was performed. These unidentified metabolites made max. 30% and 17% in low dose males and females, respectively and 13% and 15% in high dose males and females. In the repeated dose study, parent fenpyrazamine was a major component in liver (max 9.6% of liver TRR).

The metabolite pattern of kidney was similar to that of liver. The results of plasma, liver and kidney measurement indicate that there are differences between sexes in metabolic degradation pathway of fenpyrazamine, as well as in metabolite pattern depending on the dose applied.

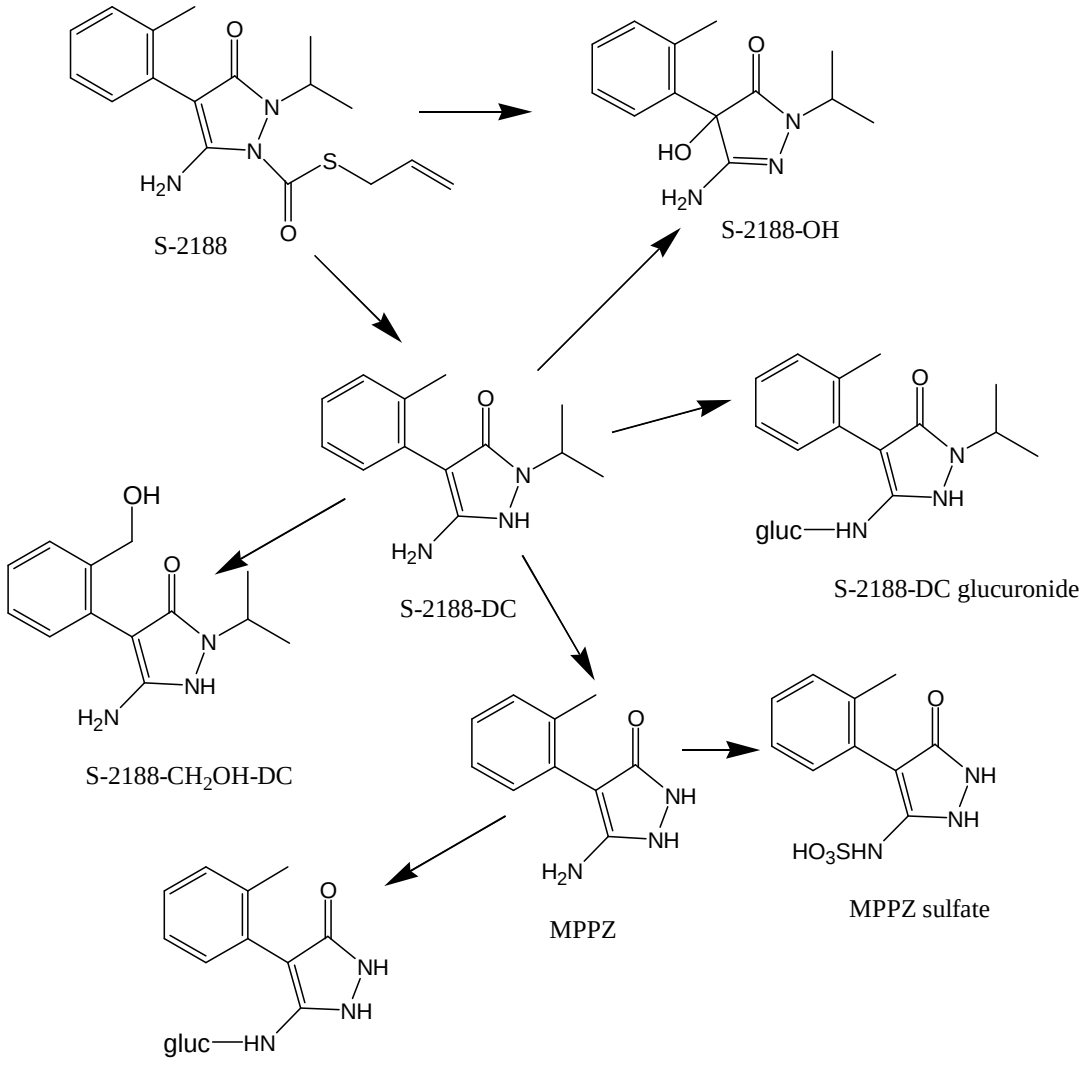
In urine of animals in the repeated dose study, the relative amounts of identified metabolites were similar for both sampling periods (days 2 to 4 and days 13 to 15) and both sexes. In males, MPPZ sulphate and S-2188-CH_2OH-DC contributed mostly to the identified radioactivity in urine, while S-2188-DC was present < 10%. In urine of females, MPPZ, MPPZ sulphate and S-2188-DC contributed evenly to the identified radioactivity. In the animals of single low dose very similar pattern as in repeated dose study was observed. In single high dose males and females S-2188-DC was by far the major component of excreta.

In faeces of animals in the repeated dose study S-2188-CH_2OH-DC was the major metabolite in males (max. 25.1%) and MPPZ (max. 26.6%) in females. MPPZ sulphate was not identified in faeces, neither in males nor in females. Considerable radiolabel was unextractable from faeces.

Elimination of the allylsulfanylcarbonyl group to produce S-2188-DC was the initial step in metabolism. This metabolite was eliminated as a significant fraction in males and females at high and low doses (particularly in females of the low dose). S-2188-DC was hydroxylised to form S-2188-OH in only small amounts. Dealkylation of S-2188-DC to MPPZ was important in both sexes at high and low doses. MPPZ was conjugated with sulphate and with glucuronide (sulphation was more important at the low dose). The proportion of dose identified as metabolites was satisfactory (> 68% in all groups), however the unknown metabolites were not identified. The proposed metabolic pathway is shown in figure below.

Since the identified metabolites like MPPZ (including its conjugates), S-2188-DC and S-2188-CH_2OH-DC are present in rat metabolism > 10% of applied dose, these compounds are considered to contribute to the toxicological profile of fenpyrazamine (parent). S-2188-OH (hydoxylised S-2188-DC) is probably of comparable toxicity as the precursor S-2188-DC since hydroxylation on the pyrazolyl ring might be considered to be a step of detoxification. For S-2188-DC acute toxicity and mutagenicity studies were conducted, which revealed comparable toxicity to parent fenpyrazamine.

Proposed metabolic pathway of fenpyrazamine in mammals:



Dermal absorption: *In vivo* human absorption rate (%) was calculated, as recommended by the "Guidance document on dermal absorption", Sanco/222/2000rev.7, 19 March 2004. For calculations of dermal absorption rate, dermal absorption percentage values (absorbed and potentially bioavailable) were taken into account.

Table 10: Dermal absorption of the formulation (concentrate and dilution):

Study	Species	Dilution	Max. absorption measured (% applied dose)	Max. possible absorption (% applied dose)
Sugimoto K.; 2008 (*in vivo* study)	Rat (♂)	1:10	1.74	1.9
		1:4000 (spray dilution)	13.35	17.9
Hadfield N.; 2006 (*in vitro* study)	Rat skin (♂)	undiluted	0.462	0.855
		1:4000 (spray dilution)	58.6	61.6

Study	Species	Dilution	Max. absorption measured (% applied dose)	Max. possible absorption (% applied dose)
	Human skin	undiluted	0.13	0.041
		1:4000 (spray dilution)	2.19	2.666

Table 11: Dermal absorption values for human skin:

***In vitro* absorption rat skin (% applied dose)**	***In vitro* absorption human skin (% applied dose)**	**Ratio of rat/human skin absorption**	***In vivo* absorption rat skin (% applied dose)**	**Total absorbed human *in vivo* (% applied dose)**
Concentrate				
0.855	0.041	0.855/0.041 = **20.8**	1.9	1.9/20.8 = **0.09**
Spray dilution				
61.6	2.19	61.6 / 2.66 = **23.1**	17.9	17.9/ 23.1 = **0.78**

For human skin *in vivo* absorption rates of 0.09% of the applied dose for the concentrate and 0.78% for the diluted formulation can be calculated. These values have been rounded to 0.1% and 0.8% dermal absorption for the concentrate and the spray dilution, respectively.

4.1.2 Human information

A formal statement from the manufacturer is presented (Ohtsubo, T (2009)), that members of staff involved in the synthesis and development of fenpyrazamine are routinely monitored and that no indication of fenpyrazamine-related ill-health have been detected by, or reported to, medical staff. No poisoning incidents or clinical cases have been reported. Fenpyrazamine is a new chemical currently in development. There has been no exposure of the general population or epidemiology study.

4.1.3 Summary and discussion on toxicokinetics

Rate and extent of oral absorption ‡	Extensive (> 80%), almost totally metabolised after administration of single oral low and high dose
Distribution ‡	Evenly distributed, highest amount in liver and kidney
Potential for accumulation ‡	No potential for accumulation
Rate and extent of excretion ‡	> 80% via urine in males and females
Metabolism in animals ‡	Extensively metabolized; main metabolites in urine and feces: S-2188-DC, MPPZ , MPPZ sulphate, S-2188-CH_2OH-DC

4.2 Acute toxicity

Table 12: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Oral route (OECD 423)	♀ LD_{50} > 2000 mg/kg bw	Wistar rats (BrlHan:WIST@Jcl(GALAS)) Purity 94.7%	Deguchi, Y, 2007a
Dermal route (OECD 402)	♂/♀ LD_{50} > 2000 mg/kg bw	Wistar rats (BrlHan:WIST@Jcl(GALAS)) Purity 94.7%	Deguchi, Y, 2007b
Inhalation route (OECD 403)	♂/♀ LC_{50} > 4.84 mg/L (maximum attainable concentration)	Wistar rats (BrlHan:WIST@Jcl(GALAS)), 4 hours nose only dust inhalation Purity 94.7%	Deguchi, Y, 2007c

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

No deaths and no clinical signs in female rats were observed during the observation period. Body weights of the rats were not affected. Retention of fluid in the uterus was found in one animal, but was considered to be spontaneous, since it was occasionally observed in non-treated rats. No gross abnormalities at necropsy were found.

The oral LD_{50} of fenpyrazamine in female rats was > 2000 mg/kg and does not require classification and labeling for acute oral toxicity.

4.2.1.2 Acute toxicity: inhalation

No mortality was observed. No abnormal findings were observed in any animal during or after the exposure period. Wet fur was observed in both sexes of air control and treatment groups immediately after the exposure period, but disappeared within one hour post-dosing. This finding is considered to be a result of the restraint procedure and not of toxicity related to treatment. Body weights in the treatment groups were comparable to controls. Dark red foci were observed on the lung surface of both sexes of control and treatment groups. Retention of fluid in the uterus was seen in control and treated females. These findings are commonly seen in this strain and age of rat, and were observed in control as well as in treated females.

The 4-hour inhalation LC_{50} was determined to be > 4.84 mg/L (maximum attainable concentration) for male and female rats and therefore, classification and labelling for acute inhalation toxicity of fenpyrazamine is not required.

4.2.1.3 Acute toxicity: dermal

No deaths were observed during the observation period. One male rat was found with scab in the dorsal neck, but this finding was considered to be spontaneous since occasionally observed in non-treated rats. No clinical signs were observed in females. Body weights of the rats were not affected. No treatment-related gross pathological findings were observed.

The dermal LD_{50} of fenpyrazamine in male and female rats was > 2000 mg/kg and does not require classification and labelling for acute dermal toxicity.

4.2.1.4 Acute toxicity: other routes

No data on other routes available.

4.2.2 Human information

A formal statement from the manufacturer is presented (Ohtsubo, T (2009)), that members of staff involved in the synthesis and development of fenpyrazamine are routinely monitored and that no indication of fenpyrazamine-related ill-health have been detected by, or reported to, medical staff. No poisoning incidents or clinical cases have been reported. Fenpyrazamine is a new chemical currently in development. There has been no exposure of the general population or epidemiology study.

4.2.3 Summary and discussion of acute toxicity

Fenpyrazaminel has low acute oral, dermal and inhalation toxicity (oral LD_{50} > 2000 mg/kg bw, dermal LD_{50} > 2000 mg/kg bw, LC_{50} > 4.84 mg/L air) in rats.

4.2.4 Comparison with criteria

All estimated LD_{50} values are above the criteria for classification and labelling (both DSD and CLP).

4.2.5 Conclusions on classification and labelling

No classification and labelling is proposed for fenpyrazamine regarding acute toxicity.

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

No specific, non lethal, target organ toxicity after single exposure was observed in acute toxicity studies and acute neurotoxicity study. Almost no clinical effects were observed. No classification as STOT-SE under the CLP Regulation is proposed.

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

No specific target organ toxicity after single exposure was observed in acute toxicity studies and acute neurotoxicity study.

4.3.2 Comparison with criteria

No effects observed in acute toxicity studies and acute neurotoxicity study would trigger criteria for classification and labelling as STOT SE.

4.3.3 Conclusions on classification and labelling

No classification and labelling is proposed for fenpyrazamine regarding specific target organ toxicity after single exposure.

4.4 Irritation

4.4.1 Skin irritation

Table 13: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Primary skin irritation (OECD 404)	Not irritating	New Zealand White Rabbit Purity: 94.7%	Odawara, K, 2007a

4.4.1.1 Non-human information

No signs of ill health or toxicity were observed in any of the animals during the experimental period. No skin irritation reactions were observed in any animal during the observation period of 72 hours after the removal of the patches.

The irritation was calculated to be 0.0 (no irritating) and therefore fenpyrazamine does not require classification and labelling for skin irritation.

4.4.1.2 Human information

A formal statement from the manufacturer is presented (Ohtsubo, T (2009)), that members of staff involved in the synthesis and development of fenpyrazamine are routinely monitored and that no indication of fenpyrazamine-related ill-health have been detected by, or reported to, medical staff. No poisoning incidents or clinical cases have been reported. Fenpyrazamine is a new chemical currently in development. There has been no exposure of the general population or epidemiology study.

4.4.1.3 Summary and discussion of skin irritation

According to the results of the rabbit skin irritation study, fenpyrazamine is not irritant to the intact shaved rabbit skin.

4.4.1.4 Comparison with criteria

Estimated skin irritation scores (0.00) are below the criteria for triggering classification and labelling (according to both DSD and CLP).

4.4.1.5 Conclusions on classification and labelling

No classification and labelling is proposed for fenpyrazamine regarding skin irritation.

4.4.2 Eye irritation

Table 14: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Eye irritation (OECD 405)	Slight irritating	New Zealand White rabbit Purity: 94.7%	Odawara, K, 2007b

4.4.2.1 Non-human information

No signs of ill health or toxicity were observed in any of the animals during the experimental period. Two of the three animals in the unwashed group showed very slight conjunctival redness, chemosis, and discharge (all grade 1) after test compound application, but these reactions had disappeared at latest after 48 hours. Two of three animals in the washed group showed very slight conjunctival redness (grade 1), and one of three animals showed very slight conjunctival chemosis (grade 1) after test compound application, and these reactions had also disappeared at latest 48 hours after application. The scores of eye irritation test are given in tables below (unwashed and washed group).

Table 15: Eye irritation scores (unwashed group)

		Rabbit Nr.	1 hour	24 hours	48 hours	72 hours	Mean/Rabbit (24 h + 48 h + 72 h)
Conjunctiva	**Chemosis**	1	0	0	0	0	0
		2	1	0	0	0	0
		3	1	0	0	0	0
	Redness	1	0	0	0	0	0
		2	1	0	0	0	0
		3	1	1	0	0	0.3
	Discharge	1	0	0	0	0	0
		2	1	0	0	0	0
		3	1	0	0	0	0
Iris	**Congestion**	1	0	0	0	0	0

		Rabbit Nr.	1 hour	24 hours	48 hours	72 hours	Mean/Rabbit (24 h + 48 h + 72 h)
		2	0	0	0	0	0
		3	0	0	0	0	0
Cornea	**Opacity**	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0

Table 16: Eye irritation scores (washed group)

		Rabbit Nr.	1 hour	24 hours	48 hours	72 hours	Mean/Rabbit (24 h + 48 h + 72 h)
Conjunctiva	**Chemosis**	4	1	0	0	0	0
		5	0	0	0	0	0
		6	0	0	0	0	0
	Redness	4	1	1	0	0	0.3
		5	0	0	0	0	0
		6	1	0	0	0	0
	Discharge	4	0	1	0	0	0.3
		5	0	0	0	0	0
		6	0	0	0	0	0
Iris	**Congestion**	4	0	0	0	0	0
		5	0	0	0	0	0
		6	0	0	0	0	0
Cornea	**Opacity**	4	0	0	0	0	0
		5	0	0	0	0	0
		6	0	0	0	0	0

Fenpyrazamine caused only mild transient ocular irritancy and therefore does not require classification and labelling for eye irritation.

4.4.2.2 Human information

A formal statement from the manufacturer is presented (Ohtsubo, T (2009)), that members of staff involved in the synthesis and development of fenpyrazamine are routinely monitored and that no indication of fenpyrazamine-related ill-health have been detected by, or reported to, medical staff. No poisoning incidents or clinical cases have been reported. Fenpyrazamine is a new chemical currently in development. There has been no exposure of the general population or epidemiology study.

4.4.2.3 Summary and discussion of eye irritation

According to the results of the eye irritation study, fenpyrazamine is slight irritant to the rabbit eye; according to classification criteria, classification and labelling is not warranted.

4.4.2.4 Comparison with criteria

Estimated eye irritation scores (24 – 72 hours; 0.3 (conjunctival redness), 0.3 (conjuctival discharge)) are below the criteria for triggering classification and labelling (according to both DSD and CLP).

4.4.2.5 Conclusions on classification and labelling

No classification and labelling is proposed for fenpyrazamine regarding eye irritation.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

In the acute inhalation toxicity study no mortality was observed. No abnormal findings were observed in any animal during or after the exposure period. Wet fur was observed in both sexes of air control and treatment groups immediately after the exposure period, but disappeared within one hour post-dosing. This finding is considered to be a result of the restraint procedure and not of toxicity related to treatment. Body weights in the treatment groups were comparable to controls. Dark red foci were observed on the lung surface of both sexes of control and treatment groups. Retention of fluid in the uterus was seen in control and treated females. These findings are commonly seen in this strain and age of rat, and were observed in control as well as in treated females. No signs of irritation on respiratory tract were observed.

4.4.3.2 Human information

A formal statement from the manufacturer is presented (Ohtsubo, T (2009)), that members of staff involved in the synthesis and development of fenpyrazamine are routinely monitored and that no indication of fenpyrazamine-related ill-health have been detected by, or reported to, medical staff. No poisoning incidents or clinical cases have been reported. Fenpyrazamine is a new chemical currently in development. There has been no exposure of the general population or epidemiology study.

4.4.3.3 Summary and discussion of respiratory tract irritation

No respiratory tract irritation was observed in acute inhalation toxicity study in rats.

4.4.3.4 Comparison with criteria

No irritating effects on respiratory tract were observed in acute inhalation study with fenpyrazamine (according to both DSD and CLP).

4.4.3.5 Conclusions on classification and labelling

No classification and labelling is proposed for fenpyrazamine regarding respiratory tract irritation.

4.5 Corrosivity

Fenpyrazamine did not show any corrosive properties in rabbit skin and eye irritation studies (see 4.4.1 and 4.4.2).

4.6 Sensitisation

4.6.1 Skin sensititsation

Table 17: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Skin sensitization (M&K-test) (OECD 406)	Not sensitising	SIc:Hartley Guinea pig Purity: 94.7%	Odawara, K.; 2007c

4.6.1.1 Non-human information

No signs of ill health of toxicity were observed in any of the animals during the experimental period. The body weights of all animals increased normally during the experimental period.

Slight erythema was observed in two out of 20 animals (sensitisation rate: 10%) sensitised with the test substance. In the control group, skin reactions were not observed in any of the 10 animals. In the previously sensitised HCA group slight to moderate erythema were observed in 4 out of 5 animals. In the HCA control group no skin reactions were observed.

The results of the main sensitisation study are given in table below.

Table 18: Results of dermal sensitisation study

Group name		**Sensitised**				**Control**			
Test substance used for the induction treatment		S-2188 Technical Grade				-			
Test substance used for the challenge treatment		S-2188 Technical Grade				S-2188 Technical Grade			
Number of animals used		20				10			
Concentration									
Induction (intradermal)		5%[a]				-			
Induction (topical)		50%[b]				-			
Challenge (topical)		25%[b]				25%[b]			
Observation time[c]		24 hours		48 hours		24 hours		48 hours	
Skin reaction[d]		E	S	E	S	E	S	E	S
Grade[e]	**0**	18	20	18	20	10	10	10	10

Group name		Sensitised				Control			
	1	2	0	2	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0

a – Corn oil was used as the vehicle
b – Acetone was used as the vehicle
c – Time after removal of the patch
d – E: erythema, S: swelling
e – 0: no reaction, 1: slight, 2: moderate, 3: severe

In the dermal sensitisation test using an adjuvant, fenpyrazamine gave a dermal response of minimal grade in 2/20 animals (10%). This level of response does not meet the criterion for classification as a sensitizer within the EU (30% response required using an adjuvant method).

4.6.1.2 Human information

A formal statement from the manufacturer is presented (Ohtsubo, T (2009)), that members of staff involved in the synthesis and development of fenpyrazamine are routinely monitored and that no indication of fenpyrazamine-related ill-health have been detected by, or reported to, medical staff. No poisoning incidents or clinical cases have been reported. Fenpyrazamine is a new chemical currently in development. There has been no exposure of the general population or epidemiology study.

4.6.1.3 Summary and discussion of skin sensitisation

According to the results of the skin sensitisation study in guinea pig (Maximisation test), fenpyrazamine is not sensitising to guinea pig skin; according to classification criteria, classification and labelling is not warranted.

4.6.1.4 Comparison with criteria

Effects observed in the skin sensitisation study on guinea pig are below the criteria for triggering classification and labelling (according to both DSD and CLP).

4.6.1.5 Conclusions on classification and labelling

No classification and labelling is proposed for fenpyrazamine regarding skin sensitisation.

4.6.2 Respiratory sensitisation

No data on respiratory sensitisation available.

4.7 Repeated dose toxicity

Table 19: Summary table of relevant repeated dose toxicity studies

Method	Cut off values according to DSD and CLP [mg/kg bw/d]	Dose range / NOAEL	Remarks	Reference
Rat, 90-days (oral)	DSD: 50	0, 300, 600, 1000, 3000	Wistar;	Sommer,

(OECD 408)	CLP: STOT RE 1: 10 CLP: STOT RE 2: 100	ppm equivalent to: 0, 19.1, 37.7, 64.0 and 196.1 mg/kg bw/d in males and 0, 20.5, 42.0, 68.6 and 207.3 mg/kg bw/d in females NOAEL: 1000 ppm 64 mg/kg bw/d (males) 68.6 mg/kg bw/d (females) Main effects at 3000 ppm (196.1 mg/kg bw/d in males) and 207.3 mg/kg bw/d in females): - ↓ body weight ♂ - ↓ body weight gain ♂ - ↑ abs and rel liver weight ♂ ♀ - hepatocellular hypertrophy ♂ ♀ - thyroid follicular cell hyperthrophy ♂ - urinary bladder congestion and dilation ♂	HanRcc:WIST(SPF) rats Purity: 94.7%	E.W., Flade, D., Gretener, P., Krinke, G, 2006
Mouse, 90-days (oral) (OECD 408)	DSD: 50 CLP: STOT RE 1: 10 CLP: STOT RE 2: 100	0, 200, 2000, 4000, 6000 ppm equivalent to: 0, 28.0, 296.2, 639.5 and 1022.6 mg/kg bw/d in males and 0, 33.5, 363.3, 719.6 and 1098.3 mg/kg bw/d in females NOAEL: 200 ppm 28.0 mg/kg bw/d (males) 33.5 mg/kg bw/d (females) Main effects at 2000 ppm (296.2 mg/kg bw/d in males and 363.3 mg/kg bw/d in females): - ↓ body weight gain ♂ - ↑ abs and rel liver weight ♂ ♀	CD-1 (ICR)BR (SPF) mouse Purity: 94.7%	Sommer, E.W., Flade, D., Gretener, P., Weber, K, 2007

		- hepatocellular hypertrophy ♂ ♀ - ↓ bilirubin ♂		
Dog, 90-days (oral) (OECD 409)	?*	0, 25, 50, 150 mg/kg bw/d NOAEL: 25 mg/kg bw/d (males and females) Main effects at 50 mg/kg bw/d: - ↑ rel liver weight ♂ - hepatocellular hypertrophy ♂ ♀	Beagle dogs Purity: 94.7%	Sato, S, 2008
Dog, 1-year (oral) (OECD 452)	?*	0, 5, 25, 100 mg/kg bw/d NOAEL: 25 mg/kg bw/d (males and females) Main effects at 100 mg/kg bw/d: - ↑ liver weight ♂ ♀ - hepatocellular hypertrophy ♂ ♀ - changes in haematology and clinical chemistry parameters ♂ ♀	Beagle dogs Purity: 94.7%	Sato, S, 2009
Rat, 28-days (dermal) (OECD 410)	DSD: 300 CLP: STOT RE 1: 60 CLP: STOT RE 2: 600	0, 100, 300, 1000 mg/kg bw/d NOAEL: 300 mg/kg bw/d (males) 1000 mg/kg bw/d (females) Main effects at 1000 mg/kg bw/d (males): - ↓ haemoglobin (-2.6%) and haematocrit (-3%) ♂ - shortened prothrombin time (-14.9%) ♂	Crl:CD (Sprague Dawley) rats Purity: 94.7%	Ogata, H, 2008
Rat oral via diet, 104 weeks (OECD 453)	DSD: 25 CLP: STOT RE 1: < 5 CLP: STOT RE 2: < 50	0, 100, 300, 1200, 2400 ppm equivalent to 0, 4.25, 12.7, 51.9 and 107 mg/kg bw/day (males)	HanRcc:WIST(SPF) rats Purity: 94.7%	Sommer, E.W., 2009

		and 0, 5.29, 15.6, 63.6 and 130 mg/kg bw/d (females) <u>NOAEL</u>: 12.7 mg/kg bw/d (males) 15.6 mg/kg bw/d (females) <u>Main effects at 51.9 mg/kg bw/d (males) and 63.6 mg/kg bw/d (females)</u>: - ↑ hepatocellular hypertrophy ♂ - ↑ relative liver weight ♂ - ↓ MCV and neutrophils ♂ - ↑ Albumin ♂ - GGT ♂ - Shortened prothrombin time and decreased MCH and MCV ♀		
Mouse oral via diet, 78 weeks (OECD 451)	DSD: 25 CLP: STOT RE 1: < 5 CLP: STOT RE 2: < 50	0, 100, 1500 and 3000 ppm in males and 0, 100, 2000 and 4000 ppm in females equivalent to 0, 11.1, 176 and 349 mg/kg bw/d in males and 0, 13.9, 283 and 552 mg/kg bw/d in females <u>NOAEL</u>: 176 mg/kg bw/d (males) 283 mg/kg bw/d (females) <u>Main effects at 349 mg/kg bw/d (males) and 552 mg/kg bw/d (females)</u>: - ↑ absolute and relative liver weight ♂♀	CD-1, Crl:CD1(Icr) mice Purity: 94.7%	Sommer, E.W., 2009b

		- ↑ hepatocellular hypertrophy ♂♀ - ↑ MCV and MCH ♂ - ↓ RBC ♂ - ↓ RBC, HB and HCT ♀		

*For cut off values in dog studies, the only available document is ECBI/64/06 "Dose limits for classification with R48 based on dogs studies", 2006. In this document it is proposed that the cut off values for dog studies should be below the limit dose for the rat, but no further information is found. Since no cut off values for dog studies are available until now, we took the information from dog studies just as supporting information.

*+ For extrapolation from subchronic to chronic studies in rodents regarding cut off values for effects observed, different approaches were found: whereas in the ECBI/64/06 "Dose limits for classification with R48 based on dogs studies", 2006, the cut off value for chronic studies in rodents of 6.25 mg/kg bw/d is found, in the REACH guidance on information requirements and chemical safety assessment, chapter R8 is stated that factor of 2 should be applied resulting in the cut off value of 25 mg/kg bw/d in chronic studies in rodents.

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Rat:

13 week feeding study

Based on reduced mean body weight and body weight gain, increased absolute and relative liver weight with hepatocellular hypertrophy in males and females at 3000 ppm (196.1 mg/kg bw/d in males and 207.3 mg/kg bw/d in females):, additionally thyroid follicular cell hypertrophy and urinary bladder congestion and dilation in males at 3000 ppm, the NOAEL was determined to be 1000 ppm for both genders, equivalent to 64 mg/kg bw/d for males and 68.6 mg/kg bw/d for females. No neurotoxic effects were observed in the study. Taking into consideration the cut off values for repeated dose toxicity (DSD: 50 mg/kg bw/d, CLP, STOT RE 1: 10 mg/kg bw/d, CLP, STOT RE 2: 100 mg/kg bw/d) and the nature of the observed effects, no classification and labelling for repeated dose toxicity is triggered for fenpyrazamine.

104 weeks feeding study

In the combined chronic and carcinogenicity study (2 years) in rat, the NOAEL is set at 300 ppm (15.6 mg/kg bw/d) in females, based on shortened prothrombin time and decreased MCH and MCV at 1200 ppm. In males, the NOAEL is set also at 300 ppm (12.7 mg/kg bw/d), based on statistically significantly increased hepatocellular hypertrophy accompanied by increased relative liver weight at 1200 ppm at week 52 in satellite group and hepatocellular hypertrophy (but without change in relative liver weight) in main group males at week 104. In addition, in males of 1200 ppm group and above (week 52, satellite group), MCV and neutrophils were statistically significantly decreased and albumin increased. Taking into consideration the cut off values for repeated dose toxicity (DSD: 25 mg/kg bw/d, CLP, STOT RE 1: < 5 mg/kg bw/d, CLP, STOT RE 2: < 50 mg/kg bw/d) and the nature of the observed effects, no classification and labelling for repeated dose toxicity is triggered for fenpyrazamine.

Mouse:

13 week feeding study

In the 90 days study in mice, the NOAEL for fenpyrazamine is determined at 200 ppm for males (equivalent to 28.0 mg/kg bw/d) and females (equivalent to 33.5 mg/kg bw/d) based on significantly reduced body weight gain, significantly increased absolute and relative liver weight, significantly increased hepatocellular hypertrophy and significantly decreased bilirubin level in males at 2000 ppm (296.2 mg/kg bw/d) and significantly increased relative liver weight and hepatocellular hypertrophy in females at 2000 ppm (363.3 mg/kg bw/d). Taking into consideration the cut off values for repeated dose toxicity (DSD: 50 mg/kg bw/d, CLP, STOT RE 1: 10 mg/kg bw/d, CLP, STOT RE 2: 100 mg/kg bw/d) and the nature of the observed effects, no classification and labelling for repeated dose toxicity is triggered for fenpyrazamine.

78 weeks feeding study

In the carcinogenicity study in CD-1 mouse (78 weeks), the NOAEL for males is set at 1500 ppm (176 mg/kg bw/d), based on statistically significantly higher relative liver weight in satellite and main group animals accompanied by higher incidence of hepatocellular hypertrophy observed in main group animals, and supported by statistically significantly increased MCV and MCH and decreased RBC at 3000 ppm. In females, NOAEL is set at 2000 ppm (283 mg/kg bw/d), based on statistically significantly higher relative liver weight in satellite and main group animals accompanied by statistically significantly increased hepatocellular hypertrophy observed in main group animals, and supported by statistically significantly decreased RBC, HB and HCT at 4000 ppm. Taking into consideration the cut off values for repeated dose toxicity (DSD: 25 mg/kg bw/d, CLP, STOT RE 1: < 5 mg/kg bw/d, CLP, STOT RE 2: < 50 mg/kg bw/d), no classification and labelling for repeated dose toxicity is triggered for fenpyrazamine.

Dog:

3 months feeding study

In the 90 days study in dog the NOAEL for males and females is proposed at 25 mg/kg bw/d, based on increased mean relative liver weight (12.9% compared to control) and hepatocyte hypertrophy in two males at 50 mg/kg group and on hepatocellular hypertrophy (but without changes in liver weight) in females. Since no cut off values for dog studies are available, neither according to DSD not to CLP, the information from dog studies can be taken only as supportive information.

1 year feeding study

In the 1-year dog study the NOAEL for males is proposed at 25 mg/kg bw/d, based on significantly increased mean absolute liver weight and slight hepatocyte hypertrophy in three males at 100 mg/kg group, as well as based on statistically significant increase in MCHC and ALP and decrease in MCV. In females, the NOAEL is proposed at 25 mg/kg bw/d, based on increased relative liver weight (+10.4%) and slight hepatocyte hypertrophy in two animals at 100

mg/kg, supported by increased platelet count. Since no cut off values for dog studies are available, neither according to DSD not to CLP, the information from dog studies can be taken only as supportive information.

4.7.1.2 Repeated dose toxicity: inhalation

No repeated dose inhalation studies are available.

4.7.1.3 Repeated dose toxicity: dermal

28 days dermal study

In the dermal 28-days study in rats dosed at 100, 300, and 1000 mg/kg, only statistically significantly lower values for hemoglobin (-2.6%) and hematocrit (-3%) and shortened prothrombin time (-14.9%) were seen in males at 1000 mg/kg bw/d. No effects in females were noted. Based on the observed results, the NOAEL was considered to be 300 mg/kg bw/day for male and 1000 mg/kg bw/d for female rats. Taking into consideration the cut off values for repeated dose toxicity (DSD: 300 mg/kg bw/d, CLP, STOT RE 1: 60 mg/kg bw/d, CLP, STOT RE 2: 600 mg/kg bw/d) and the severity of observed effects, no classification and labelling for repeated dose toxicity is triggered for fenpyrazamine.

4.7.1.4 Repeated dose toxicity: other routes

No data on other routes available.

4.7.1.5 Human information

A formal statement from the manufacturer is presented (Ohtsubo, T (2009)), that members of staff involved in the synthesis and development of fenpyrazamine are routinely monitored and that no indication of fenpyrazamine-related ill-health have been detected by, or reported to, medical staff. No poisoning incidents or clinical cases have been reported. Fenpyrazamine is a new chemical currently in development. There has been no exposure of the general population or epidemiology study.

4.7.1.6 Other relevant information

No other relevant information.

4.7.1.7 Summary and discussion of repeated dose toxicity

A series of studies were carried out to investigate the effects of orally administered fenpyrazamine in rats (one 90-day study), mice (one 90-day study) and dogs (one 90-day and one 1-year study) following repeated exposure via the oral route over subchronic periods. In addition, the effects after repeated exposure via the dermal route were also investigated in the rat (one 28-day study).
In the 90 days rat study, based on reduced mean body weight and body weight gain, increased

absolute and relative liver weight with hepatocellular hypertrophy in males and females at 3000 ppm (196.1 mg/kg bw/d in males and 207.3 mg/kg bw/d in females), additionally thyroid follicular cell hypertrophy and urinary bladder congestion and dilation in males at 3000 ppm, the NOAEL was determined to be 1000 ppm for both genders, equivalent to 64 mg/kg bw/d for males and 68.6 mg/kg bw/d for females. No neurotoxic effects were observed in the study. Taking into consideration the cut off values for repeated dose toxicity (DSD: 50 mg/kg bw/d, CLP, STOT RE 1: 10 mg/kg bw/d, CLP, STOT RE 2: 100 mg/kg bw/d) and the nature of the observed effects, no classification and labelling for repeated dose toxicity is triggered for fenpyrazamine.

In the 90 days study in mice, the NOAEL is determined at 200 ppm for males (equivalent to 28.0 mg/kg bw/d) and females (equivalent to 33.5 mg/kg bw/d) based on significantly reduced body weight gain, significantly increased absolute and relative liver weight, significantly increased hepatocellular hypertrophy and significantly decreased bilirubin level in males at 2000 ppm (296.2 mg/kg bw/d) and significantly increased relative liver weight and hepatocellular hypertrophy in females at 2000 ppm (363.3 mg/kg bw/d). Taking into consideration the cut off values for repeated dose toxicity (DSD: 50 mg/kg bw/d, CLP, STOT RE 1: 10 mg/kg bw/d, CLP, STOT RE 2: 100 mg/kg bw/d) and the nature of the observed effects, no classification and labelling for repeated dose toxicity is triggered for fenpyrazamine.

In the 90 days study in dog the NOAEL for males and females is proposed at 25 mg/kg bw/d, based on increased mean relative liver weight (12.9% compared to control) and hepatocyte hypertrophy in two males at 50 mg/kg group and on hepatocellular hypertrophy (but without changes in liver weight) in females. Since no cut off values for dog studies are available, neither according to DSD not to CLP, the information from dog studies can be taken only as supportive information.

In the 1-year dog study the NOAEL for males is proposed at 25 mg/kg bw/d, based on significantly increased mean absolute liver weight and slight hepatocyte hypertrophy in three males at 100 mg/kg group, as well as based on statistically significant increase in MCHC and ALP and decrease in MCV. In females, the NOAEL is proposed at 25 mg/kg bw/d, based on increased relative liver weight (+10.4%) and slight hepatocyte hypertrophy in two animals at 100 mg/kg, supported by increased platelet count. Since no cut off values for dog studies are available, neither according to DSD not to CLP, the information from dog studies can be taken only as supportive information.

In the dermal 28-days study in rats dosed at 100, 300, and 1000 mg/kg, only statistically significantly lower values for hemoglobin (-2.6%) and hematocrit (-3%) and shortened prothrombin time (-14.9%) were seen in males at 1000 mg/kg bw/d. No effects in females were noted. Based on the observed results, the NOAEL was considered to be 300 mg/kg bw/day for male and 1000 mg/kg bw/d for female rats. Taking into consideration the cut off values for repeated dose toxicity (DSD: 300 mg/kg bw/d, CLP, STOT RE 1: 60 mg/kg bw/d, CLP, STOT RE 2: 600 mg/kg bw/d) and the severity of observed effects, no classification and labelling for repeated dose toxicity is triggered for fenpyrazamine.

In the combined chronic and carcinogenicity study (2 years) in rat, the NOAEL is set at 300 ppm (15.6 mg/kg bw/d) in females, based on shortened prothrombin time and decreased MCH and MCV at 1200 ppm. In males, the NOAEL is set at 300 ppm (12.7 mg/kg bw/d), based on statistically significantly increased hepatocellular hypertrophy accompanied by increased relative liver weight at 1200 ppm at week 52 in satellite group and hepatocellular hypertrophy (but without change in relative liver weight) in main group males at week 104. In addition, in males of 1200 ppm group and above (week 52, satellite group), MCV and neutrophils were statistically significantly decreased and albumin increased. Taking into consideration the cut off values for repeated dose toxicity (DSD: 25 mg/kg bw/d, CLP, STOT RE 1: < 5 mg/kg bw/d, CLP, STOT

RE 2: < 50 mg/kg bw/d) and the nature of the observed effects, no classification and labelling for repeated dose toxicity is triggered for fenpyrazamine.

In the carcinogenicity study in CD-1 mouse (78 weeks), the NOAEL for males is set at 1500 ppm (176 mg/kg bw/d), based on statistically significantly higher relative liver weight in satellite and main group animals accompanied by higher incidence of hepatocellular hypertrophy observed in main group animals, and supported by statistically significantly increased MCV and MCH and decreased RBC at 3000 ppm. In females, NOAEL is set at 2000 ppm (283 mg/kg bw/d), based on statistically significantly higher relative liver weight in satellite and main group animals accompanied by statistically significantly increased hepatocellular hypertrophy observed in main group animals, and supported by statistically significantly decreased RBC, HB and HCT at 4000 ppm. Taking into consideration the cut off values for repeated dose toxicity (DSD: 25 mg/kg bw/d, CLP, STOT RE 1: < 5 mg/kg bw/d, CLP, STOT RE 2: < 50 mg/kg bw/d), no classification and labelling for repeated dose toxicity is triggered for fenpyrazamine.

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

Effects observed in the subchronic (oral and dermal) and chronic (oral) studies in rat, mouse and dog do not trigger the criteria for classification and labelling for repeated dose toxicity (for more details please see 4.7.1.1, 4.7.1.3 and 4.7.1.7).

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

Effects observed in the subchronic (oral and dermal) and chronic (oral) studies in rat, mouse and dog do not trigger the criteria for classification and labelling for repeated dose toxicity (for more details please see 4.7.1.1, 4.7.1.3 and 4.7.1.7).

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

Effects observed in the subchronic (oral and dermal) and chronic (oral) studies in rat, mouse and dog do not trigger the criteria for classification and labelling for repeated dose toxicity.

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

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

Effects observed in the subchronic (oral and dermal) and chronic (oral) studies in rat, mouse and dog do not trigger the criteria for classification and labelling for repeated dose toxicity (for more details please see 4.7.1.1, 4.7.1.3 and 4.7.1.7).

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

Effects observed in the subchronic (oral and dermal) and chronic (oral) studies in rat, mouse and dog do not trigger the criteria for classification and labelling for repeated dose toxicity (for more details please see 4.7.1.1, 4.7.1.3 and 4.7.1.7).

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

Effects observed in the subchronic (oral and dermal) and chronic (oral) studies in rat, mouse and dog do not trigger the criteria for classification and labelling for repeated dose toxicity.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 20: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Dose range	Results	Reference
***In vitro* studies**			
Bacterial assay for gene mutation (OECD 471)	156, 313, 625, 1250, 2500, 5000 µg/plate (dissolved in DMSO)	negative (+/- S-9 mix)	Kitamoto, S, 2006a
In vitro mammalian chromosome aberration test in Chinese hamster lung cells (CHL/IU) (OECD 473)	Cytotoxicity testing: 19.5, 39.1, 78.1, 156*, 313*, 625*, 1250*, 2500*, 5000* µg/mL (* Precipitate seen at and above this concentration) CA assay 6 hours: -S9: : 60, 75, 90, 105, 120,135,150 µg/mL +S9: 20, 40, 80, 120, 160 µg/mL CA assay 24 hours: -S9: 2.81, 5.63, 11.3, 22.5, 45, 90 µg/mL +S9: 40, 80, 120, 160 µg/mL	negative (+/- S-9 mix)	Kitamoto, S, 2006b
In vitro Chinese hamster V79/HPRT locus gene mutation assay (OECD 476)	Mutation assay I (4 hours): - S9 mix: 2.5, 5.0, 10, 20, 30, 40, 50, 60 µg/mL + S9 mix: 12.5, 25, 50, 75, 100, 150, 175, 200 µg/mL Mutation assay II: -S9 (24 hours): 25, 40, 55, 70, 85, 100, 115, 130 µg/mL +S9 (4 hours): 20, 40, 60, 80, 100, 120, 140, 160 µg/mL	negative (+/- S-9 mix)	Wollny, H-E., 2007
***In vivo* studies**			
Mouse micronucleus test (CD mice) (OECD 474)	0, 500, 1000, 2000 mg/kg bw (suspended in Methylcellulose; single oral treatment)	negative	Kitamoto, S, 2007

4.9.1 Non-human information

4.9.1.1 In vitro data

Bacterial assay for gene mutation

Cytotoxicity (range-finding assay):
Cytotoxicity to TA100 was seen at the top dose of 5000 µg/plate, with and without S9 mix, but not at the second highest dose, 2500 µg/plate. Precipitation was observed at and above 1250 µg/plate without S9 mix and at and above 2500 µg/plate with S9 mix. The top dose for each strain was defined as 5000 µg/plate or the dose at which cytotoxicity first occurred. Therefore, 5000 µg/plate was chosen as the top dose for all strains in the main study.

Mutation assay:
The number of revertant colonies, with or without S9 mix, was never twice the number of colonies in that of the solvent control plate. Negative results were consistent between the range-finding and main tests. Positive controls all induced marked increases in the numbers of revertant colonies.

Table 21: Results of reverse mutation test in bacterial systems

Treat-ment	**Dose (µg/ plate)**		**Revertant colonies/plate (mean±SD)**									
Without S9 mix												
			TA100		TA1535		WP2*uvrA*		TA98		TA1537	
			DF[a]	Main	DF	Main	DF	Main	DF	Main	DF	Main
S-2188	0		97±15.6	104±11.0	6±2.9	9±2.0	21±5.5	16±1.0	25±1.7	27±6.2	11±3.8	11±3.0
	156		100±12.3	99±11.5	7±2.6	7±4.0	22±4.0	19±4.7	27±6.4	27±3.2	7±1.5	8±3.8
	313		93±8.0	94±12.9	7±3.5	7±1.2	23±4.2	17±4.4	29±6.0	24±3.2	10±3.8	13±6.4
	625		90±8.7	94±2.6	7±1.5	7±3.1	19±2.5	21±7.1	30±9.3	30±8.4	12±3.5	6±2.5
	1250[p]		91±2.6	90±4.0	5±2.1	6±0.6	16±3.6	19±5.3	30±2.6	24±2.5	7±1.5	7±0.0
	2500[p]		66±13.1	71±4.0	3±1.5	6±1.2	20±1.0	19±3.2	25±9.5	20±3.2	3±0.6	5±4.5
	5000[p]		49±6.4[*]	56±4.7[*]	6±4.9	8±1.0	15±1.7	21±4.6	25±4.6	21±2.3	4±1.2	10±6.1
2-FNFA	0.01		579± 27.2	763± 28.6			88±4.4	103±2.1				
	0.1								335± 16.0	372± 11.9		
Sodium azide	0.5				312± 30.4	326± 24.4						
9-amino-acridine	80										520± 58.9	595± 89.1
With S9 mix												
			TA100		TA1535		WP2*uvrA*		TA98		TA1537	
			DF	Main	DF	Main	DF	Main	DF	Main	DF	Main
S-2188	0		87±11.1	90±21.6	8±6.6	6±2.3	25±7.6	24±4.0	33±2.5	45±3.6	10±3.2	11±4.7
	156		81±2.5	90±7.5	11±5.6	10±3.6	29±4.9	24±2.1	41±6.7	34±8.3	8±4.0	12±0.6
	313		80±10.4	90±12.5	10±4.2	7±4.0	33±1.5	22±3.8	42±6.4	42±10.5	13±3.6	9±4.0
	625		78±5.0	82±10.4	10±2.5	6±0.6	30±6.7	21±0.6	46±9.9	32±7.0	7±0.0	10±4.7

Treat-ment	Dose (µg/ plate)		Revertant colonies/plate (mean±SD)									
	1250		74±13.5	80±6.1	9±3.6	5±2.3	25±7.8	29±4.9	42±2.3	35±1.2	9±1.7	8±0.6
	2500[p]		52±11.0	56±14.0	10±2.1	8±2.5	20±2.5	26±7.2	36±4.6	26±5.9	3±0.6	3±1.2
	5000[p]		38±5.0[*]	42±10.5[*]	5±1.0	5±1.2	23±4.0	19±3.0	32±8.4	21±4.4	2±0.6	3±0.6
2-amino anthracene	0.5								261± 34.0	231± 5.5		
	1		696± 47.5	653± 12.5								
	2				227± 16.5	202± 15.4					107± 13.9	114± 6.8
	10						445± 13.0	424± 31.7				

[a] – DF, Dose finding study

[p] – Precipitate

* - Toxic effects observed

Fenpyrazamine was not mutagenic in the acceptable bacterial reverse mutation assay under the test conditions.

Test for clastogenicity in mammalian cells

Preliminary cytotoxicity assay:

Marked growth inhibition was seen after exposure of CHL cells (+/- S9) to fenpyrazamine. The dose-response was very steep without S9, from 94.8% growth at 78.1 µg/mL to 16.2% growth at 156 µg/mL for 6 hours treatment, from 51.3% growth at 78.1 µg/mL to 3.8% growth at 156 µg/mL, for 24 hours treatment and somewhat less steep with S9, from 79.8% growth at 78.1 µg/mL to 56.7% at 156 µg/mL, to 6.5% at 313 µg/mL.Precipitation of test compound was observed at the beginning of treatment at 156 µg/mL, and throughout treatment at 313 µg/mL and above (6 of 9 doses).

Table 22: Results of cytotoxicity test

Chemical	Dose	Growth rate (%) [a)]		
		-S9 (6-18h)[b)]	+S9 (6-18h)[b)]	-S9 (24-0h)[b)]
DMSO	1 %	100	100	100
fenpyrazamine	19.5 µg/mL	104.1	89.8	71.8
	39.1 µg/mL	100.7	83.5	59.9
	78.1 µg/mL	94.8	79.8	51.3
	156 µg/mL#	16.2	56.7	3.8
	313 µg/mL##	2.0	6.5	0.4
	625 µg/mL##	0.7	6.6	0.2
	1250 µg/mL##	0.8	6.8	0.5

Chemical	Dose	Growth rate (%) [a]		
		-S9 (6-18h)[b]	+S9 (6-18h)[b]	-S9 (24-0h)[b]
	2500 µg/mL##	0.6	4.9	0.3
	5000 µg/mL##	32.3	31.3	7.1

a) Percentage of the control value
b) Treatment period – recovery period
Precipitate seen at the beginning of the treatment
Precipitate seen at the beginning and at the end of the treatment

Cytogenicity and chromosome analysis:

In the initial 6 hour exposure assay, in the absence of S9 a concentration of 135 µg/ml caused slightly greater than 50% cell growth inhibition so this concentration and two lower concentrations were analysed. In the presence of S9 a concentration of 160 µg/ml caused slightly greater than 50% growth inhibition so this and two lower concentrations were analysed. A marginal increase in the incidence of structurally aberrant cells was observed at 160 µg/mL in the presence of S9 mix. There was no other increase in the incidence of chromosomally aberrant cells (structural or numerical) observed with or without S9.

In a 24 hour exposure assay, CHL cells without S9 were treated with 6 doses, starting with 90 ·µg/mL and decreasing sequentially by a factor of 2. Slides from 90, 45, and 22.5 ·µg/mL were analysed, and showed no increases in the incidence of structurally aberrant cells or polyploid cells. This study was considered confirmatory of the negative response seen in the absence of S9 after the 6-hour exposure.

In a repeated 6 hour exposure assay with S9 mix using exposures from 40 – 160 µg/mL, no increases in the incidence of structurally aberrant cells or polyploid cells was observed.

In a repeated 6 hour exposure assay with S9 mix using exposures from 40 – 160 µg/mL, no increases in the incidence of structurally aberrant cells or polyploid cells was observed.

A second repeated (confirmatory) 6 hour exposure with S9 mix was performed to check reproducibility of results. CHL cells were exposed to 40, 80, 120, and 160 µg/mL, and slides from the three highest doses analysed. Growth inhibition was comparable to previous exposure, and no increase in the incidence of structurally aberrant cells or polyploidy cells was observed. The initial observation of a marginal increase was not reproduced in two consecutive experiments, and was therefore considered an accidental event with no biological relevance.

Table 23: Results of chromosomal aberration test

Group	Dose	Rel growth	N	Structural Aberrations								Polypl
				No. of Aberrations							Cells with abs	
	(µg/mL)	(%)		gap	ctb	cte	csb	cse	mul	Tot-gp	(%)	(%)
6-Hour Exposure, without S9												
Control	0	100	200	3	0	0	0	0	0	0	0.0-	0.5-
fenpyrazamine	105	71.8	200	2	2	0	0	1	0	3	1.5-	2.0-
	120	67.5	200	1	0	0	0	0	0	0	0.0-	2.5-
	135	41.9	200	4	5	1	0	0	0	6	3.0-	1.5-

Group	Dose	Rel growth	N	Structural Aberrations								Polypl
				No. of Aberrations							Cells with abs	
	(µg/mL)	(%)		gap	ctb	cte	csb	cse	mul	Tot-gp	(%)	(%)
MMC	0.06	88.9	200	8	52	20	0	0	0	72	24.0+	0.0-
6-Hour Exposure, with S9												
Control	0	100	200	1	0	0	0	0	0	0	0.0-	1.5-
fenpyrazamine	80	76.1	200	2	1	0	0	1	0	2	1.0-	0.5-
	120	65.9	200	1	1	0	0	0	0	1	0.5-	2.5-
	160[p]	42.8	200	3	6	9	0	0	1	25	6.5±	0.5-
CP	10	66.6	200	6	42	50	0	1	0	93	35.0+	3.0-
24-Hour Exposure, without S9												
Control	0	100	200	1	0	0	0	0	0	0	0.0-	0.5-
fenpyrazamine	22.5	73.3	200	1	2	0	0	0	0	2	1.0-	1.0-
	45	62.6	200	2	2	0	0	0	0	2	1.0-	0.5-
	90	47.7	200	4	3	0	0	0	0	3	1.5-	0.5-
MMC	0.02	80.7	200	10	23	14	0	2	0	39	16.0+	1.0-
6-Hour Exposure, with S9, first repeat												
Control	0	100	200	0	1	0	0	1	0	2	1.0-	1.0-
fenpyrazamine	40	86.3	200	2	2	3	0	0	0	5	1.0-	0.0-
	80	78.3	200	1	4	0	0	0	0	4	1.5-	0.5-
	120	69.7	200	2	2	0	0	0	0	2	1.0-	0.5-
	160[p]	39.3	165#	3	1	4	0	0	0	5	3.0-	1.2#-
CP	10	65.0	200	6	38	38	0	0	1	86	28.5+	0.0-
6-Hour Exposure, with S9 (Confirmatory Assay)												
Control	0	100	200	1	4	0	0	0	0	4	2.0-	0.0-
fenpyrazamine	80	74.7	200	2	3	0	0	0	0	3	1.5-	3.0-
	120	60.9	200	2	2	1	0	0	0	3	1.5-	1.0-
	160[p]	33.9	200	4	6	6	0	0	0	12	4.5-	0.0-
CP	10	54.4	200	8	103	86	0	0	0	189	52.5+	0.0-

- One hundred cells were not available because of toxicity
- Negative, + Positive, ± Marginal as determined by criteria of Ishidate
[p] – Precipitation observed at the beginning of the experiment
Rel growth – % of controls, cytotoxicity
Ctb – chromatid break
Cte – chromatid exchange
Csb – chromosome break
Cse – chromosome exchange
Mul – multiple aberrations (scored as 10 aberrations)
Tot – total aberrations including gaps
Polypl – polyploid cells and cells with endoreduplication

Fenpyrazamine has no potential to induce chromosomal aberrations in Chinese hamster lung cells in culture under the conditions tested.

Gene mutation assay with mammalian cell

Preliminary cytotoxicity assay

Fenpyrazamine caused significant cytotoxicity, which was stronger without S9 mix. In the pre-test 1 cell survival was reduced to 10% at 27.3 µg/mL without S9 mix and to less than 10% at 109.4 µg/mL with S9 mix after 4 hour treatment. After 24 hours of treatment cell survival was reduced to less than 10% at 109.4 µg/mL without S9. Pre-test 2 was conducted to determine consistency and set doses for the main study. After 4 hours of treatment cell survival exceeded 10% using 40.8 µg/mL as the maximum dose without S9 mix, and 150 µg/mL with S9 mix. After 24 hours 25% cell survival was observed at 100 µg/mL without S9 mix. Precipitation or turbidity was noted at and above 218 µg/mL.
Mutation assays:
Main assay I (4 hour treatment): Eight doses from 2.5 – 60 µg/mL were chosen for treatment with S9 mix, and the five treatments from 10 – 50 µg/mL were selected for evaluation. Eight doses from 12.5 – 200 µg/mL were chosen for treatment without S9 mix and the five treatments from 12.5 – 100 µg/mL were selected for evaluation. The maximum dose was selected based on cytotoxicity.

Main assay II (4 and 24 hour treatment): Eight doses from 25 – 130 µg/mL were chosen for treatment with S9 mix, and doses from 25-85 µg/mL were selected for evaluation. Eight doses from 20 – 160 µg/mL were chosen for treatment without S9 mix, and doses from 20 – 100 µg/mL were selected for evaluation. The maximum dose was again selected based on cytotoxicity.

Negative and positive controls were within the expected historical range. Additionally, mutant frequencies for all treatment groups remained well within the historical range of negative and solvent controls. The induction factor exceeded the threshold of three times comparing to the corresponding solvent control in culture 2 of the Main assay II at 40 and 85 µg/mL (but not at the concentrations of 55 and 70 µg/mL) without S9 mix. This effect was judged to be based on a low solvent control value since no concentration-related increase was found, and the mutation frequency for these cultures was within the historical range of controls (year 2000 to 2005). While in the historical control data for solvent the number of mutant colonies per 10^6 cells ranges between 0.6 and 31.8, the number of mutant colonies per 10^6 cells at 40µg/mL fenpyrazamine in this study was 14.3 and at 85.0 µg/mL it was 17.6. Based on a lack of consistency with the first culture, and the lack of increase in mutation frequency, the increased induction factor in the second culture was not considered treatment-related.

Table 24: Results of In Vitro Mammalian Gene Mutation (V79-HPRT)

Substance	**Dose**	**Relative cloning efficiency I - survival**		**Relative cloning efficiency II - viability**		**Mutant colonies per 10^6 cells**		**Induct. factor**	**Induct. factor**
	(µg/mL)	**Culture 1**	**Culture 2**	**Culture 1**	**Culture 2**	**Culture 1**	**Culture 2**	**Culture 1**	**Culture 2**
Main Assay I (without S9, 4 hours)									
Negative control	0	100	100	100	100	5.4	5.7	-	-
Solvent control (DMSO)	0	100	100	100	100	8	4.9	1.0	1.0
Positive control (EMS)	150	87.1	91.7	59.1	123.8	75.4	62.7	14.0	11.1
fenpyrazamine	2.5	100.1	96.5	#	#	#	#	#	#
	5	107.3	101.3	#	#	#	#	#	#

Substance	Dose	Relative cloning efficiency I - survival		Relative cloning efficiency II - viability		Mutant colonies per 10^6 cells		Induct. factor	Induct. factor
	(µg/mL)	Culture 1	Culture 2	Culture 1	Culture 2	Culture 1	Culture 2	Culture 1	Culture 2
	10	98.9	100.9	84.1	102.2	8.1	4.8	1.0	1.0
	20	74.6	78.2	104.7	88.3	2.8	5.1	0.4	1.0
	30	43.7	36.1	71.1	103.8	8.9	2.3	1.1	0.5
	40	19.3	19.6	65.6	94.8	7.4	3.2	0.9	0.6
	50	2.8	0.8	19.7	73.4	1.6	0.7	0.2	0.1
	60	0	0	##	##	##	##	##	##
Main Assay I (with S9, 4 hours)									
Negative control	0	100	100	100	100	6.3	9.7	-	-
Solvent control (DMSO)	0	100	100	100	100	8	9.1	1.0	1.0
Positive control (DMBA)	2	43.4	33.7	32.2	75	2362.4	568	294.6	62.4
fenpyrazamine	12.5	95.9	96.5	109.1	102.8	3.1	6.9	0.4	0.8
	25	97.6	101.1	116.4	121.6	6.1	9.4	0.8	1.0
	50	94.6	100.1	119.9	108.1	6.2	9.1	0.8	1.0
	75	88.2	92.1	130.3	138.1	7.5	7.7	0.9	0.8
	100	11.1	16.1	129.1	100.5	10.5	12.3	1.3	1.4
	150	0.1	1.3	##	##	##	##	##	##
	175[p]	0	0.2	##	##	##	##	##	##
	200[p]	0	0	##	##	##	##	##	##
Main Assay II (without S9, 24 hours)									
Negative control	0	100	100	100	100	7	4.2	-	-
Solvent control (DMSO)	0	100	100	100	100	4.5	3.8	1.0	1.0
Positive control (EMS)	75	102.8	96.3	86	92.7	151.2	128.3	21.7	30.8
fenpyrazamine	25	93.5	103	110	99.6	12.3	10.4	2.7	2.7
	40	112.1	102.6	105.4	107.1	7.6	14.3	1.7	3.7
	55	84.5	85.2	100.3	117.5	1.8	4.6	0.4	1.2
	70	101	84.2	111.7	126.8	5.1	2.2	1.1	0.6
	85	18.9	30.5	105.9	95.7	10.1	17.6	2.2	4.6
	100	0	0	##	##	##	##	##	##
	115	0	0	##	##	##	##	##	##
	130	0	0	##	##	##	##	##	##
Main Assay II (with S9, 4 hours)									
Negative control	0	100	100	100	100	3.4	5.6	-	-

Substance	Dose	Relative cloning efficiency I - survival		Relative cloning efficiency II - viability		Mutant colonies per 10^6 cells		Induct. factor	Induct. factor
	(µg/mL)	Culture 1	Culture 2	Culture 1	Culture 2	Culture 1	Culture 2	Culture 1	Culture 2
Solvent control (DMSO)	0	100	100	100	100	7.7	5.4	1.0	1.0
Positive control (DMBA)	2	35.8	38.2	58.8	46.7	1246.8	1380	162.6	254.1
fenpyrazamine	20	92.1	107.8	80.3	80	12.8	4.8	1.7	0.9
	40	99.4	107.2	110.7	94.9	5	5.3	0.6	1.0
	60	92	103.1	98.8	74.9	8.5	1.5	1.1	0.3
	80	66.8	88.6	114	85.4	8.5	3.8	1.1	0.7
	100	16.7	16.4	124.4	78.9	5.6	3.2	0.7	0.6
	120	0	0	##	##	##	##	##	##
	140	0	0	##	##	##	##	##	##
	160	0	0	##	##	##	##	##	##

Relative: as % of controls
[p] – Precipitate
- Culture was not continued, five higher concentrations were selected to be evaluated at the end of the experiment
- Culture was not continued due to strong toxic effects

In the *in vitro* mutagenicity study fenpyrazamine did not induce gene mutations at the HPRT locus in V79 cells.

4.9.1.2 In vivo data

Micronucleus test in mice after oral administration

In confirmation of the initial toxicity test, no mice died as a result of administration of Fenpyrazamine and the only treatment-related effect was decreased spontaneous activity and ptosis at the highest dose in males 2.5 hours after dosing.

There was no dose-related increase in micronuclei as a result of fenpyrazamine administration. The positive control showed appropriate increase in micronuclei formation to validate the sensitivity of the assay.

There was no decrease in the PCE/(PCE+NCE) ratio after exposure to fenpyrazamine or the positive control. Although no change in the PCE/NCE ratio was found, observation of clinical signs in top-dose animals infers systemic absorption. Additionally the exposure of bone marrow to test substance was confirmed in ADME studies in rat, showing presence of fenpyrazamine in bone marrow after administration of low (3mg/kg bw) and high (300 mg/kg bw/d) dose from 0.25 hours to 72 hours post administration.

Table 25: Results of *In Vivo* Micronucleus Test

Treatment	Dose (mg/kg)	Sampling time (hr)	Micronucleated PCE (%, mean ± SD)	PCE ratio (%, mean ± SD)
Control	0	24	0.20±0.071	48.0±4.95

fenpyrazamine	500		0.18±0.091	50.3±6.57
	1000		0.13±0.076	52.8±4.47
	2000		0.13±0.057	54.5±4.32
Cyclophosphamide	60		2.45±0.966**	49.2±2.06
Control	0	48	0.16±0.089	48.4±5.69
fenpyrazamine	2000		0.23±0.057	47.9±5.47

PCE - polychromatic erythrocytes; NCE – normochromatic erythrocytes
**p≤0.01 significantly different from control
Micronuclei: 2000 PCE were examined from each animal
PCE ratio: PCE/(PCE+NCE), 1000 erythrocytes examined from each animal

Fenpyrazamine did not cause an increase in number of micronucleated PCE in male mice.

4.9.2 Human information

No human information.

4.9.3 Other relevant information

No other relevant information.

4.9.4 Summary and discussion of mutagenicity

Fenpyrazamine was tested in a sufficient range of *in vitro* and *in vivo* mutagenicity assays measuring different mutagenic endpoints like gene mutation in bacterial and mammalian cells and clastogenicity *in vitro* as well as an *in vivo* micronucleus test in mice.

The results of all these studies mentioned show that **no mutagenic potential** attributed to fenpyrazamine under the test conditions used can be derived.

4.9.5 Comparison with criteria

No genotoxic effects were observed in studies with fenpyrazaminen, neither in *in vivo* nor *in vitro* studies (according to both DSD and CLP).

4.9.6 Conclusions on classification and labelling

There is no evidence of genotoxic potential of fenpyrazamine, therefore, no classification and labelling is proposed.

4.10 Carcinogenicity

Table 26: Summary table of relevant carcinogenicity studies

Method	Dose levels / NOAEL /Effects	Remarks	Reference
Rat oral via diet, 104 weeks (OECD 453)	0, 100, 300, 1200, 2400 ppm equivalent to 0, 4.25, 12.7, 51.9 and 107 mg/kg bw/day (males) and 0, 5.29, 15.6, 63.6 and 130 mg/kg bw/d (females) NOAEL: 12.7 mg/kg bw/d (males) 15.6 mg/kg bw/d (females) Main effects: - ↑ hepatocellular hypertrophy ♂ - ↑ relative liver weight ♂ - ↓ MCV and neutrophils ♂ - ↑ Albumin ♂ - ↑ GGT - Shortened prothrombin time and decreased MCH and MCV ♀ Hepatocarcinoma and thyroid follicular carcinoma at 2400 ppm (107 mg/kg bw/d) in males, but no human relevance	HanRcc:WIST(SPF) rats Purity: 94.7%	Sommer, E.W., 2009a
Mouse oral via diet, 78 weeks (OECD 451)	0, 100, 1500 and 3000 ppm in males and 0, 100, 2000 and 4000 ppm in females equivalent to 0, 11.1, 176 and 349 mg/kg bw/d in males and 0, 13.9, 283 and 552 mg/kg bw/d in females NOAEL: 176 mg/kg bw/d (males) 283 mg/kg bw/d (females) Main effects: - ↑ absolute and relative liver weight ♂♀ - ↑ hepatocellular hypertrophy	CD-1, Crl:CD1(Icr) mice Purity: 94.7%	Sommer, E.W., 2009b

	♂♀ - ↑ MCV and MCH ♂ - ↓ RBC ♂ - ↓ RBC, HB and HCT ♀ **No oncogenic potential**		
Study for Mode of Action Analysis for Rat Liver and Thyroid Tumors by S-2188: Evaluation for time course alteration mainly focusing on hepatocellular proliferation, liver enzyme induction and thyroid hormone (No appropriate guideline existing)	2400 ppm: ↑ abs. and rel. liver weight after 3, 7 and 14 days Enlarged liver after 3, 7 and 14 days ↑ centrilobular hepatocellular hypertrophy after 3, 7 and 14 days ↑ hepatic CYP2B activity after 3, 7 and 14 days ↑ hepatic T4-UGT after 3, 7 and 14 days ↑ replicative DNA synthesis after 3 days and ↓ after 7 and 14 days ↑ abs. and rel. thyroid weight after 7 and 14 days treatment ↑ thyroid follicular cell hypertrophy after 3, 7 and 14 days ↑ TSH after 3, 7 and 14 days ↓ T_3 after 3 and 7 days ↓ T_4 after 3, 7 and 14 days	Wistar (BrlHan:WIST@Jcl(GALAS) male rats Purity: 94.7%	Kondo, M., 2010
In vitro evaluation for role of nuclear receptor CAR in S-2188-induced mRNA expression of CYP2B1, UGT1A, and UGT2B1 (No appropriate guideline existing)	↑ expression of CAR, CYP2B1, UGT1A, and UGT2B1	Primary hepatocytes from single male Wistar (Han/RCC) Purity: 94.7%	Yamada, T., 2010a
An evaluation of the human relevance of the liver and thyroid tumors observed in male rats treated with fenpyrazamine (S-2188) based on mode of action Position paper	Position paper on non-relevance of liver and thyroid tumours in rats for humans (Phenobarbital MOA)	-	Yamada, T., 2010b

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Rats

Reference:	Combined chronic toxicity/oncogenicity (feeding) study in the Wistar rat
Author(s), year:	Sommer, E.W.; 2009a

Report/Doc. number:	Sumitomo Chemical Co. Ltd. Report No. QNT-0042
Guideline(s):	OECD 453, EPA OPPTS 870.4300, Japanese MAFF 12 Nousan 8147, Directive 88/302/EC Part B "Combined chronic toxicity/carcinogenicity"
GLP:	Yes (laboratory certified by National Authority)
Deviations:	No
Validity:	Yes

Material and methods:

Test Material:	S-2188 Technical Grade
Lot/Batch:	030-050914-1G
Purity:	94.7%
Stability of test compound:	Expiry date after the end of the study
Vehicle:	None. Test material was mixed directly into diet.

Test animals:

Species:	Rat
Strain:	HanRcc:WIST(SPF)
Age:	6 weeks at start of treatment
Weight at dosing:	87.8 – 124.6 g (males) and 76.2 – 103.8 g (females)
Source:	Harlan Laboratories Ltd, Füllinsdorf, Switzerland
Diet:	Kliba Nafag 3433 rat maintenance pellet diet
Group allocation:	Main groups (A): each 50 males and 50 females Satellite groups (B): each 20 males and 20 females

The purpose of the study was to assess the chronic toxicity as well as carcinogenic potential of fenpyrazamine when administered to rats in their feed for at least 52 weeks in the satellite groups and at least 104 weeks in the main study groups.

Animal assignment and treatment:

Diet containing fenpyrazamine was offered to 50 rats per sex in the main groups for 104 weeks and to 20 rats per sex in the satellite groups for 52 weeks. Animals were caged in groups of 5.

Diet preparation and analysis:

Fresh batches of the feed pellets for this study were prepared weekly up to week 17 and every 2 week thereafter, with a constant concentration (ppm) throughout the feeding duration. The test item was weighed into a tared glass beaker on a suitable precision balance, briefly milled and mixed with microgranulated feed. Water was added to aid pelleting. The pellets were dried with air for approximately 48 hours before storage. Feed for the animals of control group was prepared similarly without the test item.

Stability of the test item in pelleted rodent feed was investigated during a previous 90-day feeding study. The test item was found to be stable for a period of 21 days.

Table 27: Mean test substance intakes (mg/kg bw/d)

Group	Diet concentration (ppm)	Daily intake (mg/kg bw/d)	
		Male	Female
1 (Control)	0	-	-
2	100	4.25	5.29
3	300	12.7	15.6
4	1200	51.9	63.6
5	2400	107	130

Clinical observations:

Animals were checked for viability/mortality twice daily. Moribund animals were sacrificed and subjected to necropsy. Clinical signs were observed at least once daily and a detailed clinical examination including palpation for tissue masses was performed weekly on each animal outside the home cage.

Food consumption and body weight:

Food consumption and body weight was measured once weekly during acclimatization and treatment up to week 13, and every two weeks thereafter.

Functional observational battery:

A functional observation battery based on a modified Irwin screen was recorded in all rats of satellite groups during week 49. Rats were observed for twenty-nine parameters covering appearance, motor activity, behaviour, respiration, and reflexes. In addition, quantitative data on grip strength (strain gauge) were measured.

Following parameters were examined:

Appearance – piloerection, salivation, hunched posture

Motor – ataxia, tremor/twitching, prostration, circling, spasm

Behaviour – hyperactivity, somnolence, increased exploration, reduced grooming, vocalisation

Respiration – dyspnea, tachypnea, bradypnea

Reflexes – blink, pinna, iridic light reflex, push-off (hind leg), pain response, startle/hearing

Miscellaneous – lacrimation, limbs cyanotic, mydriases, miosis, exophthalmos, reduced muscle tone

Locomotor activity was measured quantitatively in week 49. Activity of the animals was recorded for 10-minute intervals over a period of 60 minutes and as total activity over 60 minutes.

Ophthalmoscopy:

Ophthalmoscopic examinations were performed on all animals during acclimatization and on all surviving satellite group animals of the control and high dose group during week 51. As no test item related lesions were detected, animals of the low and intermediate dose groups were not examined at week 51.

Haematology, clinical chemistry and urine analysis:

Satellite group:

Blood and urine samples for clinical laboratory investigations (haematology, clinical biochemistry, and urine analysis) were collected from all satellite groups animals in weeks 13, 26 and 52. These animals were fasted for approximately 18 hours before blood sampling but water was provided. The samples were collected early in the working day to reduce biological variation caused by circadian rhythms. Blood samples were drawn from the retro-orbital plexus under isoflurane anesthesia.

The following parameters were examined in satellite group rats: erythrocyte count, haemoglobin(HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), reticulocyte count, total and differential leukocyte count, (neutrophils, eosinophils, basophils, lymphocytes, monocytes, large unstained cells), and thrombocyte count. Coagulation parameters of prothrombin time and activated partial thromboplastin time were measured.

Samples for clinical chemistry parameters included: glucose, urea, creatinine, bilirubin (total), cholesterol (total), triglycerides, phospholipids, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), sodium, potassium, chloride, calcium, phosphorus (inorganic), total protein, albumin, globulin, and albumin/globulin ratio. Data on bilirubin (total) in females and phospholipids in both sexes at 52 weeks were missing.

Urine samples were collected using metabolism cages, during the overnight fasting period. Volume, relative density and osmolality were measured and colour and appearance recorded. The following urine components were investigated semi-quantitatively using a test strip analyzer: pH, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes and leukocytes. After centrifugation, the urine sediment was examined microscopically and classified as to number of blood cells (RBC and WBC), epithelial cells (squamous, renal, transitional), crystals (triple phosphate, calcium oxalate, uric acid, unidentified), and casts.

Main group:

Blood samples were collected from all main group animals during weeks 78 and 104, without fasting, for haematology investigations only (reduced number of parameters: erythrocyte count, total leukocyte count and differential leukocyte count).

Sacrifice and pathology:

Animals found in poor health or moribund were sacrificed once found. Surviving satellite group animals were sacrificed on completion of 52 weeks of treatment. Surviving main group animals were sacrificed after completion of 104 weeks of treatment. Animals were sacrificed by exsanguinations under pentobarbitone anaesthesia.

All animals, either found dead or sacrificed, were subject to detailed necropsy and collection of tissues. The following tissues were collected from all animals and fixed in neutral phosphate buffered formaldehyde:

Adrenal glands	Ovaries
Aorta	Pancreas

Bone (sternum, femur including joint)
Bone marrow (femur, sternum)
Bone marrow smear*
Brain (cerebrum, cerebellum, medulla/pons)
Carcass*
Cecum
Colon
Duodenum
Epididymides (fixed in Bouin's solution)
Esophagus
Eyes w/optic nerve (fixed in Davidson's solution)
Harderian gland (fixed in Davidson's solution)
Head, remaining
Heart
Ileum, with Peyer's patches
Jejunum, with Peyer's patches
Kidneys
Larynx
Lacrimal gland, exorbital*
Liver
Lungs, inflated with formalin at necropsy
Lymph nodes (mesenteric, mandibular)
Mammary gland area
Nasal cavity with nasopharynx & paranasal sinus
Pituitary gland
Preputial/clitoral gland*
Prostate gland
Rectum
Salivary glands – mandibular, parotid*, sublingual
Sciatic nerve
Seminal vesicles
Skeletal muscle
Skin
Spinal cord – cervical, midthoracic, lumbar
Spleen
Stomach
Testes (fixed in Bouin's solution)
Thymus
Thyroid (including parathyroid)
Tongue
Trachea
Urinary bladder, inflated w/formalin at necropsy
Uterus with cervix
Vagina
Gross lesions and tissue masses (tumours)

*Not processed for histopathology

Adrenals, brain, heart, liver, thymus, kidneys, spleen, testes, epididymides, prostate gland, ovaries, uterus, and thyroid were weighed from all animals of the satellite groups at interim sacrifice and from all surviving animals of main group at terminal sacrifice.

For histological examination samples of all organs and tissues were processed and embedded, and cut at a thickness of 2-4 microns. Sections were stained with haematoxylin and eosin or special stains as noted. Histological examination was conducted on:

- All organs and tissues from animals which died or were found moribund during the study;
- All organs and tissues from control and high dose animals at scheduled sacrifice (satellite and main group)
- All gross lesions (in any animals)
- Target tissues in all groups

In the liver and thyroid of both sexes at the high dose group test substance related morphologic changes were detected. Therefore, liver and thyroid from the mid- and low dose groups were also examined.

Findings:

Mortality and clinical observations:

In general, mortality data did not reveal any statistically significant differences between control and treated groups. However, the mortality of males in 2400 ppm at the end of the study was slightly higher than in control group (the survival was 85% of control group).

In the satellite group only one female of 100 ppm group died, but the cause of death could not be determined (not considered treatment related).

Table 28: Mortality in satellite and main group

	Males					**Females**				
Diet concentration (ppm)	**0**	**100**	**300**	**1200**	**2400**	**0**	**100**	**300**	**1200**	**2400**
Mortality (satellite group)	0/20	0/20	0/20	0/20	0/20	0/20	1/20	0/20	0/20	0/20
Mortality (main group)	10/50	13/50	13/50	8/50	16/50	11/50	13/50	14/50	11/50	8/50
- % survived until sacrifice	80%	74%	74%	84%	68%	78%	74%	72%	78%	84%
- % of control (survival rate)					(85%)					

There was no treatment-related distribution of clinical signs, neither in satellite nor in main group animals. All clinical signs recorded were those typically seen in rats in studies of this nature. The incidence, onset and location of palpable nodules and masses did not distinguish treated groups from their respective controls.

Food consumption, body weight and body weight gain:
In the satellite groups, no effects on food consumption, body weight and body weight gain were observed during the study and after 52. In the main group, food consumption did not appear disturbed by treatment after 104 weeks but the overall mean body weight at 2400 ppm was significantly decreased in males and females (10.1% and 12.6%, respectively). The mean body weight in males and females was statistically significantly lower compared to control group from week 4 and week 5, respectively, throughout the study. Statistically significant impairment of body weight gain up to week 104 was seen among females receiving 2400 ppm (-17.3% compared to control) but was less pronounced in males of 2400 ppm group (-7.3% compared to control). For males, no statistically significant reduction in body weight gain was observed during the study and at week 104, while the significant reduction in body weight gain in females of 2400 ppm group was observed from week 5 to the end of the study but was transient in females of 1200 ppm group.

Table 29: Food consumption, body weight and body weight gain in satellite and main group animals

Diet concentration (ppm)	**Males**					**Females**				
	0	**100**	**300**	**1200**	**2400**	**0**	**100**	**300**	**1200**	**2400**
Satellite group										
Overall food consumption (g/animal/d)	21.1	21.8	22.1	21.3	21.7	15.5	16.0	15.8	15.6	15.4

Mean body weight, week 52 (g)	576	606	618	583	577	315	319	317	297	296
Weight gain, weeks 0 – 52 (%)	283	300	300	289	290	153	150	155	138	133
Main group										
Overall food consumption (g/animal/d)	21.0	21.7	21.2	21.4	20.9	15.5	16.0	15.8	15.6	15.4
Mean body weight, week 104 (g) - % of control	693	710 (+2.5)	674 (-2.7)	703 (+1.4)	**623**** (-10.1)	412	399 (-3.2)	412 (0.0)	391 (-5.1)	**360**** (-12.6)
Weight gain, weeks 0 – 104 (%) - % of control	354	356 (+0.6)	346 (-2.3)	363 (+2.5)	328 (-7.3)	226	221 (-2.2)	221 (-2.2)	209 (-7.5)	**187**** (-17.3)

* P<0.05 different from control by Dunnett's test ** P<0.01 different from control by Dunnett's test

Functional observational battery:

FOB performed in week 49 in all satellite group animals revealed no substance related behavioural effects. Grip strength in the hind limbs of females in 2400 ppm group was statistically significantly decreased. No effects were seen at grip strength of fore limbs, neither in males, nor in females. The motor activity in females of 300, 1200 and 2400 ppm was statistically significantly increased.

Table 30: Grip strength and locomotor activity at week 49

	Males					**Females**				
Diet concentration (ppm)	**0**	**100**	**300**	**1200**	**2400**	**0**	**100**	**300**	**1200**	**2400**
Grip strength (hind), kg	0.76	0.74	0.73	0.75	0.66	0.58	0.60	0.55	0.52	**0.49***
Grip strength (fore), kg	0.87	0.85	0.87	0.80	0.91	0.88	0.71	0.83	0.77	0.73
Motor activity, (distance, arbitrary units)	516	526	594	**317***	512	724	886	**990***	**996***	**1058****

* P<0.05 different from control by Dunnett's test ** P<0.01 different from control by Dunnett's test

Ophthalmoscopy:

Ophthalmoscopic examination performed in week 51 in satellite animals of the control and high dose groups did not reveal any treatment related effects.

Haematology, clinical chemistry and urine analysis:

Haematology - Satellite group:

Week 13:

13 weeks after treatment no effects on haematology parameters in males of any treatment group were observed. In females, a statistically significant decrease in MCV and MCH and a statistically significant increase in platelets and shortening of prothrombin time in 1200 and 2400

group were observed. At 300 ppm in females, decrease in MCV and increase in platelets were within the HCD from 2002 to 2007 from the same laboratory. Decrease in MCH and shortening of prothrombin time were within the HCD from 2002 to 2007 from the same laboratory but were above the HCD at 1200 and 2400 ppm.

Week 26:

26 weeks after treatment a statistically significant decrease in eosinophiles and neutrophiles was observed in males of 2400 ppm group, but no such effects were seen in females. A statistically significant increase in platelets was observed in males of 2400 ppm, but this effect, seen in females after 13 weeks was not further observed in females after 26 weeks. Prothrombin time was statistically significantly longer in males of 2400 ppm group but statistically significantly shortened in females of 300, 1200 and 2400 ppm group. The significantly shortened prothrombin time at 300 ppm in females was within the HCD from 2002 to 2007 from the same laboratory, but above the HCD at 1200 and 2400 ppm.

Week 52:

Haemoglobin and haematocrit were statistically significantly decreased in females of 2400 ppm group after 52 weeks of treatment. Mean corpuscular volume (MCV) was reduced in males of 1200 and 2400 ppm groups, while this effect was not seen as dose dependent effect in females. Mean corpuscular haemoglobin (MCH) was statistically significantly reduced in males of 2400 ppm and in females of 1200 and 2400 ppm group. Neutrophils were reduced in males of 1200 and 2400 ppm but no such effect was observed in females. Platelets were increased in females of 300, 1200 and 2400 ppm and prothrombin time shortened in females of 1200 and 2400 ppm groups, however, all these values were within the HCD from from 2002 to 2007 from the same laboratory . No effects on platelets and prothrombin time were observed in males after 52 weeks of treatment.
All other changes were temporary and/or lacking in dose response relationship, and therefore not substance related.

Table 31: Haematology parameters in the satellite groups at weeks 13, 26 and 52

	Males					**Females**				
Diet concentration (ppm)	**0**	**100**	**300**	**1200**	**2400**	**0**	**100**	**300**	**1200**	**2400**
Haematology (week 13)										
MCV (fl)	51.0	50.4	50.5	50.0	49.6	53.8	53.4	52.7	**52.0****	**52.5***
MCH (fmol)	1.08	1.08	1.08	1.06	1.04	1.18	1.17	1.15	**1.13****	**1.14***
Platelets (g/l)	966	936	952	963	1026	914	956	1003	**1022***	**1049****
Prothrombin time (rel. 1)	0.81	0.82	0.82	0.83	0.76	0.84	0.85	0.85	**0.90+**	**0.92+**
Haematology (week 26)										
Neutrophiles (g/l)	1.68	1.53	1.52	1.35	**1.23***	0.63	0.77	0.82	0.62	0.75
Eosinophiles (g/l)	0.14	0.13	0.11	0.11	**0.10***	0.06	0.07	0.07	0.07	0.07
Platelets (g/l)	905	938	953	992	**1032****	909	939	1003	992	991
Prothrombin time (rel. 1)	0.88	0.86	0.87	0.87	**0.83+**	0.81	0.84	**0.87+**	**0.93+**	**0.94+**
Haematology (week 52)										
HGB (mmol/l)	10.1	10.2	9.9	9.9	10.1	9.9	9.7	9.8	9.7	**9.5****
HCT (%)	42	42	41	41	43	40	40	41	40	**39***

	Males					Females				
Diet concentration (ppm)	**0**	**100**	**300**	**1200**	**2400**	**0**	**100**	**300**	**1200**	**2400**
MCV (fl)	47.9	47.0	47.8	**45.9***	**45.4****	51.5	51.2	50.7	**49.7****	50.3
MCH (fmol)	1.14	1.13	1.15	1.10	**1.08****	1.26	1.25	1.23	**1.21****	**1.22***
Neutrophiles (g/l)	2.06	1.86	1.57	**1.44***	**1.44***	0.90	0.78	0.92	0.86	0.84
Platelets (g/l)	859	862	884	933	892	758	859	**891****	**900****	**875***
Prothrombin time (rel. 1)	0.80	0.78	0.78	0.78	0.82	0.75	0.73	0.74	**0.79+**	**0.82+**

* P<0.05 different from control by Dunnett's test + P<0.05 different from control by Steel's test
** P<0.01 different from control by Dunnett's test ++ P<0.01 different from control by Steel's test

Haematology - Main group:

In main group animals only erythrocyte count, total leukocyte count and differential leukocyte count were performed after 78 and 104 weeks of treatment.
No differences in any measured haematology parameter were observed between control and treated groups at the end of the study (week 104).
At week 78, lymphocyte count was statistically significantly decreased in all female treated groups, while no effects on males were observed. However, at termination of the study (week 104) no statistical differences were observed any more. Changes in leucocyte, basophiles and monocyte count are considered not substance related based on lacking in dose response relationship.

Table 32: Haematology parameters in the main groups at week 78

	Males					Females				
Diet concentration (ppm)	**0**	**100**	**300**	**1200**	**2400**	**0**	**100**	**300**	**1200**	**2400**
Haematology (week 78)										
Leucocytes (g/l)	6.43	6.51	6.25	6.57	6.62	4.15	3.89	**3.43****	**3.71***	3.73
Basophiles (g/l)	0.04	0.04	0.04	**0.03***	0.03	0.02	0.02	**0.02****	0.02	**0.02****
Lymphocytes (g/l)	4.41	4.22	4.15	4.34	4.25	2.83	**2.45***	**2.17****	**2.43****	**2.42****
Monocytes (g/l)	0.15	0.19	0.15	0.18	0.18	0.11	0.11	0.09	0.10	**0.09***

* P<0.05 different from control by Dunnett's test ** P<0.01 different from control by Dunnett's test

Clinical chemistry – Satellite group only:

Week 13:

13 weeks after begin of the treatment glucose concentration and urea was significantly decreased in females of 2400 ppm, without any effects in males. Total bilirubin was statistically significantly increased in males of 1200 and 2400 ppm. Triglycerids were significantly increased in females of 2400 ppm, while the significant decrease in males of 1200 ppm only is regarded as incidental finding. ASAT and ALAT were decreased in all male treated groups and achieved a statistical significance in males of 2400 ppm group. Sodium and chloride concentration was statistically significantly higher in males of 300, 1200 and 2400 ppm groups, where in females the increase in sodium concentration was shown to be statistically different from 1200 ppm indicating dose relationship.

Week 26:

26 weeks after begin of treatment glucose concentration in females of 2400 ppm group was still significantly decreased. Creatinine was significantly reduced in males and females of 2400 ppm groups. While total bilirubin was reduced in all treated males but without statistical significance,

it was significantly decreased in females of 300, 1200 and 2400 ppm groups. ASAT and ALAT were still lower in all treated groups and significantly reduced in males of 1200 and 2400 ppm, and in 100, 300 and 2400 ppm females (ASAT) and in 300 and 2400 ppm females (ALAT). Calcium, sodium and albumin were significantly increased in males of 2400 ppm, without effects in females. Globulin concentration was significantly increased and A/G ratio significantly decreased in females of 2400 ppm.

Week 52:

At week 52 glucose concentration was significantly increased in all treated female groups and in males of 100 and 1200 ppm and significantly decreased in males of 2400 ppm group (no dose relationship could be observed). As after 26 weeks, the creatinine was significantly reduced in males and females at 2400 ppm. Cholesterol level was significantly increased in females at 1200 and 2400 ppm, but no effects were observed in males. GGT and ALP were statistically significantly increased in males of 2400 ppm, indicating hepatobiliary toxicity. Sodium, chloride and potassium concentrations were significantly increased in all treated male groups (sodium), in males of 300, 1200 and 2400 ppm (chloride) and in males of 2400 ppm (potassium). Total protein was significantly increased in males of 2400 ppm and in females of 300, 1200 and 2400 ppm, indicating severe hepatocellular dysfunction. Albumin concentration was significantly higher in males and females of 1200 and 2400 ppm. Globulin concentration was statistically significantly higher in females of 2400 ppm group comparing to control animals (indicating dose relationship) but not in males.

Table 33: Clinical chemistry parameters in the satellite groups at weeks 13, 26 and 52

	Males					**Females**				
Diet concentration (ppm)	**0**	**100**	**300**	**1200**	**2400**	**0**	**100**	**300**	**1200**	**2400**
Clinical Biochemistry (week 13)										
Glucose (mmol/l)	4.79	5.04	4.95	4.60	4.36	4.54	4.88	4.52	4.49	**3.92****
Urea (mmol/l)	5.21	5.17	5.13	5.26	5.54	6.80	6.18	**5.66****	6.12	**5.75****
Total bilirubin (µmol/l)	1.83	**1.57+**	1.69	**1.45+**	**1.25+**	2.01	1.84	1.83	1.82	1.72
Triglycerides (mmol/l)	0.38	0.41	0.39	**0.28***	0.36	0.33	0.34	0.32	0.31	**0.39****
ASAT (U/l)	98.9	92.0	89.8	86.4	**79.2****	98.9	90.5	90.6	102.5	94.3
ALAT (U/l)	29.9	29.7	29.1	27.2	**25.4***	28.6	26.3	26.7	26.6	24.6
Sodium (mmol/L)	143.8	144.8	**145.8****	**146.1****	**145.8****	142.4	**141.3***	142.0	**143.9****	**144.3****
Chloride (mmol/L)	102.6	103.3	**103.7***	**104.8****	**103.8***	103.1	102.8	103.4	**104.3***	104.1
Clinical Biochemistry (week 26)										
Glucose (mmol/l)	5.11	5.09	5.48	5.33	4.84	5.24	5.46	5.49	5.55	**4.58***
Creatinine (µmol/l)	28.0	28.7	29.1	28.2	**25.4***	34.9	34.2	33.8	32.7	**31.8***
Total bilirubin (µmol/l)	1.61	1.38	1.55	1.34	1.36	2.25	2.03	**1.78+**	**1.74+**	**1.77+**
ASAT (U/l)	94.9	86.6	84.2	**81.3****	**76.8****	144.3	**92.9***	**89.4***	102.7	**78.8****
ALAT (U/l)	36.8	34.4	34.4	**32.5***	**30.8****	55.4	33.1	**30.8***	40.9	**27.3***
Calcium (mmol/L)	2.76	2.78	2.76	2.76	**2.88****	2.79	2.81	2.83	2.82	2.81
Sodium (mmol/L)	145.8	**145.2***	145.5	146.1	**147.1****	145.4	145.2	145.8	146.6	146.8
Albumin (g/L)	42.95	43.10	42.57	43.61	**45.19****	52.01	51.55	51.29	51.16	51.90
Globulin (g/L)	27.40	24.31	25.78	26.33	26.83	20.87	22.77	22.12	22.60	**23.18***

	Males					Females				
Diet concentration (ppm)	**0**	**100**	**300**	**1200**	**2400**	**0**	**100**	**300**	**1200**	**2400**
A/G ratio	1.58	1.44	1.66	1.66	1.69	2.51	**2.27+**	2.34	2.36	**2.26+**
Clinical Biochemistry (week 52)										
Glucose (mmol/l)	4.94	**5.47****	5.30	**5.45****	**4.53***	4.67	**5.66****	**5.49****	**5.78****	**5.34****
Creatinine (µmol/l)	31.8	32.8	33.3	30.9	**28.3****	35.9	36.5	36.7	35.2	**31.8***
Cholesterol (mmol/l)	2.49	2.50	2.28	2.26	2.48	1.96	2.08	2.12	**2.52****	**2.71****
GGT (U/L)	0.0	0.0	0.1	0.7	**5.5****	0.0	0.0	0.0	0.2	0.2
ALP (U/L)	51.5	46.5	48.9	47.4	**61.6***	14.0	14..4	14.7	13.0	14.7
Sodium (mmol/L)	144.0	**145.0****	**145.3****	**145.0****	**146.2****	144.1	144.5	**145.7***	145.1	143.2
Chloride (mmol/L)	103.7	104.3	**105.1****	**105.1****	**105.3****	104.8	105.4	**106.6***	105.7	104.0
Potassium (mmol/l)	3.62	3.59	3.50	3.54	**3.83***	3.18	3.05	3.16	3.21	2.99
Total protein (g/L)	73.00	**75.25***	72.43	74.02	**74.95***	73.31	75.86	**76.11***	**76.96****	**78.25****
Albumin (g/L)	41.44	**42.84***	42.15	**43.23****	**43.91****	48.38	48.89	48.98	**50.97****	**50.57***
Globulin (g/L)	31.56	32.41	30.28	30.79	31.03	24.94	**26.97***	**27.13***	25.99	**27.68****

* P<0.05 different from control by Dunnett's test + P<0.05 different from control by Steel's test
** P<0.01 different from control by Dunnett's test

Urine analysis – Satellite group only:

Week 13:

In females, urine volume and pH was statistically significantly increased and the relative density decreased in females of 1200 ppm and 2400 ppm. Osmolality was decreased in females of 300, 1200 and 2400 ppm group and achieved statistical significance at 2400 ppm. In males, only leucocyte count was significantly increased at 1200 and 2400 ppm.

Week 26:

26 weeks after the start of the treatment, no glucose was measured in urine of males in 2400 ppm group and in 1200 and 2400 ppm female groups. Leucocyte count and WBC score were significantly increased in females of 2400 ppm group.

Week 52:

At week 52, only difference to control was significantly increased leucocyte count in females of 2400 ppm group.

Table 34: Urine parameters in the satellite groups at weeks 13, 26 and 52

	Males					Females				
Diet concentration (ppm)	**0**	**100**	**300**	**1200**	**2400**	**0**	**100**	**300**	**1200**	**2400**
Urine parameters (week 13)										
Volume/18h ml	5.9	6.8	6.7	6.2	5.3	8.3	9.1	14.0	**15.4+**	**16.2+**
Rel. density (rel. 1)	1.048	1.046	1.044	1.046	1.049	1.029	1.033	1.025	**1.020+**	**1.015+**
Osmolality (mosmol/kg)	1471	1429	1366	1369	1451	929	1042	813	631	**457***
pH	6.6	6.7	6.6	6.7	6.8	6.2	6.2	6.5	**6.6+**	**6.8+**
Leucocytes (per µl)	51	43	51	**96+**	**140+**	8	31	6	20	25

	Males					Females				
Diet concentration (ppm)	**0**	**100**	**300**	**1200**	**2400**	**0**	**100**	**300**	**1200**	**2400**
Urine parameters (week 26)										
Glucose (mmol/l)	2	2	1	3	**0+**	1	1	1	0	0
Leucocytes (per µl)	78	75	74	100	180	14	14	18	28	**31+**
WBC (score 0/3)	1	0	1	1	1	0	0	0	1	**1+**
Urine parameters (week 52)										
Leucocytes (per µl)	206	165	205	194	229	33	47	73	85	**161+**

* P<0.05 different from control by Dunnett's test + P<0.05 different from control by Steel's test

Sacrifice and pathology (interim sacrifice):

At week 52 no differences in body weight between control and treated animals in the satellite groups were observed. Absolute liver weight was significantly decreased in males and females of 2400 ppm group. Relative liver weight was significantly reduced in males of 1200 and 2400 ppm group and females of 2400 ppm group. Relative heart and kidney weight were statistically significantly increased in females of 2400 ppm group (Table B.6.5.1-9).

Non-neoplastic findings:

Histologically, at interim sacrifice statistically significant centrilobular hepatocellular hypertrophy was recorded at minimal to slight severity in males at 300 (without liver weight change) and 1200 ppm (with liver weight change) and in both sexes at 2400 ppm, This finding was present along with an increased fatty change in animals at 2400 ppm in both sexes.

Neoplastic findings:

No differences in neoplastic findings were observed between control and treated groups at interim sacrifice.

Sacrifice and pathology (terminal sacrifice):

At week 104, terminal bodyweight of males and females in 2400 ppm groups was significantly lower than in controls. Relative liver weights were significantly increased in males and females at 2400 ppm. Relative brain and heart weight were significantly increased in males and females at 2400 ppm. Relative kidney and testes weight was statistically increased in males of 2400 ppm, while relative spleen weight was statistically significantly decreased in females of 2400 ppm group. However, in the absence of any histopathologic correlate, the changes in the relative organ weights other than liver are not considered to represent substance related effects (Table B.6.5.1-9).

Non-neoplastic findings:

At terminal sacrifice histological findings revealed centrilobular hepatocellular hypertrophy at minimal to slight severity in animals of both sexes at 1200 and 2400 ppm. The incidence was below historical control data (2000 to 2006) from nine comparable feeding studies from the same laboratory (max. males: 20%, max. females: 27%). Centrilobular hepatocellular hypertrophy was accompanied with an increased fatty change in animals at 2400 ppm in both sexes and an increased incidence of vacuolated foci in males at 2400 ppm.

Diffuse thyroid follicular hyperplasia was increased in males (300, 1200 and 2400 ppm) and females (1200 and 2400). Incidence of focal thyroid follicular hyperplasia was slightly higher in males and females of 2400 ppm than in the control animals. Historical control data from nine

comparable feeding studies from the same laboratory (2000 to 2006) do not distinguish between diffuse and focal hyperplasia, nevertheless, the highest incidence of total hyperplasia in males was max. 12.5% and max. 21.3% in females. Therefore, the incidence of diffuse thyroid follicular hyperplasia in males at 300 (16%), 1200 (14%) and 2400 (22.4%) ppm is above historical control data for total thyroid follicular hyperplasia. For females, even if combining the difuse and focal follicular hyperplasia in current study, the incidence is below total thyroid follicular hyperplasia in historical controls.

Diffuse thyroid follicular hypertrophy was slightly higher in males and females of 2400 ppm group than in the control. Historical control data from nine comparable feeding studies from the same laboratory (2000 to 2006) do not distinguish between types of hypertrophy. In these studies the maximum incidence of total thyroid hypertrophy was 36% in males and 24.7% in females, while in the current study the maximum incidence of diffuse follicular hypertrophy was 4.1% in males and 6.1% in females. In case of diffuse thyroid follicular hypertrophy the comparability of study findings and HCD is hardly given (table below).

Table 35: Organ weights and histopathology findings in the satellite and main groups

	Males					**Females**				
Diet concentration (ppm)	**0**	**100**	**300**	**1200**	**2400**	**0**	**100**	**300**	**1200**	**2400**
Organ weights – Interim sacrifice (week 52)										
Terminal bodyweight (g)	562	587	603	561	558	305	309	305	286	289
Absolute liver weight (g)	14.0	14.3	14.3	15.0	**16.6****	8.48	8.42	8.17	8.33	**9.44***
Relative liver weight (%)	2.49	2.43	2.38	**2.69***	**2.98****	2.78	2.72	2.69	2.92	**3.28****
Relative heart weight (%)	0.22	0.22	0.21	0.22	0.23	0.28	0.27	0.27	0.29	**0.30***
Relative kidneys weight (%)	0.44	0.42	**0.40****	0.43	0.46	0.53	0.54	0.52	0.56	**0.59****
Histopathology - Interim sacrifice (week 52)										
Hepatocyte hypertrophy (minimal)	1/20	3/20	**7/20#**	**10/20##**	**8/20##**	2/20	0/20	4/20	6/20	**12/20##**
Liver fatty change	8/20	11/20	14/20	8/20	**16/20****	5/20	2/20	7/20	10/20	11/20
Organ weights – Terminal sacrifice (week 104)										
Terminal bodyweight (g)	683	699	675	694	**613***	413	386	409	382	**353****
Absolute liver weight (g)	16.0	16.5	16.1	16.8	17.4	10.9	10.0	10.5	**9.7***	10.2
Relative liver weight (%)	2.34	2.38	2.38	2.44	**2.85****	2.64	2.59	2.58	2.55	**2.90****
Relative brain weight (g)	0.33	0.32	0.33	0.33	**0.37***	0.51	0.54	0.52	0.54	**0.59****
Relative heart weight (%)	0.20	0.19	0.20	0.20	**0.22****	0.24	0.25	0.25	0.26	**0.28****
Relative kidneys weight (%)	0.42	0.42	0.44	0.43	**0.54****	0.50	0.53	0.51	0.52	0.53
Relative spleen weight (%)	0.21	0.22	0.22	0.20	0.21	0.25	0.25	0.26	0.23	**0.22***
Relative testes weight (%)	0.60	0.61	0.62	0.60	**0.71****	-	-	-	-	-
Histopathology – Terminal sacrifice (week 104)										
Hepatocyte hypertrophy (minimal) - %	0/50 (0)	2/50 (4)	2/50 (4)	**7/50##** (14)	**6/50#** (12)	1/50 (2)	2/50 (4)	1/50 (2)	**7/50#** (14)	5/50 (10)
Fatty change	30/50	14/50	21/50	34/50	38/50	13/50	12/50	10/50	16/50	**26/50****
Vacuolated foci	6/50	13/50	7/50	7/50	**15/50#**	6/50	1/50	1/50	3/50	3/50
Thyroid follicular hyperplasia, diffuse[1)] - %	5/50 (10)	5/50 (10)	8/50 (16)	7/50 (14)	11/49 (22.4)	3/49 (6.1)	1/49 (2)	1/50 (2)	8/50 (16)	5/49 (10.2)

	Males					Females				
Diet concentration (ppm)	**0**	**100**	**300**	**1200**	**2400**	**0**	**100**	**300**	**1200**	**2400**
Thyroid follicular hyperplasia, focal[1)] - %	1/50 (2)	0/50 (0)	0/50 (0)	1/50 (2)	3/49 (6.1)	3/49 (6.1)	0/49 (0)	0/50 (0)	1/50 (2)	5/49 (10.2)
Thyroid follicular hypertrophy, diffuse[1)] - %	1/50 (2)	0/50 (0)	1/50 (2)	1/50 (2)	2/49 (4.1)	0/49 (0)	0/49 (0)	0/50 (0)	0/50 (0)	3/49 (6.1)

Different from control according to Fisher's Exact Test
* P<0.05 different from control by Dunnett's test ** P<0.01 different from control by Dunnett's test
[1)] no statistical evaluation performed

Neoplastic findings:

In general, the number of animals with neoplasma showed no differences between control and treated groups. Furthermore, no difference was observed for malign and benign neoplasma (tables below).

Table 36: Number of animals with neoplasma

Diet concentration (ppm)	**0**		**100**		**300**		**1200**		**2400**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number affected	36	44	32	46	37	38	24	35	38	38
- %	51.4	62.9	45.7	65.7	52.9	54.3	34.3	50.0	54.3	54.3

Table 37: Number of animals with benign and malign neoplasma

Diet concentration (ppm)	**0**		**100**		**300**		**1200**		**2400**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Benign neoplasma	31	43	22	45	29	33	20	34	35	35
Malign neoplasma	8	8	15	7	9	12	7	4	11	11

For all organs investigated, no increase of neoplastic findings was observed, with exception of liver and thyroid. The incidence of hepatocellular carcinoma of 4% (2 out of 50 animals) in males at 2400 ppm was above historical control data (in seven comparable feeding studies, conducted between 2002 and 2006, the range for hepatocellular carcinoma in males was between 0.0% to 2.8%). Hepatocarcinoma seem to be an uncommon finding in male Wistar rats, showing very low incidences in many studies (Poteracki, J & Walsh[1], K.M., 1998; Deralanko, M.J., 2000[2]).

Additionally, the higher incidence of thyroid follicular carcinoma in males of 2400 ppm group (6.1%, 3 out of 49 animals) was above the historical control data (in seven comparable feeding studies, conducted between 2002 and 2006, the incidence of follicular carcinoma in thyroids in males was between 0.0% and 4%). No mechanistic studies including hormone level measurement were provided for clarification of mechanism behind follicular thyroid tumours. Considering the observed thyroid hyperplasia, a hypothesis might be set, that the factors responsible for the increase in mitotic activity/organ enlargement may act as promotional events which enhance the probability of tumour development from these cells.

[1] Poteracki, J & Walsh, K.M., 1998: Spontaneous neoplasms in Control Wistar Rats: a Comparison of Reviews. Toxicological Sciences 45, 1-8

[2] Deralanko, M.J., 2000: Toxicologist's pocket handbook, CRC Press LLC

Table 38: Incidence of neoplastic findings (including deaths) in liver and thyroid at study termination

Diet concentration (ppm)	**0**		**100**		**300**		**1200**		**2400**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of animals affected/animals examined										
Liver										
- Adenoma hepatocell.	1/50	2/50	1/50	-	1/50	-	1/50	-	1/50	-
- Carcinoma hepatocell.	-	-	-	-	-	-	-	-	2/50 *(4%)#*	-
- Cholangioma	1/50	1/50	-	-	-	-	-	1/50	-	-
- Cholangiocarcinoma	-	-	-	-	-	-	1/50	-	-	-
- Metastatic sarcoma	-	-	-	-	-	1/50	-	-	-	-
Thyroid gland										
- C-cell adenoma	4/50	6/49	2/50	4/49	2/50	4/50	8/50	5/50	3/49	1/49
- C-cell carcinoma	-	-	4/50	1/49	-	1/50	-	2/50	3/49	2/49
- Follicular adenoma	2/50	1/49	4/50	-	2/50	1/50	3/50	1/50	3/49	4/49
- Follicular carcinoma	-	-	1/50	2/49	1/50	-	-	-	3/49 *(6.1%)##*	-
.- Ductal remnant carcinoma	-	-	-	-	-	-	-	-	-	1/49

- : no finding, # above historical control data (range from 0 – 2.8%), ## above historical control data (range from 0 – 4%)

Conclusion:

In the combined chronic and carcinogenicity study (2 years) in rat, the NOAEL is set at 300 ppm (12.7 mg/kg bw/d) in females, based on decreased MCV and MCH at weeks 13 and 52 (termination) and shortened prothrombin time at weeks 13, 26 and 52. The effects observed at 100 ppm were within the historical control data from studies conducted between 2002 and 2006. In males, the NOAEL is set also at 300 ppm (12.7 mg/kg bw/d), based on statistically significantly increased hepatocellular hypertrophy accompanied by increased relative liver weight at 1200 ppm at week 52 in satellite group and hepatocellular hypertrophy (but without change in relative liver weight) in main group males at week 104, as well as on increase in albumin and gamma-glutamyl-transferase. In addition, in males of 1200 ppm group and above (week 52, satellite group), MCV and neutrophils were statistically significantly decreased and albumin increased.

Regarding carcinogenic potential of fenpyrazamine, no increase of neoplastic findings was observed in any organ of treated animals, with exception of liver and thyroid. The incidence of hepatocellular carcinoma of 4% in males at 2400 ppm was above historical control data (0.0% to 2.8%) from comparable feeding studies. The higher incidence of thyroid follicular carcinoma in males of 2400 ppm group (6.1%) was higher than the historical control data (max. 4% in males) from comparable feeding studies. At the dose causing higher incidence of liver and thyroid tumours (2400 ppm) no excessive toxicity was observed. The animals with tumours survived until scheduled sacrifice. A clear threshold dose (1200 ppm) could be estimated where no increased incidence of liver and thyroid carcinoma was seen.

By default, carcinogenic effects in experimental animals are considered relevant to humans and are considered for classification as carcinogens (*Guidance on the Application of CLP Criteria, Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, 2009*). Only if there is sufficient evidence showing non-relevance of a

certain type of tumours to humans, this tumour type should be excluded for classification. Fenpyrazamine is not genotoxic. In the provided carcinogenicity study, malignant tumours were observed in male rats in multiple sites (liver and thyroid), which incidence was above historical control data of well comparable studies. No exceeding of MTD (maximum tolerated dose) was observed in males (body weight loss was 10.1% compared to control, but no signs of severe toxicity including reduced survival rate was seen). Both affected tissues, liver and thyroid, have their equivalent in humans. Two mechanistic studies provided (one *in vivo* and one *in vitro*, see Chapter 6.8.1) could show that fenpyrazamine has a comparable MOA to phenobarbital (perturbation of homeostasis of pituitary-thyroid-axis by extrathyroidal mechanism). Phenobarbital MOA is considered to be not relevant for humans[3]. No liver and thyroid tumours were observed above historical control data in mouse carcinogenicity study or in female rats (male rats are frequently found to be more sensitive with respect to induction of thyroid tumours than female rats and mice) showing that even if fenpyrazamine may have a MOA comparable to phenobarbital, it is of much lower potency (shown also by low incidences of liver and thyroid tumours in male rats).

Combining all these criteria for classification of a substance as a carcinogen, according to Dangerous Substance Directive (67/548/EC) and Regulation 1272/2008, no classification and labelling is proposed for fenpyrazamine.

Mice

Reference:	78-Week oncogenicity (feeding) study in the CD-1 mouse
Author(s), year:	Sommer, E.W.; 2009b
Report/Doc. number:	Sumitomo Chemical Co. Ltd. Report No. QNT-0043
Guideline(s):	OECD 451, EPA OPPTS 870.4200, Japanese MAFF 12 NohSan 8147, Directive 88/302/EEC Part B.32
GLP:	Yes (laboratory certified by National Authority)
Deviations:	Yes: thyroid was not weighed although it is one of the organs which is mandatory to investigate according to OECD 453
Validity:	Yes

Material and methods:

Test Material:	S-2188 Technical Grade
Lot/Batch:	030-050914-1G
Purity:	94.7%
Stability of test compound:	Expiry date after the end of the study
Vehicle:	None. Test material was mixed directly into diet
Test animals:	
Species:	Mouse
Strain:	CD-1, Crl:CD1(Icr) SPF quality
Age:	5 weeks at delivery
Weight at acclimatisation:	21.8-29.6 g (males) and 19.4 – 25.6 g (females)
Source:	Charles River, Sulzfeld/ Germany
Diet:	Kliba 3433 maintenance pellet diet, *ad libitum*

[3] IPCS, 2007: Part 1: IPCS Framework for analysing the relevance of a cancer mode of action for humans and case studies

Group allocation: Main groups (A): each 52 males and 52 females
Satellite groups (B): each 12 males and 12 females

The purpose of the study was to assess the carcinogenic potential of fenpyrazamine when administered to mice in their feed for at least 52 weeks in the satellite groups and at least 78 weeks in the main study groups.

Animal assignment and treatment:

Diet containing fenpyrazamine was offered to 52 mice per sex in the main groups for 78 weeks and to 12 rats per sex in the satellite groups for 52 weeks. Animals were caged individually.

Diet preparation and analysis:

Fresh batches of the feed pellets for this study were prepared weekly up to week 13 and every 2 week thereafter, with a constant concentration (ppm) throughout the feeding duration. The test item was weighed into a tared glass beaker on a suitable precision balance, briefly milled and mixed with microgranulated feed. Water was added to aid pelleting. The pellets were dried with air for approximately 48 hours before storage. Feed for the animals of control group was prepared similarly without the test item.

Stability of the test item in pelleted rodent feed was investigated during a previous 90-day feeding study. The test item was found to be stable for a period of 21 days. Therefore, stability of the test item was not reanalyzed in this study. Concentration and homogeneity of the actual test item batch in the feed were determined with the first feed preparations. The concentration and homogeneity were determined every three months thereafter.

Table 39: Mean test substance intakes (mg/kg bw/d)

Group	Diet preparation (ppm)		Daily intake (mg/kg bw/d)	
	Male	Female	Male	Female
1 (Control)	0	0	0	0
2	100	100	11.1	13.9
3	1500	2000	176	283
4	3000	4000	349	552

Clinical observations:

Animals were checked for viability/mortality twice daily. Clinical signs were observed at least once daily and a detailed clinical examination including palpation for tissue masses was performed weekly on each animal outside the home cage.

Food consumption and body weight:

Food consumption and body weight was measured once weekly during acclimatization and treatment up to week 13, and every two weeks thereafter.

Haematology, clinical chemistry and urine analysis:

Blood samples for haematology were collected under isoflurane anesthesia from all satellite group animals at week 52 and from all main group animals at week 78. The animals were not fasted before blood collection. Blood samples were collected early in the working day to reduce

biological variation caused by circadian rhythms. Blood samples were also drawn from any moribund animal, when feasible. Blood samples were drawn from the retroorbital plexus and tri-potassium-EDTA was used as anticoagulant for investigation of the hematological parameters.

The following haematology parameters were determined: erythrocyte count, haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, reticulocyte count, total leukocyte count and differential leukocyte count (neutrophils, eosinophils, basophils, lymphocytes, monocytes, large unstained cells).

Blood smears were prepared, but not evaluated further.

Sacrifice and pathology:

Animals found in poor health or moribund were sacrificed once found. Surviving satellite group animals were sacrificed on completion of 52 weeks of treatment. Surviving main group animals were sacrificed after completion of 78 weeks of treatment. Animals were sacrificed by exsanguinations under pentobarbitone anaesthesia.

All animals, either found dead or sacrificed, were subject to detailed necropsy and collection of tissues. The following tissues were collected from all animals and fixed in neutral phosphate buffered formaldehyde:

Adrenal glands
Aorta
Bone (sternum, femur including joint)
Bone marrow (femur, sternum)
Brain (cerebrum, cerebellum, medulla/pons)
Carcass*
Cecum
Colon
Duodenum
Epididymides (fixed in Bouin's solution)
Esophagus
Eyes w/optic nerve (fixed in Davidson's solution)
Harderian gland (fixed in Davidson's solution)
Head, remaining
Heart
Ileum, with Peyer's patches
Jejunum, with Peyer's patches
Kidneys
Larynx
Lacrimal gland, exorbital*
Liver, with gall bladder
Lungs, inflated with formalin at necropsy
Lymph nodes (mesenteric, mandibular)
Mammary gland area
Nasal cavity with nasopharynx & paranasal sinus
Ovaries
Pancreas
Pituitary gland
Prostate gland
Rectum
Salivary glands – mandibular, parotid, sublingual
Sciatic nerve
Seminal vesicles and coagulating gland
Skeletal muscle
Skin
Spinal cord – cervical, midthoracic, lumbar
Spleen
Stomach
Testes (fixed in Bouin's solution)
Thymus
Thyroid (including parathyroid)
Tongue
Trachea
Urinary bladder, inflated w/formalin at necropsy
Uterus with cervix
Vagina
Zymbal gland with external ear*
Gross lesions and tissue masses (tumours)

*Not processed for histopathology

Adrenals, brain, heart, liver with gallbladder, kidneys, spleen, testes, epididymides, ovaries and uterus were weighed from all animals of the satellite groups at interim sacrifice and from at least 10 animals per sex and group of main group at terminal sacrifice. Thyroid was not included in organ weight measurements, although mandatory according to OECD 453, but no explanation was given.

For histological examination samples of all organs and tissues were processed and embedded, and cut at a thickness of 2-4 microns. Sections were stained with haematoxylin and eosin or special stains as noted. Histological examination was conducted on:

- All organs and tissues from animals which died or were found moribund during the study;

- All organs and tissues from control and high dose animals at scheduled sacrifice (satellite and main group)
- All gross lesions (in any animals)
- Target tissues in all groups
-

Findings:

Mortality and clinical observations:

There was no effect on mortality, neither in satellite groups after week 52 nor in main groups after week 78. Survival at the end of the study was higher than 50% in each group, thus number of surviving animals was adequate for carcinogenicity evaluation. No treatment-related change in the incidence of clinical findings was detected. All clinical signs detected affected single animals of any dose group including control and were those commonly seen in mice of this strain and age.

Table 40: Mortality in satellite and main groups

Diet concentration (ppm)	**Males**				**Females**			
	0	**100**	**1500**	**3000**	**0**	**100**	**2000**	**4000**
Mortality (satellite groups), after week 52	1	0	0	0	0	2	1	5
Mortality (main groups), after week 78 - survival rate (%)	23 (55.8%)	14 (73.1%)	15 (71.2%)	15 (71.2%)	15 (71.2%)	24 (53.8%)	16 (69.2%)	18 (65.4%)

Food consumption, body weight and body weight gain:

Overall food consumption was not disturbed at the end of treatment, neither in satellite group animals nor in main group animals. While body weight and body weight gain of males in satellite group was lower in all treated groups, no such effect was observed in the males of main group after week 78. Overall food consumption, body weight and body weight gain of females did not show dose response, neither in satellite nor in main groups.

Table 41: Food consumption, body weight and body weight gain in satellite and main group animals

	Males				**Females**			
Diet concentration (ppm)	**0**	**100**	**1500**	**3000**	**0**	**100**	**2000**	**4000**
Interim sacrifice (satellite group)								
Overall food consumption (g/animal/day) after week 52	5.2	4.8	5.2	5.0	4.9	4.5	5.2	4.8
Mean body weight (g) after week 52	52.5	49.4	48.1	47.6	34.6	**39.4****	35.1	33.6
Body weight gain (%)	73.4	70.1	64.9	55.9	47.5	**69.3***	49.9	49.5
Terminal sacrifice (main group)								
Overall food consumption (g/animal/day) after week 78	4.8	4.6	5.0	4.7	4.6	4.5	4.5	4.4

	Males				Females			
Diet concentration (ppm)	**0**	**100**	**1500**	**3000**	**0**	**100**	**2000**	**4000**
Mean body weight (g) after week 78	45.7	45.9	47.5	46.1	37.1	36.4	36.8	35.9
Body weight gain (%) after week 78	55.4	55.9	59.6	52.0	53.7	55.3	57.5	53.9

* P<0.05 different from control by Dunnett's test ** P<0.01 different from control by Dunnett's test

Haematology analysis:

At week 52, there was no test item-related effect on any hematology parameters in both sexes.

At week 78, a statistically significant decrease in the red blood cell counts (RBC), haemoglobin (HB) concentration and haematocrit (HCT) was observed in females at 4000 ppm. In males, a statistically significant decrease in the red blood cell counts (RBC) at 3000 ppm, an increase in mean corpuscular volume (MCV) at 1500 and 3000 ppm and increase in mean corpuscular haemoglobin (MCH) at 3000 ppm were observed. Changes in haematology parameters in males and females indicated signs of anaemia in animals of high dose groups.

Table 42: Haematology parameters after week 78

	Males				Females			
Diet concentration (ppm)	**0**	**100**	**1500**	**3000**	**0**	**100**	**2000**	**4000**
Haematology (week 78)								
RBC (10^6/µL)	8.12	8.45	7.98	**7.53***	7.93	8.20	7.70	**7.28***
HB (mmol/L)	8.0	8.2	8.0	7.8	8.2	8.1	7.9	**7.4****
HCT (%)	37	39	38	37	38	38	37	**35***
MCV (fl)	45.7	46.1	**47.2***	**48.5***	48.1	46.5	48.1	48.5
MCH (fmol)	0.99	0.96	1.00	**1.04***	1.03	0.99	1.03	1.01

* P<0.05 different from control by Dunnett's test ** P<0.01 different from control by Dunnett's test

Sacrifice and pathology:

At interim sacrifice (week 52), significantly increased absolute liver weights were found in males and females of the high dose group, and in females of the mid-dose group. When expressed relative to bodyweight, the increase in liver weight was statistically significant in males and females of the mid- and high-dose groups. The degree of increase was marked (up to 43% in high dose females). At the low dose, organ weights were undisturbed.

At terminal sacrifice (week 78) statistically significantly increased absolute and relative liver weights were confirmed in males and females of the high dose group only. However, in males and females of mid-dose groups, relative liver weight was also higher compared to control (12.1% and 12.6%, respectively). Among females of the high dose group, increased absolute and relative kidney weights achieved statistical significance. However, in the absence of histological changes in kidneys the apparent organ weight change was not considered to be of toxicological significance.

Gross observation at necropsy revealed no abnormalities that were attributed to treatment.

Histologically, the only finding to show relationship to treatment was an increase in the incidence and degree of hepatocellular hypertrophy in the liver, detected among main group males and

females of the high dose only. At the low and mid-dose groups there was no evidence of any effect on organ pathology.

In general, there was no effect on the incidence of tumours, either benign or malignant. The higher incidence of hepatocellular carcinoma in males of 1500 and 3000 ppm groups is considered spontaneous and in range of historical control data (5 comparable feeding studies from 2003 to 2008; incidence of hepatocellular carcinoma between 0.0% and 5.8% in males).

Table 43: Organ weights and histopathology findings

	Males				**Females**			
Diet concentration (ppm)	**0**	**100**	**1500**	**3000**	**0**	**100**	**2000**	**4000**
Organ weights								
Absolute liver weight (g), week 52	2.25	2.15	2.38	**2.64***	1.49	1.58	**1.90****	**2.03****
Relative liver weight (%), week 52 - % of control	4.36	4.58 (+5.0%)	**5.17*** (+18.6 %)	**5.77**** (+32.3 %)	4.53	4.18 (-7.7%)	**5.61**** (+23.8 %)	**6.48**** (+43.0 %)
Absolute liver weight (g), week 78	2.09	2.28	2.38	**2.71****	1.83	1.64	1.93	**2.61****
Relative liver weight (%), week 78 - % of control	4.78	5.19 (+8.6%)	5.36 (+12.1 %)	**6.17**** (+29.1 %)	4.92	4.69 (-4.7%)	5.54 (12.6%)	**7.52**** (+52.8 %)
Absolute kidney weight (g), week 78	0.724	0.732	0.708	0.676	0.436	0.450	0.443	**0.487***
Relative kidney weight (%), week 78	1.653	1.673	1.598	1.549	1.216	1.301	1.277	**1.417****
Histopathology, week 78								
Hepatocyte hypertrophy	3/52	4/52	5/52	7/52	0/52	1/51	2/51	**9/52##**
Adenoma hepatocell.	4/52	5/52	9/52	5/52	1/52	-	-	1/52
Carcinoma hepatocell. - %	-	1/52 (1.9%)	2/52 (3.8%)	2/52 (3.8%)	-	-	-	-

* P<0.05 different from control by Dunnett's test ** P<0.01 different from control by Dunnett's test
# Different from control according to Fisher's Exact Test

Conclusion:
In the carcinogenicity study in CD-1 mouse (78 weeks), the NOAEL for males is set at 1500 ppm, based on statistically significantly higher relative liver weight in satellite and main group animals accompanied by higher incidence of hepatocellular hypertrophy observed in main group animals, and supported by statistically significantly increased MCV and MCH and decreased RBC at 3000 ppm. In females, NOAEL is set at 2000 ppm, based on statistically significantly higher relative liver weight in satellite and main group animals accompanied by statistically significantly increased hepatocellular hypertrophy observed in main group animals, and supported by statistically significantly decreased RBC, HB and HCT at 4000 ppm. In the mouse carcinogenicity study, no carcinogenic potential of fenpyrazamine could be observed.

4.10.1.2 Carcinogenicity: inhalation

No data available.

4.10.1.3 Carcinogenicity: dermal

No data available.

4.10.2 Human information

A formal statement from the manufacturer is presented (Ohtsubo, T (2009)), that members of staff involved in the synthesis and development of fenpyrazamine are routinely monitored and that no indication of fenpyrazamine-related ill-health have been detected by, or reported to, medical staff. No poisoning incidents or clinical cases have been reported. Fenpyrazamine is a new chemical currently in development. There has been no exposure of the general population or epidemiology study.

4.10.3 Other relevant information

With respect to the clarification of MOA of fenpyrazamine regarding liver and thyroid tumours in male rats, two mechanistic studies (one *in vitro*, one *in vivo*) have been performed showing that fenpyrazamine has a MOA comparable to Phenobarbital, which is considered not relevant for humans[4].

Reference:	Study for Mode of Action Analysis for Rat Liver and Thyroid Tumors by S-2188: Evaluation for time course alteration mainly focusing on hepatocellular proliferation, liver enzyme induction and thyroid hormone
Author(s), year:	Kondo, M.; 2010
Report/Doc. number:	Sumitomo Chemical Co. Ltd. Report No. QNT-0048
Guideline(s):	No appropriate guideline
GLP:	No. Conducted in a GLP-compliant facility, but standard procedures are not generally available for studies of this nature
Deviations:	-
Validity:	Yes

Material and methods:

Test Material: S-2188 Technical Grade

Lot/Batch:	030-050914-1G
Purity:	94.7%
Stability of test compound:	not documented
Vehicle:	None. Test material was mixed directly into diet.

Test animals:

Species:	Rat (males only)
Strain:	Wistar (BrlHan:WIST@Jcl(GALAS)
Age at start of treatment:	approximately 5 weeks

[4] IPCS, 2007: Part 1: IPCS Framework for analysing the relevance of a cancer mode of action for humans and case studies

Weight at start of tretment:	165- 195 g
Source:	CLEA Japan, Inc (Shizuoka, Japan)
Diet:	CRF-1 powdered feed (Oriental Yeast Co. Ltd, Tokyo) provided *ad libitum*
Housing:	Two rats per cage in suspended aluminium cages with wire-mesh front and floor
In life dates:	29 March 2007 – 23 April 2007

Animal assignment and treatment:

Groups of 10 male rats received a dietary concentration of 2400 ppm fenpyrazamine in diet for either 3, 7 or 14 days of treatment. Control groups for each treatment period received untreated diet. Diet levels were chosen to match dietary concentrations in the rat carcinogenicity study. The required amounts of test substance and basal diet for each dose level were weighed. The test substance was mixed with a portion of the basal diet in a mortar to prepare a premix. The premix was mixed with the remaining portion of the basal diet using an automatic mixer, for approximately 15 minutes. The test diets were prepared once for all two weeks and kept in cold and dark conditions until used for feeding the animals.

Table 44: Test substance intake

	Diet concentration (ppm)	**Number of animals (males only)**			**Achieved intakes (mg/kg bw/day)**		
Days of Treatment		**3**	**7**	**14**	**3**	**7**	**14**
Control	**0**	10	10	10	-	-	-
Fenpyrazamine	**2400**	10	10	10	216.5	222.6	216.6

Food consumption and body weight:

Food consumption for each cage was measured over a period of 72 consecutive hours weekly or more often throughout the study. Body weights were measured on Days 1, 4, 8 and 15.

Clinical observations:

Animals were observed for clinical signs at least once daily.

Sacrifice and pathology:

After the treatment period, rats were given an i.p. injection of bromodeoxyuridine (BrdU; 100 mg/kg bw) dissolved in saline. Two hours later animals were sacrificed by decapitation without prior fasting, and blood collected. BrdU labelling indices for the livers were calculated by dividing the number of labeled hepatocellular nuclei by the total number of hepatocellular nuclei (%).
All animals were subject to detailed necropsy examination. The following samples were collected:
- Blood samples for hormone analysis were collected at sacrifice by decapitation. Serum was prepared from the collected blood and stored at -90 to -70 degrees centigrade until analysis.
- After liver weight was recorded, samples of liver were collected. Samples were fixed in buffered formalin for histological examination, or in RNA stabilisation solution for potential

future gene expression analysis. Remaining liver was frozen in liquid nitrogen and stored at -80°C until analyzed for enzyme activity.
- A sample of duodenum from all animals was stored in buffered formalin and dehydrated for immunohistochemical staining, as a quality control measure.

Organ weights:

Liver (before fixation) and thyroid (after fixation) were weighed while wet from all animals. Relative organ weight (organ weight to body weight ratio) was calculated on the basis of the body weight on the day of sacrifice.

Histopathology:

Liver and thyroid were dehydrated, embedded in paraffin wax, and sectioned. Sections were stained with haematoxylin and eosin and examined using light microscopy. The severity of lesions was graded from - to 3+ (- none, +- slight, + mild, 2+ moderate, or 3+ severe).

Hormone analysis:

Serum hormone concentrations were determined by immunoassay (Access2 Immunoassay System, Beckman Coulter Inc., California, U.S.A.) for total T_3 and T_4, or by commercially available kit (Rat thyroid stimulating hormone (rTSH)[^{125}I] assay system, GE Healthcare Bio-Sciences Corp., New Jersey, U.S.A.) for TSH.

Hepatic enzyme analysis:

A portion of fresh liver (approximately 2 g) was homogenized in 4 volumes of Tris/HCl buffer (pH7.4; 50 mM; containing 154 mM KCl) with a Potter homogenizer. Liver homogenate was centrifuged at 9,000 × g for 20 min at 4 °C to separate S9 fractions. The protein level in the S9 fraction was determined using the DC protein assay kit (Bio-Rad, CA) with bovine serum albumin as the protein standard.

a) 7-Pentoxyresorufin O-depentylase activity (PROD)
The reaction mixture (200 µL) consisted of 3 µM 7-pentoxyresorufin, 10 µM dicoumarol, 1 mM NADPH, 1 µL S9 fractions in Tris/HCl buffer (pH7.4) and was added to 96-well microplates. After incubation for 10 min at 37 ºC, the reaction was stopped by addition of 100 µL acetonitrile. Activity of 7-pentoxyresorufin O-depentylase was determined by fluorometric analysis. The fluorescence of the sample was measured with a microplate reader (Saffire II, Tecan) with an excitation wavelength of 550 nm and an emission wavelength of 589 nm. The activity was expressed as the rate of resorufin formation, and was calculated based on the fluorescence of a standard curve of resorufin.

b) UDP-glucuronosyltransferase activity to T_4 (T_4-UGT)
The reaction mixture consisted of 5 µL of liver S9, 10 mM MgCl2, 0.05% Brij58, 1.4 mM D-saccharic acid-1,4-lactone, 4 mM UDP-glucuronic acid and 1 µM 125I-thyroxine (30,000 cpm, GE Healthcare, UK) in 200 µL of 66 mM Tris-HCl Buffer (pH 7.4). After incubation for 2 hours at 37 ºC, 200µL of methanol was added to the reaction mixture. T_4-UGT activity was determined by autoradiography with a fluorescent image analyzer (FLA-5000, FujiFilm, Japan) after separation by TLC with a solvent system of ethyl acetate/ methyl ethyl ketone/ formic acid/ water (5:3:1:1,v/v/v/v as the TLC solvent system has four components)

Statistics:

A comparison between two groups procedure was used for analysis of data for bodyweight, body weight gains, food consumption, absolute and relative organ weights, BrdU labeling index, microsomal protein level, hepatic enzyme activities, and serum hormone levels. The F-test was applied to compare the fenpyrazamine -treated group with the control group. If the variance was homogeneous, Student's t-test was used. If the variance was heterogeneous, Aspin-Welch-test was used. The Mann-Whitney U test was used for analysis of the gradable histopathological findings, comparing test substance and control groups. The Fisher's Exact probability test was similarly employed for analysis of gross pathological findings. Each evaluation was by 2-tailed tests with 0.05 and 0.01 as the levels of significance. Fisher's exact probability test was by 1-tailed tests with 0.05 and 0.01 as the levels of significance.

Findings:

Mortality and clinical signs:

No treatment-related abnormalities were detected. No animals died before terminal sacrifice.

Food consumption and body weight:

Food consumption was not negatively affected by the treatment. No treatment-related and biologically relevant disturbance in body weight and body weight gain was seen.

Table 45: Food consumption, terminal body weight and body weight gain (mean values ± SD)

Diet concentration (ppm)	**0**	**2400**	**0**	**2400**	**0**	**2400**
Treatment days	**3**		**7**		**14**	
Food consumption (g/animal/day)	18±1.0	19±1.2	20±1.4	21*±0.8	20±1.5	20±0.7
Terminal body weight (g)	202±7.9	204±8.8	226±10.1	230±9.0	255±12.7	259±8.8
Body weight gain (g)	22±3.4	25**±3.7	25±2.8	24±4.0	32±6.7	30±4.5

* statistically significant from the control group ($p<0.05$); ** statistically significant from the control group ($p<0.01$)

Organ weights:

At all three timepoints, the absolute and relative liver weights of rats receiving fenpyrazamine at 2400 ppm were significantly higher than those of controls (17.6%, 22.6% and 29.6% higher liver weight in treated rats after 3, 7 and 14 days, respectively) . Absolute and relative thyroid weights were significantly higher than those of controls at day 7 (+ 28.6%) and higher than controls at day 14 (+16.7%).

Histopathology:

At all three timepoints (3, 7 and 14 days), the incidence of livers appearing macroscopically enlarged was significantly increased among rats receiving fenpyrazamine. Thyroids did not appear macroscopically changed.

Slight to mild centrilobular hypertrophy of hepatocytes was observed in the fenpyrazamine treated group after 3, 7 and 14 days of treatment and the incidence was increased significantly. Increased incidence of necrosis in the liver was not observed at any treatment period.

After 3 day treatment, 30 % (3 out of 10 animals) in the fenpyrazamine treated group showed higher values of BrdU labeling indices than the highest value of controls, although statistical significance was not achieved. Statistically significant decreases of BrdU labeling indices were observed after 7 and 14 day treatment with fenpyrazamine.

Table 46: Organ weights (liver, thyroid) and histopathological findings

Treatment days	**3**		**7**		**14**	
Fenpyrazamine diet concentration (ppm)	**0**	**2400**	**0**	**2400**	**0**	**2400**
Organ weights						
Final bodyweight (g)	203	200	228	231	255	259
Absolute liver weight (g) - % of control	9.34	**10.98**** (+17.6)	10.16	**12.45**** (+22.6)	10.40	**13.48**** (+29.6)
Relative liver weight (%)	4.6	**5.49****	4.45	**5.38****	4.07	**5.20****
Absolute thyroid weight (mg) - % of control	13	13 (0)	14	**18**** (+28.6)	18	21 (+16.7)
Relative thyroid weight (%)	6.2	6.3	6.0	**7.8****	7.2	8.1
Histopathology						
Liver: enlarged	0/10	**5/10***	0/10	**9/10****	0/10	**6/10****
Liver: hepatocyte hypertrophy	0/10	**5/10***	1/10	**6/10****	0/10	**9/10****
Thyroid: follicular cell hypertrophy	0/10	**7/10****	0/10	**9/10****	0/10	**9/10****
Liver: BrdU staining index (%)	2.38	3.36	1.93	**0.57****	1.25	**0.57****

* $p < 0.05$ in comparison to controls, ** $p<0.01$ in comparison to controls

Serum hormones:

At all three timepoints (3, 7 and 14 days) TSH values of fenpyrazamine treated animals were higher than controls, without achieving statistical significance but having biological relevance (+39.8%, + 59.8% and +74.7% after 3, 7 and 14 days, respectively). At day 3, the levels of both T_3 and T_4 were significantly lower in fenpyrazamine treated rats than those of controls. At day 7 T_3 was significantly lower than those of controls and T_4 was lower but without statistical significance. At day 14, no statistically significant difference was seen between treated and control animals regarding both, T_3 and T_4 levels (although T_4 was lower in treated animals).

Table 47: Serum hormones

Treatment days	**3**		**7**		**14**	
Fenpyrazamine diet concentration (ppm)	**0**	**2400**	**0**	**2400**	**0**	**2400**
Serum Hormones						
TSH (ng/ml) - % of control	12.8	17.9 (+39.8)	11.7	18.7 (+59.8)	10.7	17.1 (+74.7)
T_3 (ng/ml) - % of control	0.8	**0.5**** (-37.5)	0.7	**0.5**** (-28.6)	0.6	0.6 (0)
T_4 (µg/dl) - % of control	3.95	**2.72**** (-31.1)	3.49	3.02 (-13.5)	3.31	3.26 (-1.5%)

** p<0.01 in comparison to controls

Hepatic anzyme activity:

The liver content of S9 protein was slightly increased, achieving statistical significance at day 7. At all three timepoints (3, 7 and 14 days) PROD activity (a measure of CYP2B) was markedly increased (3 to 5-fold) over controls. At all three timepoints, the T_4 conjugating activity of UGT was statistically significantly increased.

Table 48: Hepatic enzyme activity

Treatment days	**3**		**7**		**14**	
Fenpyrazamine diet concentration	**0**	**2400**	**0**	**2400**	**0**	**2400**
Hepatic enzyme activity						
S9 protein content (mg/g liver)	126.3	132.5	143.3	**160.6***	145.6	153.7
PROD (pmol/min/mg S9 protein)	4.3	**20.3****	3.8	**13.9****	3.7	**13.9****
T_4-UGT (pmol/min/mg S9 protein)	0.40	**0.53****	0.41	**0.62****	0.53	**0.66***

* p < 0.05 in comparison to controls, ** p<0.01 in comparison to controls

Conclusion:

This study was conducted to evaluate the time course of hepatic and thyroid alterations, mainly focusing on hepatocellular proliferation, liver enzyme induction and thyroid hormone levels. Male rats were fed diet containing 0 (control) and 2400 ppm fenpyrazamine (the highest dose level in the rat 2-year bioassay) for periods of 3, 7 and 14 days. Treatment with fenpyrazamine caused no deaths and abnormalities, and did not effect body weight or food consumption, demonstrating that treatment with fenpyrazamine at 2400 ppm did not show excess toxicity.

Liver

Treatment with 2400 ppm fenpyrazamine for 3 to 14 days resulted in increased liver weights at all three timepoints, which were associated with macroscopically enlarged liver with centrilobular hepatocellular hypertrophy. A statistically significant increase of hepatic CYP2B activity (PROD) and hepatic T_4-UGT activity were observed at 2400 ppm after 3 to 14-day treatments. CYP2B induction has been shown to be associated with liver tumor formation in rodents for phenobarbital and related compounds. Hepatic CYP2B and UGT were both induced by phenobarbital *via* the constitutive androstane receptor (CAR). Therefore, it is assumed that the findings in this study might be associated with the activation of CAR in rat liver (shown in the mechanistic *in vitro* study).

For hepatocyte proliferation, 2400 ppm fenpyrazamine increased replicative DNA synthesis (determined as BrdU labeling indices) after 3-day treatment but not after 7- and 14-day treatment, indicating a peak effect in less than one week of treatment. BrdU incorporation as a marker of S-phase activity DNA synthesis is a sensitive measure of cell proliferation. Studies with phenobarbital in rodents have demonstrated that the increase in relative liver weight after short term treatment is due to both hepatocellular hypertrophy and hyperplasia, and have also demonstrated that the initial elevation of hepatocyte labeling index values in rodent liver induced by phenobarbital is not sustained during chronic administration. In addition, reduction of BrdU labeling indices after administration of phenobarbital was observed after peak, which was also observed in fenpyrazamine dosed rats. Therefore, the findings in this study demonstrate that fenpyrazamine marginally enhances hepatocellular proliferation with the peak effect in less than one week, which is similar to phenobarbital. It is assumed that the findings in this study might be associated with the activation of CAR (as shown in the *in vitro* study) in rat liver, comparable to phenobarbital, althought fenpyrazamine seems to have lower potency.

Althought Phenobarbital group was not included in the MOA study with fenpyrazamine, in comparison with the publication on phenobarbital (Deguchi et al. 2009, *Mode of action analysis for the synthetic pyrethroid metofluthrin-induced rat liver tumors: evidence for hepatic CYP2B induction and hepatocyte proliferation Toxicol. Sci., 108*), the activity of fenpyrazamine is much less potent than that of phenobarbital; i.e., degree of increased hepatocellular proliferation after 1-week treatment in rat, phenobarbital vs fenpyrazamine is approximately 3-fold v.s. 1.3-fold of control value.

Thyroid

Another feature of some CYP enzyme inducers such as phenobarbital in rats are the effects on thyroid due to increased catabolism of thyroid hormones (T_3 and T_4) driven by increased hepatic UGT activity, leading to increased thyroid stimulating hormone (TSH) and resulting in thyroid hypertrophy, hyperplasia and tumors. Hepatic UGT is induced by phenobarbital *via* CAR. In the present study, histopathological examination by light microscopy revealed diffuse follicular cell hypertrophy in thyroids of rats treated with fenpyrazamine. It is well accepted, that TSH stimulation results in increased thyroid weight, thyroid follicular cell hypertrophy, and proliferation. In this study, serum TSH showed a tendency of increase during the treatment period without statistical significance. However, it is known that small increases in serum TSH (Hood, A., Liu, Y. P., Gattone, V. H., 2nd and Klaassen, C. D. (1999). *Sensitivity of thyroid gland growth to thyroid stimulating hormone (TSH) in rats treated with antithyroid drugs. Toxicol. Sci. 49*) can be sufficient to stimulate thyroid follicular cell proliferation. Therefore, the slight TSH increases even without statistical significance seems to be a biologically meaningful change. Significantly increased thyroid weight was observed after 7-day treatment and an increased weight (without statistical significance) after 14 days treatment. Fenpyrazamine treatment resulted in decreases in serum T_3 and T_4 levels in the present study. Thyroid gland histopathology, serum T_4 and TSH are considered as the most reliable parameters for identifying chemicals with thyroid hormone modulating activity. Therefore, the effects of fenpyrazamine on rat thyroid are considered secondary to perturbation of the thyroid hormone axis, by a comparable mode of action as phenobarbital

The findings in the present study indicate that fenpyrazamine induces CYP2B and T_4-UGT activities, and marginally enhances hepatocellular proliferation at an early stage of treatment (in less than one week) and perturbs the thyroid hormone axis. Bnd both effects are comaparable to phenobarbital, which is a CAR activator.

Reference:	*In vitro* evaluation for role of nuclear receptor CAR in S-2188-induced mRNA expression of CYP2B1, UGT1A, and UGT2B1
Author(s), year:	Yamada, T.; 2010a
Report/Doc. number:	Sumitomo Chemical Co. Ltd. Report No. QNT-0049
Guideline(s):	No appropriate guideline
GLP:	No. Conducted in a GLP-compliant facility, but standard procedures are not generally available for studies of this nature
Deviations:	-
Validity:	Yes

Material and methods:

Test Material:	S-2188 Technical Grade
Lot/Batch:	030-050914-1G
Purity:	94.7%
Stability of test compound:	not documented
Vehicle:	None (Williams E medium, as used for cell culture)
Test cells:	Primary hepatocytes from a single male Wistar (Han/RCC) rat, 9 weeks of age, by a modified two-step collagenase digestion method. Rat liver was perfused and hepatocytes were dispersed from digested liver and washed with William's E medium (GIBCO) three times by centrifugation. The hepatocytes were cultured in supplemented William's E medium at a density of approximately 4 $x10^5$ cells/well, and allowed to attach for 3 hours at 37 ºC in a humidified chamber. After 3 hours, the culture dishes were gently swirled and fresh medium was added after removing unattached hepatocytes
Culture medium:	Williams E medium; supplemented with 5% fetal calf serum, 100U/ml penicillin, 100 µg/ml streptomycin, 2mM L-glutamine, 0.1 µM insulin, 1 µM dexamethasone, 0.2 µM ascorbic acid, and 10mM nicotinamide, except during transfection. MATra-si Reagent (IBA) was added to medium during the transfection process
Transfection reagents:	a) siRNA for CAR (Hayashi-Kasei Co., Ltd.) or b) Negative Control (Stealth RNAi Negative Control with Medium GC, Code No. 12935-300, Invitrogen)
Transfection procedure:	On day 1, cells were rinsed and supplemented with serum/antibiotics free medium (2mL). siRNA (1µg) for CAR (Hayashi-Kasei Co.,Ltd.) or Negative Control (Stealth RNAi Negative Control with Medium GC, Code No.; 12935-300, Invitrogen), and 1 µL of MATra-si Reagent (IBA) were each diluted with 200µL of serum/antibiotics free medium according to the manufacture's instructions, and the two solutions were gently mixed. After 20 min, the transfection mixtures (200µL) were added to the cells, and the culture plates were placed on a magnet plate (IBA) for 15 min. After 4 hours, the mediums were changed to the supplemented Williams E medium containing serum and antibiotics
Performance date:	11 - 30 November 2010

Test method:

The influence of the Constitutive Androstene Receptor (CAR) on fenpyrazamine hepatic effects was investigated by the technique of RNA-interference gene silencing ("CAR-knockdown"). In this method, cells are transfected with interference RNA (si-RNA) to impair CAR synthesis, then exposed to the test agent. Test cultures of untransfected cells, and of cultures transfected either with siRNA (CAR) or with a siRNA (used as negative control (NC)) were incubated in medium containing 50µM fenpyrazamine. Since fenpyrazamine concentration in liver after administration of 300 mg/kg bw in rat metabolism study (*Quistad & Kovatchev, 2007a,* QNM-0023) was estimated to be about 11.18-14.8, corresponding to 33.7 – 44.65 µM[5], 50 µM of fenpyrazamine is considered to be reliable for evaluation of *in vivo* situation. Additionally, 50 µM fenpyrazamine was selected on the basis of a range-finding assay (using concentrations of 10, 25, 50, 75, 100, 150, 300 and 450 µM fenpyrazamine) as the highest concentration without cytotoxicity but demonstrating sufficient induction of CYP2B, UGT1A and UGT2B1.
The dose level used in the tissue distribution study (300 mg/kg bw/d) is remarkable higher comparing to the highest dose level used in the long term rat study (107 – 130 mg/kg bw/d). Considering that the fenpyrazamine level in the liver after administration of 107 – 130 mg/kg bw/d is not known (while usually not measured in the carcinogenicity studies) and that the duration of dosing in the carcinogenicity study (2 years) is much longer comparing to the metabolism study (3 days), the dose used in the in vitro mechanistic study is considered justified.
An additional culture of untransfected cells, not exposed to fenpyrazamine, served as an untransfected control. Four wells per culture were conducted.

Test procedure:

Cultures of transfected or non-transfected cells were incubated under test conditions for 3 days. On day 3, cultures were terminated and total RNA extracted. RNA was purified to remove DNA contamination. From the RNA, cDNA was prepared by reverse transcription. cDNA was then assayed by real-time PCR using specific primers for rat CAR, CYP2B1, UGT1A, and UGT2B1.

Total RNA extraction:

On day 3, hepatocytes were washed with PBS and the total RNA were extracted using Isogen solution (Wako). Total RNA was purified using RNeasy Mini kit (Qiagen) with on-column DNase treatment to avoid genomic DNA contamination. Total RNA was quantified by UV analysis at 260 nm and 280 nm using a UV spectrometer (Shimadzu). The total RNA solution was stored at –80 ºC until needed for complementary DNA (cDNA) generation.

Reverse transcription:

cDNA was prepared from total RNA by reverse transcription using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Solution (20µL) containing 10x RT Buffer (2µL), 25x NTP Mix (0.8 µL), 10x RT Random Primers (2µL), MultiScribe™ Reverse Transcriptase (50U), RNase Inhibitor (1µL), DEPC treated water (11.2µL), total RNA (400 ng)

[5] Calculation: 300 mg/kg bw, 6 hours exposure: in males 140.2 µg/g total radioactivity in liver; females: 115.3 µg/g total radioactivity in liver; in males fenpyrazamine (parent) made 10.6% of total radioactivity in liver, while in females, fenpyrazamine (parent) made 9.7% of total radioactivity in liver; this allows the calculation that fenpyrazamine (parent) was present at 14.8 µg/g in male liver and 11.18 µg/g in females liver; MW of fenpyrazamine is 331, 43 g/mol which leads to the calculation that 14.8 µg/g (ppm) corresponds to 33.7 µM and 11.18 µg/g (ppm) corresponds to 44.65 µM

was incubated at 25 ºC for 10min, 37 ºC for 120 min, and 85 ºC for 5sec. The resultant cDNA was stored at –20 ºC until needed for real-time PCR assays.

Quantitative real-time PCR:

Quantitative real-time PCR assays for rat CAR, CYP2B1, UGT1A and UGT2B1 were performed following the instruction manual of the PCR system (GeneAmp 7500 Sequence Detection System, Applied Biosystems). In addition, the mRNA level of Glyceraldehyde 3 phosphate dehydrogenase (GAPDH) of rat was measured as an internal control. Phenobarbital induces CYP2B1 mRNA, therefore, mRNA levels of CYP2B1 were determined to evaluate the effects of fenpyrazamine on CYP induction. UGT enzymes play a major role in thyroid hormone metabolism in the liver; several members of the UGT family of enzymes can metabolize T_4 as well as T_3. T_4 is glucuronidated by the phenol/bilirubin UGT (UGT1A1), whereas T_3 is glucuronidated by the androsterone UGT (UGT2B1). Induction of these enzymes increases T_4 and T_3 glucuronidation and their subsequent excretion into bile. Therefore, in this study, mRNA levels of UGT1A and UGT2B1 were determined to evaluate the effects of fenpyrazamine on UGT induction. For the quantitation of mRNA of CAR, UGT1A, UGT2B1, and GAPDH, the reaction mixture (25µL) containing 2x TaqMan Universal Master Mix (Applied Biosystems) (12.5µL), each primer (Forward and Reverse, 0.2µM each), probe (0.2µM) and cDNA (2µL of ¼ diluted solution) were used. After incubation at 50 ºC for 2min and 95 ºC for 10min, the PCR reaction was performed for 40 cycles: denaturation at 95 ºC for 15s, anneal and extension at 60 ºC for 1min. For the quantitation of CYP2B1 mRNA, the reaction mixture (25µL) containing 2x Power SYBR Green PCR Master Mix (Applied Biosystems) (12.5µL), each primer (Forward and Reverse, 0.4µM each), and cDNA (2µL of 1/4 diluted solution) were used. After incubation at 95 ºC for 10min, the PCR reaction was performed for 40cycles: denaturation at 95 ºC for 30s, anneal at 55 ºC for 30s, and extension at 72 ºC for 45s. The mRNA levels of CAR, CYP2B1, UGT1A, and UGT2B1 were normalized to those of the GAPDH.

Statistical analysis:
The F-test was applied for comparison. For the samples with or without homogenous variance, Student's t-test or Welch's t-test was used, respectively. The two-tailed test was employed for evaluation with $p<0.05$ and 0.01 as the levels of significance.

Findings:
In negative control hepatocytes (NC), mRNA expression levels of CYP2B1, UGT1A, and UGT2B1 were significantly increased by fenpyrazamine - degrees of the increase in each enzyme were 3.6-, 1.3-, and 30-fold of untreated control, respectively. The expression level of CAR mRNA was also significantly increased by fenpyrazamine to 4-fold of untreated control. However, it was suppressed to 55% of control by CAR-siRNA. In comparison with hepatocytes treated with siRNA as negative control, the hepatocytes treated with CAR-siRNA revealed less expression of mRNA of CYP2B1, UGT1A, and UGT2B1, suggesting that these mRNA expressions are reduced by CAR knockdown. Furthermore, in comparison with untreated control hepatocytes, the reduced levels of mRNA of these enzymes in the hepatocytes treated with CAR-siRNA plus fenpyrazamine were comparable with or were lower. These findings demonstrate that increased mRNAs of these enzymes by fenpyrazamine are mediated by CAR.

Table 49: mRNA levels for CAR enzyme induction in rat hepatocytes

Gene	**Untreated**	**Fenpyrazamine**	**Fenpyrazamine + negative control**	**Fenpyrazamine + siRNA(CAR) (CAR**

	control		siRNA	knockdown)
CAR/GAPDH	0.232	**1.025****	0.906	**0.126**##
CYP2B1/GAPDH	0.241	**0.865****	0.657	**0.266**##
UGT1A/GAPDH	0.785	**1.024****	1.031	**0.382**##
UGT2B1/GAPDH	0.049	**1.495****	1.061	**0.330**##

Quantity of each cDNA is normalized to GAPDH, so expressed as a ratio to GADPH
N= 4 wells/culture
** p<0.01 in comparison to untreated control
p<0.01 in comparison to transfected (siRNA(NC) control

Conclusion:
Incubation of rat hepatocytes with fenpyrazamine resulted in increased CAR, CYP2B1, UGT1A, and UGT2B1 expression. In transfected CAR-knockdown hepatocytes, the expression of all of these genes was reduced compared to the transfected control. Since only CAR was inhibited by transfection, changes in CYP2B1, UGT1A, and UGT2B1 are considered to be mediated by CAR in fenpyrazamine treated hepatocytes.

These findings demonstrate that fenpyrazamine activates the CAR and indicate that it probably acts by a mechanism similar to phenobarbital.

Reference:	An evaluation of the human relevance of the liver and thyroid tumors observed in male rats treated with fenpyrazamine (S-2188) based on mode of action
Author(s), year:	Yamada, T.; 2010b
Report/Doc. number:	Sumitomo Chemical Co. Ltd. Report No. QNT-0050
Guideline(s):	2006 IPCS Mode of Action framework
GLP:	No. Not appropriate for a document of this nature
Deviations:	-
Validity:	Yes

Hepatocellular carcinoma and thyroid follicular cell carcinoma were observed in 2 and 3 males respectively at the highest dose level in the 2-year rat study with fenpyrazamine but none of the concurrent control. The incidence of these tumors was above historical control data from comparable feeding studies. Therefore, the notifier submitted two mechanistic studies to show that the tumors observed in male rats are not relevant for humans (based on mode of action of fenpyrazamine in rat liver and) and a position paper on non-relevance of tumor findings in rat for humans according to IPCS Mode of Action framework[6].

The analysis followed guidance set out in the IPCS Mode of Action framework, which may be outlined as follows:

For liver tumors, the IPCS approach can be applied as follows:

[6] IPCS, 2007: Part 1: IPCS Framework for analysing the relevance of a cancer mode of action for humans and case studies

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

A. For liver, the postulated mode of action of fenpyrazamine is via CAR activation (as shown in the *in vivo* and *in vitro* study)

B. Key events of CAR activation, CYP2B induction, and enhancement of hepatocellular proliferation are experimentally verified

C. Dose-response can be demonstrated (these changes occur at doses relevant to the tumor response of concern)

D. Key events are demonstrated to occur before tumor formation is seen

E. The postulated mechanism is biologically plausible and coherent, and is well-researched for phenobarbital

F. No other mode of action is detected (a genotoxic mechanism can be excluded)

G. No key uncertainties or data gaps are identified

H. The data are judged to be adequate to explain the development of hepatocellular tumors in rats following chronic dietary exposure to fenpyrazamine. There is a clear evidence that fenpyrazamine activates the CAR which results in a pleiotropic response including stimulation of CYP2B and isoforms and increased hepatocellular proliferation

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

and

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

Although CAR is expressed in humans, and phenobarbital induces CYP enzymes in human liver, phenobarbital can act more through other receptors, such as pregnane X receptor (PXR). However, studies with human hepatocytes show that such cells are refractory to the hyperplastic and anti-apoptotic effects of phenobarbital. From many epidemiological studies with phenobarbital no evidence of liver tumors in patiens treated with phenobarbital for many years was observed. Therefore, it is assumed, that fenpyrazamine, having a MOA comparable to phenobarbital, would not be relevant for human liver tumors development.

For thyroid tumors, the IPCS approach can be applied as follows:

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

A. For thyroid, the postulated mode of action is of enhanced elimination of thyroid hormones consequent to CAR activation (as shown in the *in vivo* and *in vitro* study)
B. Key events of CAR activation, hepatic T_4-UGT induction, decreased circulating thyroid hormones, and increased circulating TSH have been experimentally verified
C. An appropriate dose-response can be demonstrated
D. Key events are demonstrated to occur before tumor formation is seen
E. The postulated mechanism is biologically plausible and coherent, and is well-researched for phenobarbital
F. No other mode of action is detected (a genotoxic mechanism can be excluded
G. No key uncertainties or data gaps are identified.

H. Due to key difference in thyroid hormone homeostasis between rats and humans, the postulated mode of action is not of relevance to humans

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

and

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

There are marked differences in pharmacokinetics of thyroid hormones between rats and humans. Phenobarbital (PB)-induced rat thyroid tumours provide an example where the qualitative processes (PB induction of thyroid hormone metabolism, T_3 feedback on TSH, and effects on thyroid proliferation) are the same in rats and humans but where sufficient quantitative differences exist between species to indicate that thyroid tumours would not occur in humans. E.g. rats have much smaller reserve/storage capacity of thyroid hormones, much shorter thyroid hormones half-life time, absence of high affinity binding globulin for T4 and T3, faster metabolic degradation and clearance of T4, 25-fold higher TSH level and activity of the pituitary-thyroid axis. Therefore, the thyroid tumours in rat, based on phenobarbital mode of action events, should not be regarded as relevant for humans. As for liver, epidemiological studies with phenobarbital do not show any increased risk of thyroid cancer in humans.

The statement of the manufacturer to the Phenobarbital MOA and fenpyrazamine is included below (which is considered conclusive by the dossier submitter):

1. Mode of action of phenobarbital-produced liver and thyroid tumours in rodent

Phenobarbital has been shown to produce liver and thyroid tumours in rodents. The current IARC classification of phenobarbital, which was concluded *in 2001*, is *Group 2B (possibly carcinogen to humans)*. Now, however, we should take into consideration recent reliable information based on results of epidemiological and mechanistic studies generated since the IARC review. An expert panel of scientists from academia, industry and government held a workshop at the EPA reviewing rodent liver carcinogenesis. It was concluded that *the phenobarbital-like MOA for liver carcinogenicity was deemed is not relevant for humans* (Holsapple *et al.*, 2006[7]).

Liver tumour

The key events for phenobarbital-produced liver effects have been well determined and include induction of CYP2B isoforms through constitutive androstane receptor (CAR), increased hepatocellular proliferation and eventually liver tumours. Since phenobarbital does not produce liver tumours in CAR knockout mice (Yamamoto *et al.*, 2004[8]), the MOA for production of liver tumours by phenobarbital in rodents involves activation of CAR.

Thyroid tumour

The MOA for promotion of thyroid follicular-cell tumours by phenobarbital involves the perturbation of homeostasis of the pituitary–thyroid axis by an extrathyroidal mechanism (Meek

[7] Holsapple, M. P., Pitot, H. C., Cohen, S. M., Boobis, A. R., Klaunig, J. E., Pastoor, T., Dellarco, V. L., and Dragan, Y. P. (2006). Mode of action in relevance of rodent liver tumours to human cancer risk. *Toxicol. Sci.* **89**, 51-56

[8] Yamamoto, Y., Moore, R., Goldsworthy, T. L., Negishi, M., and Maronpot, R. R. (2004). The orphan nuclear receptor constitutive active/androstane receptor is essential for liver tumour promotion by phenobarbital in mice. *Cancer Res.* **64**, 7197-7200

et al., 2003[9]). Specifically, phenobarbital induces hepatic T4-uridine diphosphate glucuronyl transferase (UDP-GT) activity, leading to enhanced metabolism of thyroxin (T4) by conjugation and increased biliary excretion of the conjugated hormone. The result of this enhanced liver metabolism is a decrease in serum T4 (and sometimes T3) half-life. As a result of the feedback function for decreased circulating thyroidal hormones on the hypothalamic-pituitary-thyroid axis, the pituitary gland enhances the output and serum level of thyroid-stimulating hormone (TSH). Prolonged elevation of circulating TSH levels stimulates the thyroid gland to deplete its stores of thyroid hormone and continues to induce hormone production. Thus, the thyroid follicular cells enlarge from squamous or cuboidal shape to columnar (hypertrophy) and are induced to proliferate at an increased rate and to increase in number (hyperplasia). With chronic exposure, thyroid hyperplasia eventually progresses to neoplasia. Furthermore, CAR is also required for phenobarbital-mediated disruption of thyroid hormone homeostasis and the induction of thyroid follicular cell proliferation evidenced by findings from CAR knockout mice (Qatanani *et al.*, 2005[10]).

2. Analysis for human relevance of phenobarbital-induced tumours bases on mode of action

Liver tumour

The most critical step is the effect on hepatocellular proliferation. It has been thought that there is an increase in liver size in humans after prolonged treatment with phenobarbital, and that the increased liver size in humans produced by phenobarbital appears to be due to hepatocellular hypertrophy (Holsapple *et al.*, 2006). Thus, there appears to be the same proliferation of smooth endoplasmic reticulum in human liver in response to phenobarbital that is seen in the rat and the mouse. However, studies with human hepatocytes *in vitro* suggest that the hepatocytes are refractory to the increased proliferative and anti-apoptotic effects of phenobarbital that occur in rodents (Hasmall and Roberts, 1999[11]; Parzefall *et al.*, 1991[12]).

Summary concordance table for mode of action on phenobarbital-induced liver tumours

Key events	Rodents	Humans
CAR activation	**Yes** (Moore *et al.*, 2003) (Yamamoto *et al.*, 2004) (Deguchi *et al.*, 2009[13])	**Yes** (Moore *et al.*, 2003) (Qatanani and Moore, 2005[14])

[9] Meek, M. E., Bucher, J. R., Cohen, S. M., Dellarco, V., Hill, R. N., Lehman-McKeeman, L. D., Longfellow, D. G., Pastoor, T., Seed, J., and Patton, D. E. (2003). A framework for human relevance analysis of information on carcinogenic modes of action. *Crit. Rev. Toxicol.* **33**, 591-653

[10] Qatanani, M., Zhang, J., and Moore, D. D. (2005). Role of the constitutive androstane receptor in xenobiotic-induced thyroid hormone metabolism. *Endocrinology* **146**, 995-1002

[11] Hasmall, S. C., and Roberts, R. A. (1999). The perturbation of apoptosis and mitosis by drugs and xenobiotics. *Pharmacol. Ther.* **82**, 63-70

[12] Parzefall, W., Erber, E., Sedivy, R., and Schulte-Hermann, R. (1991). Testing for induction of DNA synthesis in human hepatocyte primary cultures by rat liver tumour promoters. *Cancer Res.* **51**, 1143-1147

[13] Deguchi, Y., Yamada, T., Hirose, Y., Nagahori, H., Kushida, M., Sumida, K., Sukata, T., Tomigahara, Y., Nishioka, K., Uwagawa, S., Kawamura, S., and Okuno, Y. (2009). Mode of action analysis for the synthetic pyrethroid metofluthrin-induced rat liver tumours: Evidence for hepatic CYP2B induction and hepatocyte proliferation. *Toxicol. Sci.* **108**, 69-80

[14] Qatanani, M., and Moore, D. D. (2005). CAR, the continuously advancing receptor, in drug metabolism and disease. *Curr Drug Metab* **6**, 329-339

Induction of hepatic CYP2B	**Yes** (Price *et al.*, 2008[15]) (Hirose *et al.*, 2009[16]) (Price *et al.*, 2007[17]) : vivo	**Yes** (Pirttiaho *et al.*, 1982[18]) : vivo (Price *et al.*, 2008) (Hirose *et al.*, 2009)
Increased liver weight or size	**Yes** (Price *et al.*, 2007) (Jones *et al.*, 2009[19])	**Yes** (Pirttiaho *et al.*, 1982)
Increased hepatocellular proliferation	**Yes** (Price *et al.*, 2007) (Jones *et al.*, 2009) (Hirose *et al.*, 2009)	**Unlikely** (Parzefall *et al.*, 1991) (Hasmall and Roberts, 1999) (Hirose *et al.*, 2009) (Osimitz and Lake, 2009[20]) (Ross *et al.*, 2010[21])
Appearance of hepatocellular tumours	**Yes** (IARC, 2001[22]) (Jones *et al.*, 2009)	**Unlikely** (Holsapple *et al.*, 2006) (Graham and Lake, 2008[23]) (Lake, 2009[24]) (Osimitz and Lake, 2009) (Yamada *et al.*, 2009[25]) (Ross *et al.*, 2010) (Carmichael *et al.*, 2011[26])

15 Price, R. J., Giddings, A. M., Scott, M. P., Walters, D. G., Capen, C. C., Osimitz, T. G., and Lake, B. G. (2008). Effect of pyrethrins on cytochrome p450 forms in cultured rat and human hepatocytes. *Toxicology* **243**, 84-95

16 Hirose, Y., Nagahori, H., Yamada, T., Deguchi, Y., Tomigahara, Y., Nishioka, K., Uwagawa, S., Kawamura, S., Isobe, N., Lake, B. G., and Okuno, Y. (2009). Comparison of the effects of the synthetic pyrethroid metofluthrin and phenobarbital on CYP2B form induction and replicative DNA synthesis in cultured rat and human hepatocytes. *Toxicology* **258**, 64-69

17 Price, R. J., Walters, D. G., Finch, J. M., Gabriel, K. L., Capen, C. C., Osimitz, T. G., and Lake, B. G. (2007). A mode of action for induction of liver tumours by pyrethrins in the rat. *Toxicol. Appl. Pharmacol.* **218**, 186-195

18 Pirttiaho, H. I., Sotaniemi, E. A., Pelkonen, R. O., and Pitkanen, U. (1982). Hepatic blood flow and drug metabolism in patients on enzyme-inducing anticonvulsants. *Eur. J. Clin. Pharmacol.* **22**, 441-445

19 Jones, H. B., Orton, T. C., and Lake, B. G. (2009). Effect of chronic phenobarbitone administration on liver tumour formation in the C57BL/10J mouse. *Food Chem. Toxicol.* **47**, 1333-1340.

20 Osimitz, T. G., and Lake, B. G. (2009). Mode-of-action analysis for induction of rat liver tumours by pyrethrins: Relevance to human cancer risk. *Crit. Rev. Toxicol.* **39**, 501-511

21 Ross, J., Plummer, S. M., Rode, A., Scheer, N., Bower, C. C., Vogel, O., Henderson, C. J., Wolf, C. R., and Elcombe, C. R. (2010). Human constitutive androstane receptor (CAR) and pregnane x receptor (PXR) support the hypertrophic but not the hyperplastic response to the murine nongenotoxic hepatocarcinogens phenobarbital and chlordane in vivo. *Toxicol. Sci.* **116**, 452-466

22 IARC (2001). Some thyrotropic agents: Phenobarbital and its sodium salt. *IARC Monogr. Eval. Carcinog. Risks Hum.* **79**, 161-288

23 Graham, M. J., and Lake, B. G. (2008). Induction of drug metabolism: Species differences and toxicological relevance. *Toxicology* **254**, 184-191

Most importantly, there is also substantial epidemiological evidence for the non-carcinogenicity of phenobarbital in humans (IARC, 2001; Olsen *et al.*, 1989; Olsen *et al.*, 1995; Whysner, et al., 1996). The exposure levels in humans are similar to those in rodents, in contrast to the marked differences in exposure to environmental and occupational chemicals, and also, administration to humans occurs over a period of many years, frequently beginning in childhood and continuing for essentially the lifetime of the individual. Such studies have demonstrated that in human subjects receiving phenobarbital for many years at doses producing plasma concentrations similar to those which are carcinogenic in rodents, there is no evidence of increased liver tumour risk. Therefore, many scientists including us think that the Answer to Question 2 "Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between animals and human?" is YES. However, since detailed mechanism of such species differences of susceptibility is not fully understood, this "YES" answer has not been agreed upon worldwide; i.e., the Answer to Question 2 may be NO. This means that the possibility of increased of cell proliferation can not be ruled out in humans.

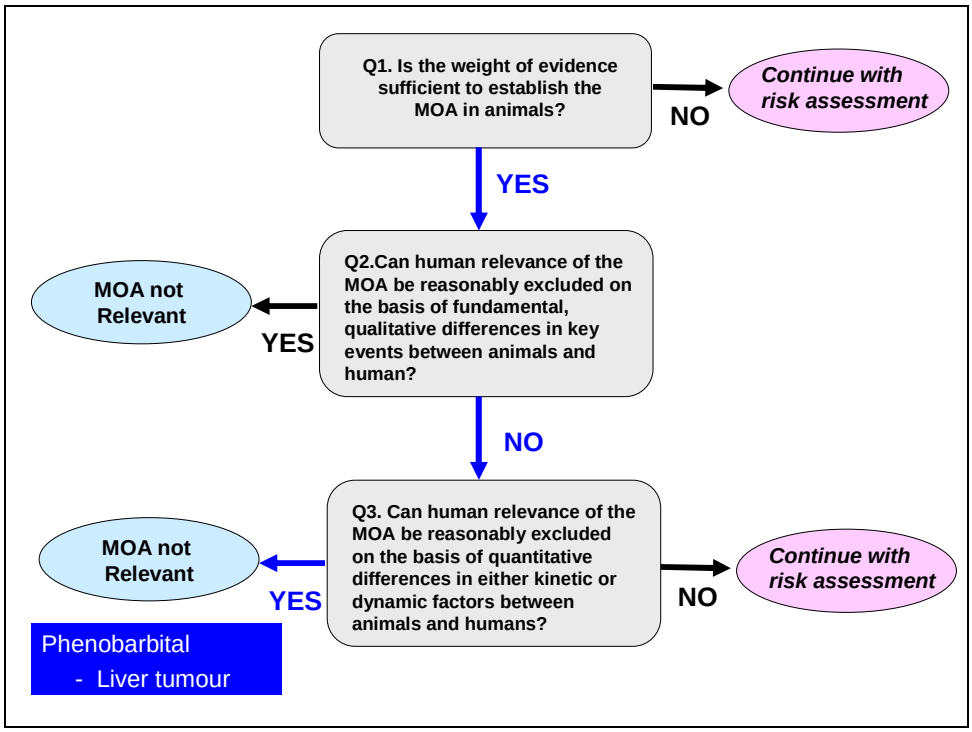
Recently, using primary culture, we confirmed that the dose-dependent increase of DNA synthesis of hepatocytes by phenobarbital was detected in rat but not in human (Hirose *et al.*, 2009). In addition, Osimitz and Lake also mentioned that pyrethrins, like phenobarbital, do not induce cell proliferation in human hepatocytes (Osimitz and Lake, 2009). Furthermore, a recent publication presented that, based on comparison among double humanized pregnane x receptor (PXR) and CAR (huPXR/huCAR), double knockout PXR and CAR (PXRKO/CARKO), and wild-type (WT) C57BL/6J mice, the human receptors are able to support the chemically induced hypertrophic responses but not the hyperplastic (cell proliferation) responses (Ross *et al.*, 2010). These findings strongly support that human hepatocytes are refractory to the increased proliferative effects of phenobarbital that occur in rodents (Hasmall and Roberts, 1999; Hirose *et al.*, 2009; Osimitz and Lake, 2009; Parzefall *et al.*, 1991; Ross *et al.*, 2010).

Therefore, the Answer to Question 3 "Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between animals and humans?" is certainly YES (Figure below). This is consistent with recent publications in which it is concluded that although the possible relevance of the MOA of phenobarbital cannot be dismissed on qualitative grounds, it is highly unlikely to be relevant to humans because of major quantitative interspecies differences in the key events for liver tumors (Carmichael *et al.*, 2011; Graham and Lake, 2008; Holsapple *et al.*, 2006; Lake, 2009; Osimitz and Lake, 2009; Yamada *et al.*, 2009).

[24] Lake, B. G. (2009). Species differences in the hepatic effects of inducers of CYP2B and CYP4A subfamily forms: Relationship to rodent liver tumour formation. *Xenobiotica* **39**, 582-596

[25] Yamada, T., Uwagawa, S., Okuno, Y., Cohen, S. M., and Kaneko, H. (2009). Case study: An evaluation of the human relevance of the synthetic pyrethroid metofluthrin-induced liver tumours in rats based on mode of action. *Toxicol. Sci.* **108**, 59-68

[26] Carmichael, N., Bausen, M., Boobis, A. R., Cohen, S. M., Embry, M., Fruijtier-Pölloth, C., Greim, H., Lewis, R., Meek, M. E., Mellor, H., Vickers, C., and Doe, J. (2011). Using mode of action information to improve regulatory decision-making: An ECETOC/ILSI RF/HESI workshop overview. *Crit. Rev. Toxicol.* **41**, 175-186



Human relevance of the rodent liver carcinogenic response of phenobarbital is evaluated based upon the human relevance framework [Adapted from Holsapple *et al.* (2006)].

Thyroid tumour

The current understanding of the regulation of thyroid hormone homeostasis in humans and of the role of increased TSH levels (as a result of altered thyroid hormone homeostasis) as a risk factor for thyroid cancer was considered in order to assess the human relevance of the key events in the animal mode of carcinogenic action by phenobarbital (Table below). The fundamental mechanisms involved in the function and regulation of the hypothalamic–pituitary–thyroid axis in rats are qualitatively similar to those in humans (Bianco *et al.*, 2002[27]). Therefore, an agent that decreases T4 levels in rats could likewise reduce T4 in humans; this, in turn, could potentially lead to an increase in TSH levels (Dellarco *et al.*, 2006[28]). However, other microsomal enzyme inducers have not been shown to increase TSH levels even when T4 is decreased (Meek *et al.*, 2003).

Although a number of pharmaceuticals (e.g., propylthiouracil, lithium, amiodarone, iopanoic acid) that disrupt thyroid homeostasis by acting directly on the thyroid gland (for example, by inhibiting hormone synthesis or release or by blocking the conversion of T4 to T3) are known to lead to hypothyroidism and increases in TSH in humans, there is no evidence of increased susceptibility to thyroid cancer in humans secondary to hypothyroidism induced by any mechanism (Dellarco *et al.*, 2006).

Summary concordance table for mode of action on phenobarbital-induced thyroid tumours

Key events	Rodents	Humans

[27] Bianco, A. C., Salvatore, D., Gereben, B., Berry, M. J., and Larsen, P. R. (2002). Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr. Rev.* **23**, 38-89

[28] Dellarco, V. L., McGregor, D., Berry, S. C., Cohen, S. M., and Boobis, A. R. (2006). Thiazopyr and thyroid disruption: Case study within the context of the 2006 IPCS human relevance framework for analysis of a cancer mode of action. *Crit. Rev. Toxicol.* **36**, 793-801

CAR activation	**Yes** (Moore *et al.*, 2003[29]) (Qatanani and Moore, 2005) (Tien *et al.*, 2007[30]) (Pakharukova *et al.*, 2010[31])	**Yes** (Moore *et al.*, 2003) (Qatanani and Moore, 2005)
Increased hepatic UDP-GT (Increase hepatic clearance of T4)	**Yes** (Qatanani *et al.*, 2005)	**Yes** (Meek *et al.*, 2003)
Decreased serum T4 levels	**Yes** (Qatanani *et al.*, 2005)	**Yes** (Meek *et al.*, 2003)
Increased serum TSH levels	**Yes** (Qatanani *et al.*, 2005)	**Unlikely** (Meek *et al.*, 2003).
Increased TSH increases thyroid cell proliferation	**Yes** (Qatanani *et al.*, 2005)	**Unlikely** (Meek *et al.*, 2003)
Appearance of thyroid tumours	**Yes** (IARC, 2001)	**Unlikely** (Meek *et al.*, 2003) (Cohen *et al.*, 2004[32]) (Dellarco *et al.*, 2006) (Graham and Lake, 2008)

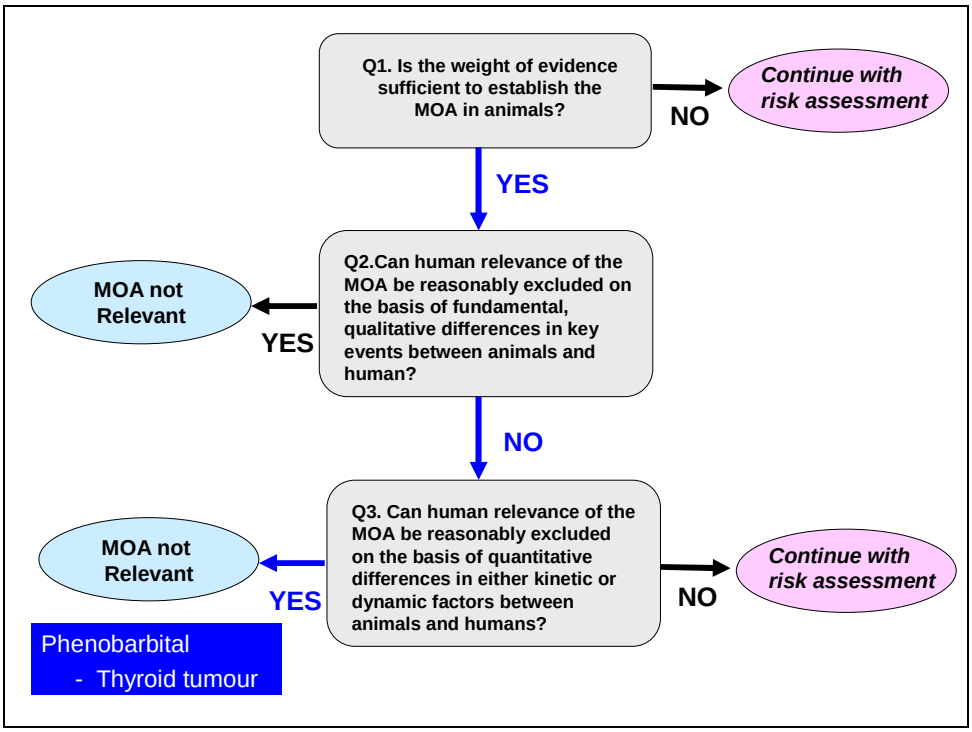
Thus, on both quantitative kinetic and dynamic bases, the response in rats does not occur in humans, even though on a qualitative basis the human has the potential (Figure below). One can thus conclude on a quantitative basis that this MOA does not apply to humans (Cohen *et al.*, 2004; Dellarco *et al.*, 2006; Graham and Lake, 2008; Meek *et al.*, 2003).

[29] Moore, J. T., Moore, L. B., Maglich, J. M., and Kliewer, S. A. (2003). Functional and structural comparison of PXR and CAR. *Biochim. Biophys. Acta* **1619**, 235-238

[30] Tien, E. S., Matsui, K., Moore, R., and Negishi, M. (2007). The nuclear receptor constitutively active/androstane receptor regulates type 1 deiodinase and thyroid hormone activity in the regenerating mouse liver. *J. Pharmacol. Exp. Ther.* **320**, 307-313

[31] Pakharukova, M., Smetanina, M., Kaledin, V., Obut, T., and Merkulova, T. (2010). The increased CAR-dependent metabolism of thyroid hormones in mice with high cancer susceptibility. *Life Sci.* **87**, 439-444

[32] Cohen, S. M., Klaunig, J., Meek, M. E., Hill, R. N., Pastoor, T., Lehman-McKeeman, L., Bucher, J., Longfellow, D. G., Seed, J., Dellarco, V., Fenner-Crisp, P., and Patton, D. (2004). Evaluating the human relevance of chemically induced animal tumours. *Toxicol. Sci.* **78**, 181-186



Human relevance of the rodent thyroid carcinogenic response of phenobarbital is evaluated based upon the human relevance framework (Cohen *et al.*, 2004; Dellarco *et al.*, 2006; Graham and Lake, 2008; Meek *et al.*, 2003).

3. Direct comparison of phenobarbital effects with effects caused by fenpyrazamine

Since the effects of phenobarbital have been extensively investigated, we did not set a phenobarbital group as a concurrent positive control in the MOA study with fenpyrazamine. Here we compare the effects caused by fenpyrazamine to those observed by phenobarbital (Table below). Data for phenobarbital, except for BrdU labeling indices, were collected in our laboratory, thus comparison of both data sets is reliable for evaluation of similarity. Regarding labeling, the method of BrdU treatment is different; intraperitoneal injection at a dosage of 100 mg/kg body weight at 2 hr before sacrifice was used in the fenpyrazamine study, while Alzet mini pumps implanted in the subcutaneous tissues on the day prior to 7 days of the scheduled sacrifice was used in the phenobarbital study. Therefore, data of labeling indices were adopted from published information using the same method (Jones and Clarke, 1993[33]). For your reference, in the study using Alzet mini pumps, BrdU labeling indices were increased 3.5-fold of control after 1-week treatment of phenobarbital, but returned to control levels after 2-week treatment (Deguchi *et al.*, 2009). Although the rate of DNA replication returns to control levels, the numbers of hepatocytes increased after phenobarbital treatment so the number of DNA replications is increased.

[33] Jones, H. B., and Clarke, N. A. (1993). Assessment of the influence of subacute phenobarbitone administration on multi-tissue cell proliferation in the rat using bromodeoxyuridine immunocytochemistry. *Arch. Toxicol.* **67**, 622-628

Table 3. Comparative table for fenpyrazamine and phenobarbital [a]

	Fenpyrazamine			Phenobarbital		
(ppm)	0	2400		0	1000	
Achieved intake of test substance (mg/kg/day)	0	216.6		0.0	62.7	
Organ weight:						
Liver: Absolute (g)	10.4	13.48	**	12.10	15.28	**
		(1.3)			(1.3)	
Liver: Relative (g/100gBW)	4.07	5.20	**	3.60	4.54	**
		(1.3)			(1.3)	
Thyroid: Absolute (mg)	18	21		18	20	
		(1.2)			(1.1)	
Thyroid: Relative (mg/100gBW)	7.2	8.1		5.3	5.9	
		(1.1)			(1.1)	
Histopathology – light microscopy:						
Centrilobular hepatocyte hypertrophy	0/10	9/10	**	0/8	8/8	**
	0%	90%		0%	100%	
Thyroid follicular cell hypertrophy	0/10	9/10	**	0/8	3/8	
	0%	90%		0%	38%	
Hepatocellular proliferation						
BrdU labeling indices (%), Day-3	2.38	3.36		2.4	6.50	**
		(1.4)			(2.7)	
BrdU labeling indices (%), Day-7	1.93	0.57		2.1	1.50	
		(0.3)			(0.7)	
Enzyme activity						
Hepatic CYP2B activity (pmol/min/mg S9 protein)	3.8	13.90	**	1.5	110.45	**
		(3.7)			(73.6)	
Hepatic UDP-GT activity toward T4 (pmol/min/mg S9 protein)	0.53	0.66	**	0.54	0.89	**
		(1.2)			(1.7)	
Serum hormone levels:						
T3 (ng/mL)	0.6	0.6		0.5	0.6	
		(1.0)			(1.2)	
T4 (µg/dL)	3.31	3.26		2.53	2.15	*
		(1.0)			(0.8)	
TSH (ng/mL)	10.7	17.1		8.0	19.1	**
		(1.6)			(2.4)	

Statistically significant, *: p<0.05, **: p<0.01.

a The phenobarbital data include published (Deguchi *et al.*, 2009) and unpublished data. All data are adopted from 2-week treatment study except for BrdU labeling indices. Alteration of BrdU labeling indices is usually appeared at early phase of treatment with enzyme inducers. Since fenpyrazamine revealed a tendency of increase of labeling at Day 3 but the value was less than that of control at Day 7, the data at Days 3 and 7 were presented. However, we do not have such data for phenobarbital, therefore these data were adopted from (Jones and Clarke, 1993). The data represent group mean value, and the value in parentheses represents proportion to control.

[illegible] he [illegible] ıould be noted that the effects by fenpyrazamine are much less potent than those of phenobarbital, and the tumour response is also less; in the rat carcinogenicity study of fenpyrazamine, the incidence of liver tumours was not statistically significant.

One possibility, therefore, is to "read across" from these findings with phenobarbital to other compounds that share its MOA in animals in inducing rodent liver tumours and to conclude that the tumours caused by such a compound are not relevant to the risk assessment of the compound in humans (Cohen, 2010[34]; Cohen *et al.*, 2004; Dellarco *et al.*, 2006; Meek *et al.*, 2003;

[34] Cohen, S. M. (2010). Evaluation of possible carcinogenic risk to humans based on liver tumours in rodent assays: The two-year bioassay is no longer necessary. *Toxicol. Pathol.* **38**, 487-501

Yamada *et al.*, 2009). Such a conclusion would be critically dependent on the reliability of the epidemiological data and the similarity between the MOA for the chemical under test to that of the compound for which there are epidemiological data available.

4.10.4 Summary and discussion of carcinogenicity

In the combined chronic and carcinogenicity study (2 years) in rat, the NOAEL is set at 300 ppm (15.6 mg/kg bw/d) in females, based on shortened prothrombin time and decreased MCV and MCH and 12.7 mg/kg bw/d in males, based on statistically significantly increased hepatocellular hypertrophy accompanied by increased relative liver weight at 1200 ppm at week 52 in satellite group and hepatocellular hypertrophy (but without change in relative liver weight) in main group males at week 104, as well as on increase in albumin and gamma-glutamyl-transferase).

Regarding carcinogenic potential of fenpyrazamine, no increase of neoplastic findings was observed in any organ of treated animals, with exception of liver and thyroid. The incidence of hepatocellular carcinoma of 4% in males at 2400 ppm was above historical control data (0.0% to 2.8%) from comparable feeding studies. The higher incidence of thyroid follicular carcinoma in males of 2400 ppm group (6.1%) was higher than the historical control data (max. 4% in males) from comparable feeding studies. At the dose causing higher incidence of liver and thyroid tumours (2400 ppm) no excessive toxicity was observed. The animals with tumours survived until scheduled sacrifice. A clear threshold dose (1200 ppm) could be estimated where no increased incidence of liver and thyroid carcinoma was seen.

By default, carcinogenic effects in experimental animals are considered relevant to humans and are considered for classification as carcinogens (*Guidance on the Application of CLP Criteria, Guidance to Regulation (EC) No 1272/2008 on classification, labeling and packaging (CLP) of substances and mixtures, 2009*). Only if there is sufficient evidence showing non-relevance of a certain type of tumors to humans, this tumor type should be excluded for classification. Fenpyrazamine is not genotoxic. In the provided carcinogenicity study, malignant tumors were observed in male rats in multiple sites (liver and thyroid), which incidence was above historical control data of well comparable studies. No exceeding of MTD (maximum tolerated dose) was observed in males (body weight loss was 10.1% compared to control, but no signs of severe toxicity including reduced survival rate was seen). Both affected tissues, liver and thyroid, have their equivalent in humans. Two mechanistic studies provided (one *in vivo* and one *in vitro*, see "other relevant information", 4.10.3) could show that fenpyrazamine has a comparable MOA to phenobarbital (perturbation of homeostasis of pituitary-thyroid-axis by extrathyroidal mechanism). Phenobarbital MOA is considered to be not relevant for humans[35]. No liver and thyroid tumors were observed above historical control data in mouse carcinogenicity study or in female rats (male rats are frequently found to be more sensitive with respect to induction of thyroid tumors than female rats and mice) showing that even if fenpyrazamine may have a MOA comparable to phenobarbital, it is of much lower potency (shown also by low incidences of liver and thyroid tumors in male rats).

Combining all these criteria for classification of a substance as a carcinogen, according to Dangerous Substance Directive (67/548/EC) and Regulation 1272/2008, no classification and labeling is proposed for fenpyrazamine.

In the carcinogenicity study in CD-1 mouse (78 weeks), the NOAEL for males is set at 1500 ppm (176 mg/kg bw/d), based on statistically significantly higher relative liver weight in satellite and

[35] IPCS, 2007: Part 1: IPCS Framework for analysing the relevance of a cancer mode of action for humans and case studies

main group animals accompanied by higher incidence of hepatocellular hypertrophy observed in main group animals, and supported by statistically significantly increased MCV and MCH and decreased RBC at 3000 ppm. In females, NOAEL is set at 2000 ppm (283 mg/kg bw/d), based on statistically significantly higher relative liver weight in satellite and main group animals accompanied by statistically significantly increased hepatocellular hypertrophy observed in main group animals, and supported by statistically significantly decreased RBC, HB and HCT at 4000 ppm. In the mouse carcinogenicity study, no carcinogenic potential of fenpyrazamine could be observed.

4.10.5 Comparison with criteria

By default, carcinogenic effects in experimental animals are considered relevant to humans and are considered for classification as carcinogens (*Guidance on the Application of CLP Criteria, Guidance to Regulation (EC) No 1272/2008 on classification, labeling and packaging (CLP) of substances and mixtures, 2009*). Only if there is sufficient evidence showing non-relevance of a certain type of tumors to humans, this tumor type should be excluded for classification. Fenpyrazamine is not genotoxic. In the provided carcinogenicity study, malignant tumors were observed in male rats in multiple sites (liver and thyroid), which incidence was above historical control data of well comparable studies. No exceeding of MTD (maximum tolerated dose) was observed in males (body weight loss was 10.1% compared to control, but no signs of severe toxicity including reduced survival rate was seen). Both affected tissues, liver and thyroid, have their equivalent in humans. Two mechanistic studies provided (one *in vivo* and one *in vitro,* see "other relevant information", 4.10.3) could show that fenpyrazamine has a comparable MOA to phenobarbital (perturbation of homeostasis of pituitary-thyroid-axis by extrathyroidal mechanism). Phenobarbital MOA is considered to be not relevant for humans. No liver and thyroid tumours were observed above historical control data in mouse carcinogenicity study or in female rats (male rats are frequently found to be more sensitive with respect to induction of thyroid tumors than female rats and mice) showing that even if fenpyrazamine may have a MOA comparable to phenobarbital, it is of much lower potency (shown also by low incidences of liver and thyroid tumors in male rats).

Combining all these criteria for classification of a substance as a carcinogen, according to Dangerous Substance Directive (67/548/EC) and Regulation (EC) 1272/2008, no classification and labelling is proposed for fenpyrazamine.

4.10.6 Conclusions on classification and labelling

No classification and labelling is proposed for fenpyrazamine regarding carcinogenicity.

4.11 Toxicity for reproduction

Table 50: Summary table of relevant reproductive toxicity studies

Method	NOAEL	Remarks	Reference
Preliminary 1-generation reproduction toxicity study (rat) (no appropriate guideline, pilot study)	Reproduction: 244.7 mg/kg bw/d (males) and 253.7 mg/kg bw/d (females) Parental: 24.8 mg/kg bw/d (males) and 75.0 mg/kg bw/d (females)	Wistar, HanRcc:WIST(SPF) rats Purity: 94.7%	Pössnecker, A., Flade, D, 2008

	Offspring: 75.0 mg/kg bw/d (females)		
Two-generation rat (OECD 416)	Reproduction: 73.7 mg/kg bw/d Parental: 20.3 mg/kg bw/d (males) and 31.6 mg/kg bw/d (females) Offspring: 28.5 mg/kg bw/d	Wistar, HanRcc:WIST(SPF) rats Purity: 94.7%	Gerspach, R., Weber, K., Flade, D, 2009
Developmental range finding study (rat) (OECD 414)	Foetal: no NOAEL established Maternal: 300 mg/kg bw/d No teratogenic potential	Wistar, HanRcc:WIST(SPF) Purity: 94.7%	Pössnecker, A, 2006
Developmental toxicity study (rat) (OECD 414)	Foetal: 125 mg/kg bw/d Maternal: 30 mg/kg bw/d No teratogenic potential	Wistar, HanRcc:WIST(SPF) Purity: 94.7%	Gerspach, R., and Flade, D, 2009
Developmental range finding study (rabbit) (guideline not stated, range finding study)	Foetal: 60 mg/kg bw/d Maternal: 60 mg/kg bw/d No teratogenic potential	New Zealand White (Kbl:NZW SPF) Rabbit Purity: 94.7%	Inakawa, K, 2008a
Developmental toxicity study (rabbit) (OECD 414)	Foetal: 30 mg/kg bw/d Maternal: 30 mg/kg bw/d No teratogenic potential	New Zealand White (Kbl:NZW SPF) Rabbit Purity: 94.7%	Inakawa, K, 2008b

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Reference:	Preliminary reproduction toxicity study in the Han Wistar rat
Author(s), year:	Pössnecker, A., Flade, D.; 2008
Report/Doc. number:	Sumitomo Chemical Co. Ltd. Report No. QNT-0020
Guideline(s):	No appropriate guideline; pilot study
GLP:	Yes (laboratory certified by National Authority)
Deviations:	Organ/body weight ratios calculated based on unverified body weight data; not considered relevant, since preliminary study only
Validity:	Yes; supporting study

Material and methods:

Test Material: S-2188
- Lot/Batch: 030-050914-1G
- Purity: 94.7%

Test animals:
- Species: Rat
- Strain: Wistar, HanRcc:WIST(SPF)
- Age: Minimum 8 weeks at delivery
- Weight at start: Males 260 – 294g, females 162 – 194 g

Source:	RCC Ltd, Laboratory Animal Services, Füllinsdorf, Switzerland
Diet:	Pelleted standard Kliba-Nafag 3433 maintenance diet; *ad libitum*

The purpose of the preliminary reproduction study was to determine suitable dose levels of the test substance for a subsequent main two-generation reproduction study. The dosing was based on a preliminary 2-week feeding study in rats, where animals of both sexes at the dose level of 3000 ppm revealed decreased body weight gain and effects on liver and thyroid.

Animal assignment and treatment

Groups of 5 male and 5 female young adult rats were offered fenpyrazamine in diet at dietary concentrations of 0 (control), 400, 1000 or 3000 ppm for 14 days pre-pairing period, and then continuously throughout pairing, gestation, and lactation. A pairing period of 14 days was allowed. The first day on which evidence of mating was seen was designated day 0 post-coitum. Females were returned to individual cages once evidence of mating was seen, and allowed to complete gestation and lactation. They were sacrificed at 21 days post-partum, at weaning of the pups. Adult males were sacrificed 43 days after pairing.

Diet preparation and analysis:

Fresh batches of the feed pellets for this study were prepared at least each 2 weeks with a constant concentration (ppm) throughout the feeding duration. The test item was mixed with granulated feed in a pelleting machine. Water was added to each feed preparation at a volume/weight ratio of approximately 1:10 to ensure pelleting. The pellets were dried with air for approximately 48 hours before storage. Feed for the control group was prepared similarly without the test item.

Accuracy of diet preparation was confirmed by analysis (GC). Stability of the test item in pelleted rodent feed was investigated over 21 days and found to be stable. Concentration and homogeneity of the actual test substance batch in the feed were determined at the start of the pre-pairing period. Mean test substance intakes (mg/kg bw/d) are given in table below.

Table 51: Mean test substance intakes (mg/kg bw/d) – P generation only

Sex	**Doses** **Period of treatment**	**400 ppm (Group 2)**	**1000 ppm (Group 3)**	**3000 ppm (Group 4)**
males	Pre-pairing period (14 days)	29.3	75.3	244.7
	After pairing period (43 days)	24.8	62.5	198.5
females	Pre-pairing period (14 days)	32.0	79.5	253.7
	Gestation period (21 days)	31.4	75.0	237.3
	Lactation period (days 1 to 14 post partum)*	73.1	177.0	490.2

*Food consumption of P females was measured only until day 14 of lactation period, but the pups were reared until weaning (day 21 post-partum). From days 21 to 28 the pups were reared for a further week on the respective diet

Clinical observations:

Animals were checked for morbidity or mortality and clinical signs at least twice daily.

Body weight:

Parental animals were weighed weekly, except during pairing. Parental females were weighed on days 0, 7, 14 and 21 post-coitum. Dams which littered were weighed on days 1, 4, 7, 14 and 21 post-partum.

Food consumption:

Food consumption was measured weekly at the same intervals as bodyweight, except during the pairing period when no food consumption was measured. During the lactation period food consumption was measured only to day 14 post-partum. Relative food consumption ratios, and test substance intake, were calculated. After weaning, food consumption of pups was measured for a further week.

Mating evaluation:

Animals were paired one male / one female for a period of maximal 14 days. A record of mating of females during the pairing period was made by daily examination of vaginal smears or appearance of a vaginal plug. The first day on which evidence of mating was found, was designated Day 0 post-coitum. Vaginal smears throughout the mating period were used to detect marked abnormalities in the oestrus cycle.

Gestation and parturition:

Approaching the end of the gestational period dams were examined twice daily for signs of parturition, and the duration of gestation was recorded.

Offsprings:

The day of birth was designated Day 0 post-partum. Litters were examined as soon as possible for litter size, live births, stillbirths and any gross abnormalities. Pups were weighed on days 0 and/or 1, 4, 7, 14, 21 and 28 post-partum and the sex ratio of live pups was determined at first litter check and on day 21. All dams and pups were observed daily for survival, and behavioural abnormalities in nesting and nursing. Dead pups were necropsied.

At 4 days post-partum, litters were standardised to 8 pups and surplus pups were necropsied and then discarded. Following weaning (day 21 post-partum), pups were maintained on treated diets for one further week to day 28 post-partum and then sacrificed and necropsied.

Gross Necropsy
All P (parent generation) animals were subject to macroscopic post-mortem examination. Examination of parental animals gave special attention to reproductive organs. In dams, the number of implantation sites was counted after staining the uterus in ammonium sulphide.

Histopathology
Tissue harvesting: From parental animals, liver, thyroid, and any tissue with gross lesions were fixed in buffered formaldehyde for possible future histological examination.

Organ weights: From pups killed at 28 days post-partum, the following weights were recorded from at least one male and one female per litter: bodyweight, brain, spleen, thymus, and uterus.

Findings:

Parental animals:

All animals survived until scheduled necropsy. No clinical signs or signs of discomfort were noted in parent animals. During the pre-pairing period the mean body weight of males and females was similar to control. During the gestation and lactation of females, males in all treated groups showed a body weight gain loss (-9.1%, -14.8% and -15.9% comparing to control). Parental female weight gain during gestation showed no consistent pattern. Only in the high dose females, body weight gain was -15.8% comparing to control animals. Mean body weight of females in the high dose at the end of gestation was -8.5% lower than in controls.

Enlarged thyroids were measured in 4 females of high dose group and in 2, 3 and 2 males of low, mid- and high dose group. No macroscopic changes were observed in any parent animal of low dose.

Reproduction:

There was no effect on time-to-mating, on numbers of females becoming pregnant, or length of gestation. There was no effect on number of implantation sites. Post-implantation loss was higher (but not statistically significant) in high dose animals than in controls (12.2% comparing to 3.8%). The number of live pups at birth was lower in high dose animals than in control (10.6 comparing to 13.5 in control), but the difference was not statistically significant.

Litter data:

No treatment-related effect on postnatal pup mortality was seen. The lower viability index in low dose group (91.4% compared to 98.1% in control) was not considered treatment related, since no effects on viability were seen in mid- and high dose animals. On day 1 post-partum mean pup weights were equal between all groups. Bodyweight of pups of the high dose group was significantly decreased from mid-lactation (effect seen starting from day 14) to day 28 post-partum. Mean food consumption in the pups of high dose between day 21 and 28 post-partum was decreased also. No effects on body weight were observed in pups of low and mid-dose. No effect on sex ration could be observed in any dose group. No treatment-related macroscopic changes were noted (thickened uterus of one female pup of low dose group was considered incidental). Absolute brain, thymus, spleen and uterus weight in female pups was significantly lower in the high dose group than in the control; for male pups, only absolute thymus weight was significantly lower in the high dose group. Relative brain weight was increased in males and females of high dose group; relative thymus weight was decreased in females.

The results of the preliminary reproduction study are given in table below.

Table 52: Results of the preliminary reproduction study

	Males				**Females**			
Diet concentration (ppm)	**0**	**400**	**1000**	**3000**	**0**	**400**	**1000**	**3000**
Parental animals								
Fenpyrazamine intake (mg/kg bw/d)								
Pre-pairing (14 days)	0	29.3	75.3	244.7	0	32.0	79.5	253.7
Gestation	0	24.8	62.5	198.5	0	31.4	75.0	237.3
Lactation					0	73.1	177.0	490.2
Bodyweight gain (g)								

	Males				Females			
Diet concentration (ppm)	**0**	**400**	**1000**	**3000**	**0**	**400**	**1000**	**3000**
Pre-pairing (14 days)	49	48	47	50	19	16	21	15
Gestation/post-partum	88	80 (-9.1%)	75 (-14.7%)	74 (-15.9%)	120	140 (+16.6%)	126 (+5%)	101 (-15.8%)
Lactation					24	60	43	50
Mean bodyweight (g)								
Pre-pairing (day 14)	326	328	324	330	203	198	202	200
Gestation/post-partum	420	415 (-1.2%)	403 (-4.0%)	405 (-3.5%)	329	347 (+5.5%)	334 (+1.5%)	301 (-8.5%)
Lactation					258	**289***	**280***	278
Mating Performance								
Mating index (%)	100	100	100	100	100	100	100	100
Mean precoital time (days)					2.8	3.6	3.0	3.2
Fertility index (%)					80	100	100	100
Gestation index (%)					100	100	100	100
Gestation Duration (days)					21.8	21.8	21.6	21.8
Implantations (mean)					13.0	15.2	14.5	12.3
Post implantation loss (%)					3.8	7.9	6.9	12.2
Viability index, days 0-4 (%)					98.1	91.4	98.5	98.1
Live pups at first litter check (mean number per litter)					12.5	14.0	13.5	10.8
F1 pup data								
Bodyweight (g) day 1 pp	5.7	6.0	6.0	5.7	5.6	5.4	5.7	5.4
Bodyweight (g) day 14 pp	30.0	31.9	30.8	**23.1****	30.1	30.4	29.8	**22.7****
Bodyweight (g) day 21 pp	48.0	51.5	48.9	**36.3****	47.1	48.8	46.8	**35.8****
Bodyweight (g) day 28 pp	84.0	83.6	81.5	**63.4****	78.7	77.0	76.5	**61.7****
Mean food consumption (g) days 21 to 28 pp	9.64	9.01 (-6.5%)	9.08 (-5.8%)	7.77 (-19.4%)	9.03	8.41 (-6.8%)	8.75 (-3.1%)	7.8 (-13.6%)
Absolute organ weights (F1) (g)								
Brain	1.45	1.59	1.54	1.41	1.50	1.54	1.50	**1.36****
Thymus	0.306	0.335	0.307	**0.214***	0.347	0.313	0.307	**0.204****
Spleen	0.28	0.35	0.34	0.27	0.32	0.31	0.29	**0.24***
Uterus					0.16	0.15	0.15	**0.11***
Organ/body weight ratio (F1) %								
Brain	1.87	2.12	2.00	**2.41****	1.98	2.15	2.11	**2.39****

	Males				Females			
Diet concentration (ppm)	**0**	**400**	**1000**	**3000**	**0**	**400**	**1000**	**3000**
Thymus	0.392	0.440	0.398	0.369	0.459	0.435	0.430	**0.358***
Spleen	0.36	0.45	0.44	0.45	0.43	0.43	0.40	0.43
Uterus					0.21	0.20	0.21	0.19

* p < 0.05 in comparison to controls, ** p<0.01 in comparison to controls

Conclusion:
In the preliminary dose-finding study fenpyrazamine was well-tolerated at all doses, resulting only in weight gain loss in parental males of mid- and high dose group (during female gestation and lactation time) and in parental females of high dose group during gestation. Mating performance and fertility appeared unaffected at all dose levels. In the high dose females, an increase in post implantation loss and reduction in litter size were noted. Pups in the high dose group had significantly lower body weight gain from day 14 and 28 post-partum, but not at birth.

At necropsy, an increased incidence of thyroid enlargement was reported in parental males and females of high dose. There were no notable necropsy findings among pups. Changes in organ weights of pups are considered secondary to body weight loss in high dose animals during lactation and weaning.

Therefore, the NOAEL for parental systemic toxicity is set at 400 ppm for males (equivalent to 24.8 mg/kg bw/d) and 1000 ppm for females (equivalent to 75 mg/kg bw/d), based on decreased body weight gain of both sexes (> 10%) during gestation period. NOAEL for offspring systemic toxicity is proposed to be set at the dose level of 1000 ppm (equivalent to 75 mg/kg bw/day during gestation) due to increased post implantation loss, decreased number of live pups at birth and significantly lower body weight of pups from day 14 to 28 post partum. Additionally, absolute and relative organ weights alterations (statistically significant) were observed in high dose pups. Reproductive NOAEL of 3000 ppm (equivalent to 244.7 mg/kg bw/d (males) and 253.7 mg/kg bw/d (females)) was derived, due to no effects observed on reproduction at highest dose tested.

On the basis of results in this study, the high dose (3000 ppm) was considered appropriate for the main study.

Reference:	Two-generation reproduction study in the Han Wistar rat
Author(s), year:	Gerspach, R., Weber, K., Flade, D.; 2009
Report/Doc. number:	Sumitomo Chemical Co. Ltd. Report No. QNT-0041
Guideline(s):	Japanese MAFF 12 Nousan 8147, EPA OPPTS 870.3800, OECD 416
GLP:	Yes (laboratory certified by National Authority)
Deviations:	-Individual pup weights of three litters from groups 1, 3 and 4 were, by error, not taken on day 7 post partum. - Not all organs from F1 pups (not selected for reproduction), by error, were sampled on the two scheduled necropsy days; therefore, the missing organs were sampled from another pups of the same litter a day after - The uterus weights were taken including the cervix and vagina All deviations are considered as not relevant for the validity of the study

Validity: Yes

Material and methods:

Test Material:	S-2188
Lot/Batch:	030-050914-1G
Purity:	94.7%
Test animals:	
Species:	Rat
Strain:	Wistar, HanRcc:WIST(SPF)
Age:	Minimum 6 weeks at delivery
Weight at start:	Males 192 - 231g, females 140 - 173g
Source:	Harlan, Laboratory Animal Services, Füllinsdorf, Switzerland
Diet:	Pelleted standard Kliba-Nafag 3433 maintenance diet; *ad libitum*

The main reproduction study was conducted in order to provide general information concerning the effects of the test substance on reproductive function as by gonadal function, estrus cycles, mating behaviour, conception, gestation, parturition, lactation, weaning and the growth and development of the off-spring. Additionally, the study provides information on the effects of the test substance on neonatal morbidity, mortality, behaviour and preliminary data on teratogenicity.

Animal assignment and treatment

Groups of 24 male and 24 female young adult rats were allocated to treatment groups (P generation). These rats were exposed to treated diets for a pre-mating period of 10 weeks (70 days) and then paired, one male to one female for a mating period of max. 14 days. Once rats had mated the males were removed, and the females monitored throughout gestation, littering, and lactation. At the end of the lactation period (21 days post-partum), 24 male and 24 female pups (ideally no more than 1 male and 1 female per litter) were selected to become the F1 parental animals. These animals were again permitted a 70 days pre-mating phase starting at approximately 28 days of age and then mated; females were allowed to rear their litters to form the F2 generation. The F2 animals were monitored until sexual maturity (preputial separation in males, vaginal opening in females) and the study then terminated. Exposure to treated diet continued throughout the study including pre-mating, mating, gestation and lactation periods of P and F1 generations. The F2 pups were indirectly exposed to test substance until weaning (day 21 post partum).

Diet preparation and analysis:

Fresh batches of the feed pellets for this study were prepared at least each 2 weeks with a constant concentration (ppm) throughout the feeding duration. The test item was mixed with granulated feed in a pelleting machine. Water was added to each feed preparation at a volume/weight ratio of

approximately 1:10 to ensure pelleting. The pellets were dried with air for approximately 48 hours before storage. Feed for the control group was prepared similarly without the test item.

Accuracy of diet preparation was confirmed by analysis (GC). Stability of the test item in pelleted rodent feed was investigated over 21 days and found to be stable. Concentration and homogeneity of the actual test item batch in the feed were determined in diets used in the first week of treatment, at the beginning of the P generation lactation period, at the start of the F1 pre-mating period, and at the start of the F1 lactation period.

Mean test substance intakes (mg/kg bw/d) are given in tables below.

Table 53: Mean test substance intakes – P generation (mg/kg bw/d)

Sex	**Doses** **Period of treatment**	**400 ppm** **(Group 2)**	**1000 ppm** **(Group 3)**	**3000 ppm** **(Group 4)**
males	Pre-pairing period (70 days)	27.4	68.6	213
	After pairing period (43 days)	21.6	55.1	172
females	Pre-pairing period (70 days)	32.0	79.9	237
	Gestation period	29.3	72.5	219
	Lactation period (days 1 to 14 post partum)*	58.9	149	449

*Food consumption was measured only until day 14 of lactation period, but the pups were reared until weaning (day 21 post-partum).

Table 54: Mean test substance intakes – F1 generation (mg/kg bw/d)

Sex	**Doses** **Period of treatment**	**400 ppm** **(Group 2)**	**1000 ppm** **(Group 3)**	**3000 ppm** **(Group 4)**
males	Pre-pairing period (70 days)	31.6	80.5	256
	After pairing period (43 days)	20.3	52.4	171
females	Pre-pairing period (70 days)	34.5	85.2	266
	Gestation period	28.5	73.7	218
	Lactation period (days 1 to 14 post partum)*	59.3	148	440

*Food consumption was measured only until day 14 of lactation period, but the pups were reared until weaning (day 21 post-partum).

Clinical observations:

Animals were checked for mortality and clinical signs twice each day at least. Additionally, a thorough physical examination was conducted on each animal on a weekly basis.

Body weight:

All animals were weighed on the first day of dosing and thereafter at weekly intervals, with the exception of the pairing period. After mating, females were weighed on days 0, 7, 14 and 21 post coitum. Dams which had littered were weighed on days 1, 4, 7, 14 and 21 post partum and on the day the animals were sacrificed. After mating, males were weighted weekly until day 43 and on the day of sacrifice.

Food consumption

Food consumption was recorded weekly together with the recording of body weights, except during the pairing period, when no food consumption was recorded. During the lactation period of the females, food consumption was recorded only until day 14 post partum, since direct exposure of the pups to the test substance (food of parents) might occur during the lactation period after day 14. Relative food consumption ratios and intake of the test item expressed as mg/kg bw/day were calculated.

Sexual maturation:

The age and body weight at which vaginal opening or preputial separation occurred, was recorded for F1 generation weanlings selected for breeding the F2 generation.

Sperm analysis:

Sperm analysis was performed for all males per group (P and F1 animals), comprising assessment of motility, morphology, and sperm count.

Motility:

At necropsy of adult males an epididymal sperm sample was obtained from the left cauda epididymidis of each male. The sample was diluted with a pre-warmed (about 35°C) physiological medium, and rapidly after being obtained, one hundred sperm were counted microscopically for determination of percentage of not motile, stationary motile and progressively motile sperm.

Morphology:

A sperm sample from the left vas deferens was used for morphological assessment after fixation and Eosin staining. 500 sperm per sample were evaluated microscopically and classified into the following categories:

A Normal, complete sperm

B Normal head only (tail detached)

C Complete sperm, misshapen hook

D Complete sperm, abnormally curved hook

E Complete sperm, reversed head

F Abnormal head only (tail detached)

Morphological sperm evaluation was performed initially only for control and high dose males. In the absence of a test item-related effect the slides for the group 2 and 3 males were not evaluated for the P generation males. As considered necessary for further evaluation, F1 parent males groups 2 and 3 were evaluated.

Sperm and spermatid count:

The left caudal epididymis and left testis were taken for determination of homogenization-resistant spermatids and caudal epididymal sperm reserve. These tissues were frozen at -20 ± 5°C pending evaluation. For evaluation, the weighed tissues were placed in Triton-X-100 solution and homogenized with a blender (Ultra Turrax) and an ultrasonic water bath. Sperm or spermatid heads were counted microscopically using a modified Neubauer chamber. These evaluations were performed only for control and high dose males. In the absence of a test item related effect the remaining frozen tissues were not evaluated.

Mating evaluation:

A record of mating of females was made by daily examination of the vaginal smears for spermatozoa and/or appearance of a vaginal plug throughout the pairing period. The day on which evidence of mating was observed was considered to be day 0 post coitum. Once evidence of mating had been noted, the females were housed individually.

The mating data were used to determine the precoital time, detect whether or not pregnancy was interrupted after mating, detect marked anomalies of the estrus cycle, and determine duration of gestation.

Gestation and parturition:

Towards the end of the gestation period, females were examined twice daily for signs of parturition. Duration of gestation was calculated. Females without litters were killed and necropsied together with the dams after weaning of the pups. Females which lost their litter were killed and necropsied either directly after litter loss or with the other dams after weaning of pups.

Lactation and litter examination:

Day 0 of lactation was the day on which a female had delivered all its pups. The litters were examined for litter size, live birth, stillbirth and any gross anomalies. Sex ratios of live pups were recorded on day 0 and/or day 1, day 4 and day 21 of lactation. Litters were caged together with the dam until weaning on day 21 of lactation. Pups were weighed individually on days 0 and / or 1, 4, 7, 14 and 21 of lactation.

Sacrifice and pathology:

All P and F1 males were sacrificed after day 43 post-coitus. All P and F1 females were sacrificed after lactation period. Excess F1 and F2 pups after standardization of litter sizes (24 male and 24 female F1 pups) were sacrificed on day 4 post-partum, examined macroscopically and preserved in neutral phosphate buffered 4% formaldehyde solution.

All parent generation animals (P and F1) were examined macroscopically for any structural abnormalities or pathological changes either at scheduled sacrifice or if death occurred during the study. Special attention was directed to the organs of the reproductive system. Implantation sites were counted for all dams. The uteri were placed in a solution of ammonium sulphide to visualize possible hemorrhagic areas of implantation sites.

The following organ weights were recorded from all P and F1 parent animals on day 21 post-partum or shortly thereafter: brain (with entire brainstem), kidney, pituitary, adrenal glands, liver, spleen, thyroid, seminal vesicles (with coagulating glands and their fluids (as one unit)), epididymides (total weight and cauda separately), testes, prostate, uterus (including cervix and vagina, excluding oviducts). Paired organs were weighed individually

Brain, spleen, thymus and uterus were recorded from one randomly selected male and female pup of each F1 and F2 litter (on day 21 post-partum).

Histopathology:

From all P and F1 animals selected for pairing and from one male and one female pup (selected for organ weight recording; not selected for breeding) of each F1 and F2 litter, samples of the following tissues and organs were collected at necropsy and fixed in neutral phosphate buffered 4% formaldehyde solution: gross lesions, pituitary, adrenal glands, liver, thyroid, vagina, prostate, seminal vesicles with coagulating gland, right testis and epididymis (in Bouin's fixative), ovaries, uterus and cervix.

Full histopathology was performed on the organs listed above for all high dose and control P and F1 animals selected for pairing and on one male and one female pup (selected for organ weight recording) of each F2 litter (high dose and control). Organs demonstrating pathology in these animals were then examined in the animals from the other dose groups.

Microscopic examination of all tissues showing gross pathological changes was made.

Ovarian histopathology, in addition to qualitative examination, included quantitative evaluation of primordial follicles, growing follicles and antral follicles and corpora lutea from 10 sections per ovary for the ten females of the F1 generations in control and high dose group. Additionally, corpora lutea were counted on one section per ovary. In absence of test item-related effects in group 4, the group 2 and 3 females were not evaluated.

Findings:

Clinical signs and mortality

There were no treated-related mortalities or clinical signs in any generation. One dam in group 4 was found dead on day 23 post coitum. This was considered related to dystocia on day 22.

Body weight and body weight gain

In the P generation, no statistical differences in body weight gain during pre-mating period, gestation and lactation were seen among the treated groups for both, males and females.

Nevertheless, in highest dose group there was a decrease in body weight gain in males and females during pre-mating period and in females during gestation comparing to control animals.

In the pups selected to form F1 generation and parental F1 animals respectively, also no statistical differences in body weight gain during pre-mating period, gestation and lactation were seen among the treated groups. Nevertheless, as well as for P parental generation, in highest dose group there was a decrease in body weight gain in males and females during pre-mating period and in females during gestation to control animals.

Food consumption

There was a statistically significant decrease of food consumption in P parental females during pre-mating period and gestation (statistical significance was not proved for mean values but was observed during themeasurments time points during the study), and F1 parental animals of the highest dose group during pre-mating period, gestation and lactation (statistical significance was not proved for mean values but was observed during themeasurments time points during the study). There was no effect on males or females of low and mid-dose group.

Reproductive performance

P parents:

No effect was seen on oestrus cycle, on number of pairs mating, in time to mating, or in the number of females becoming pregnant. There was no effect on the length of gestation. One pregnant female of the highest dose was found dead on day 23, which was considered a consequence of dystocia observed the preceding day. This single decedent was not considered associated with treatment in the absence of any effect on gestation length in other females of this group.

F1 pups:

The number of live pups born per litter was similar between groups. The increase in post natal loss and reduced viability index in the high dose group achieved statistical significance. However, the incidence only marginally exceeded the historical control data (seven studies conducted prior to current study, two studies conducted after) and was considered secondary to reduced body weight gain and reduced food consumption of maternal animals during gestation period. No breeding losses (days 5 to 21 post-partum) were observed in any group (weaning index = 100%).

The mean pup weight of F1 pups at birth and 21 days post-partum was significantly lower in pups (males and females) of the high dose compared to controls. Additionally, the female pups of the mid-dose group had significantly lower body weight comparing to control pups on day 21 post-partum. However, this finding in the mid-dose group was considered transient, since it was not seen later in the development (day 33, vaginal opening). On the other hand, male pups in mid-dose showed statistically significant body weight decrease at time of preputial separation (day 26), although their weight on day 21 post-partum was in range of control pups. In the F1 high dose group, sexual maturity was delayed in both, males and females (preputial separation, and vaginal opening respectively), but this was considered secondary to decreased body weight of pups. Sex ratios (%males / % females) were similar in all groups (45/55; 49/51; 50/50; 46/54).

F1 parents:

No effect was seen on oestrus cycle, on number of pairs mating, in time to mating, or in the number of females becoming pregnant. There was no effect on the length of gestation. However, in the high dose group there was a significant reduction in the mean number of implantations per dam and litter size, and a statistically significant increase in post-implantation loss.

F2 pups:

Bodyweights of F2 pups in the high dose group were significantly lower compared to control pups at the start of lactation and remained so throughout the lactation period. Also at the mid-dose level, bodyweights of female pups were significantly lower than those of controls from day 14 to day 21 post-partum. There was no effect on pups of low dose group. Post-natal loss during the first 4 days post-partum was significantly increased in all dose groups comparing to concurrent control but was within the historical control data. Viability index was significantly decreased in all treated groups but was still within the range of historical control data (seven studies conducted prior to current study, two studies conducted after). Sex ratios (% males / % females) were similar in all groups (45/55; 48/52; 48/52; 46/54).

Table 55: Summary of key findings (P generation) in two generation study

Diet concentration (ppm)	**Males**				**Females**			
	0	**400**	**1000**	**3000**	**0**	**400**	**1000**	**3000**
P Parental animals								
Fenpyrazamine intake (mg/kg/ bw/day)								
Pre-mating (70 days)	0	27.4	68.6	213	0	32.0	79.9	237
Gestation	0	21.6	55.1	172.3	0	29.3	72.5	219
Lactation					0	58.9	149	449
Body weight gain and food consumption								
Body weight gain (g) Pre-mating period - % of control	189	198 (+4.8%)	185 (-2.1%)	171 (-9.5%)	98	95 (-3.1%)	99 (+1%)	69 (-29.6%)
Body weight gain (g) Gestation - % of control	36	41 (+13.9%)	37 (+2.8%)	42 (+16.7%)	117	119 (+1.7%)	124 (+6.0%)	96 (-17.9%)
Body weight gain (g) Lactation - % of control					36	38 (+5.6%)	36 (0.0%)	47 (+30%)

Diet concentration (ppm)	Males				Females			
	0	**400**	**1000**	**3000**	**0**	**400**	**1000**	**3000**
Mean food consumption (g/ rat/day) Pre-mating period	23.0	23.4	22.7	22.9	17.5	17.6	17.6	**15.8****
Mean food consumption (g/ rat/day) - Gestation	23.1	23.6	23.1	23.4	22.6	23.1	22.9	**20.1***
Mean food consumption (g/ rat/day) - Lactation					46.0	44.4	45.7	39.8
Mating and Gestation Performance								
Number of pregnancies					22	23	24	23
Gestation index (%)					100	100	100	100
Gestation Duration (days)					21.8	21.6	21.4	21.5
Mean implantation/dam					13.1	13.7	14.3	12.6
Total number of post-implantantion loss (litter affected/implantations)					15/35	16/31	16/31	18/30
Post-implantation loss (%)					12.2	9.9	9.1	10.8
Mean litter size					11.5	12.3	13.0	11.2
Total number of post-natal loss (litter affected/pups)					4/4	3/11	5/6	**8/12***
Post-natal loss (%) (days 0 - 4 post-partum)					1.6	3.9	1.9	4.9[a)]
Post-natal loss (%) (days 0 – sacrifice) historical control data					0.7 - 4.8 (May 2002 – December 2007) [#)]			
Viability index, days 0-4 (%)					98.4	96.1	98.1	**95.1*[b)]**
Viability index (days 0 – sacrifice) (%) historical control data					95.2 – 99.6 (May 2002 – December 2007) [#)]			
F1 Pup data								
Bodyweight (g) pp day 1	6.2	6.0	6.0	**5.3****	6.0	5.7	5.7	**5.1****
Bodyweight (g) pp day 21	50.5	48.3	47.6	**35.8****	49.1	48.0	**46.1***	**35.1****
At preputial separation/vaginal patency:								
Age (days)	26.5	26.0	26.4	**27.7***	32.4	32.9	33.3	**35.9***
Bodyweight (g)	78.9	77.2	**73.0***	**61.0****	101.9	106.7	106.0	**94.4***

p < 0.05 in comparison to controls, ** p<0.01 in comparison to controls

a) outside historical control data

b) statistically significant and outside historical control data

#) current study was conducted from 2006 to 2007, only the study report was finalised 2009

Table 56: Summary of key findings (F1 generation) in two generation study

Diet concentration (ppm)	**Males**				**Females**			
	0	**400**	**1000**	**3000**	**0**	**400**	**1000**	**3000**
F1 Parental animals								
Fenpyrazamine intake (mg/kg/ bw/day)								
Pre-mating (70 days)	0	31.6	80.5	256	0	34.5	85.2	266
Gestation	0	20.3	52.4	171.2	0	28.5	73.7	218
Lactation					0	59.3	148	440
Body weight gain and food consumption								
Body weight gain (g) Pre-mating period - % of control	300	292 (-2.7%)	281 (-6.3%)	249 (-17.0%)	133	138 (+3.8%)	128 (-3.8%)	116 (-12.8%)
Body weight gain (g) Gestation - % of control	55	57	62	48	104	114 (+9.6%)	114 (+9.6%)	78 (-25%)
Body weight gain (g) Lactation - % of control					39	40 (+2.6%)	40 (+2.6%)	42 (7.7%)
Mean food consumption (g/ rat/day) Pre-mating period	23.8	23.6	23.0	**20.2****	16.8	17.1	16.3	**14.0****
Mean food consumption (g/ rat/day) - Gestation	23.8	23.6	23.6	**21.6****	20.4	21.8	21.8	**17.3***
Mean food consumption (g/ rat/day) - Lactation					43.5	43.8	43.1	**33.7****
Mating and Gestation Performance								
Number of pregnancies					24	23	23	24

Diet concentration (ppm)	Males				Females			
	0	**400**	**1000**	**3000**	**0**	**400**	**1000**	**3000**
Gestation index (%)					100	100	95.7	100
Gestation Duration (days)					21.7	21.7	21.6	21.5
Mean implantation/dam					12.6	13.3	12.7	**10.6***
Total number of post-implantantion loss (litter affected/implantations)					11/30	13/23	14/39	**18*/49****
Post-implantation loss (%)					9.9	7.5	13.4	19.3
Mean litter size					11.4	12.3	11.0	**8.5***
Total number of post-natal loss (litter affected/pups)					0/0	**4*/19****	**6**/15****	**10**/13****
Post-natal loss (%) (days 0 - 4 post-partum)					0.0	6.7	6.0	6.3
Post-natal loss (%) (days 0 – sacrifice) historical control data					0.0 – 8.5 (May 02 – December 2007) #)			
Viability index, days 0-4 (%)					100	**93.3**[a)]**	**94.0**[a)]**	**93.7**[a)]**
Viability index (days 0 – sacrifice) (%) historical control data					91.5 – 100 (May 02 – December 2007) #)			
F2 Pup data								
Bodyweight (g) pp day 1	6.0	5.9	5.9	**5.1****	5.7	5.6	5.6	**4.7****
Bodyweight (g) pp day 14	30.5	30.4	29.4	**22.2****	29.7	29.5	**27.9***	**21.3****
Bodyweight (g) pp day 21	50.0	50.0	47.0	**34.1****	48.2	48.0	**44.8***	**32.3****

* p < 0.05 in comparison to controls, ** p<0.01 in comparison to controls

a) statistically significant but within historical control data

#) current study was conducted from 2006 to 2007, only the study report was finalised 2009

Organ Weights and histopathological findings:

P parental animals: Significantly increased liver weights and/or liver to bodyweight ratios were seen in males and females of high and mid-dose group (also in males of low dose group). Since no histological findings in liver were observed in low dose males, the liver weight increase at the low dose was not considered adverse, although it achieved statistical significance. In high and mid-dose females and in high dose males an increased incidence of hepatocellular hypertrophy was observed. No differences in liver fatty changes could be observed between treated groups and control. Thyroid weights and/or thyroid to bodyweight ratio were significantly increased in males and females of high dose group, and females of the mid-dose group. In these animals, also histological effects (follicular cell hypertrophy and hyperplasia) were observed. Several other

changes in organ weights attaining statistical significance (kidney and testis weight in high dose males and pituitary, spleen, kidney, adrenals, ovaries and uterus in high dose females) were attributed to the bodyweight difference between control and high-dose animals. Weights of pituitary, adrenals, spleen, epididymidis and prostate in males of all treated groups were comparable to control.

Organ weight data and histological findings of P parental animals are given in table below.

Table 57: Organ weights and histopathological findings (P generation)

Diet concentration (ppm)	**Males**				**Females**			
	0	**400**	**1000**	**3000**	**0**	**400**	**1000**	**3000**
Organ weights								
Necropsy bodyweight (g)	430	443	424	413	297	287	289	**267****
Absolute liver weight (g)	11.7	**13.0****	**13.2****	**15.3****	14.5	13.6	15.3	**16.3***
Relative liver weight (%)	2.7	**2.9****	**3.1****	**3.7****	4.9	4.7	**5.3***	**6.1****
Absolute thyroid weight (mg) R/L[a)]	11/10	11/10	11/10	**14**/13***	9/8	9/10	**12*/11***	**18**/16***
Relative thyroid weight (%) R/L[a)]	0.002/ 0.002	0.002/ 0.002	0.003/ 0.002	**0.003**/ 0.003****	0.003/ 0.003	0.003/ 0.003	**0.004*/ 0.004***	**0.007**/ 0.006****
Histopathological findings (incidence)								
Liver, hepatocellular hypertrophy								
	2/24	0/24	2/24	**11/24**	12/24	13/24	**19/24**	**24/24**
Liver, fatty change								
	10/24	15/24	18/24	14/24	10/24	6/24	1/24	2/24
Thyroid, follicular cell hypertrophy								
	1/24	2/24	**5/24**	**20/24**	0/24	0/24	**14/24**	**22/24**
Thyroid, follicular cell hyperplasia								
	0/24	0/24	0/24	**3/24**	0/24	0/24	0/24	**5/24**

p < 0.05 in comparison to controls, ** p<0.01 in comparison to controls; No statistical analysis on histopathological findings reported

a) R = right lobe; L = left lobe

F1 pups: Relative body, brain, thymus and spleen weight were significantly decreased in male and female pups of high dose group (additionally relative uterus weight in females). Liver and thyroid weights were not measured in F1 pups. No histological examination was performed.

F1 parental animals: Increased liver weights and/or relative liver weights were found at the top dose level in both males and females. Relative liver weights in males of low and mid-dose level were also statistically significantly increased. Since no histological findings in liver were observed in low dose males, the liver weight increase at the low dose was not considered adverse,

although it achieved statistical significance. Hepatocellular hypertrophy, mainly centrilobular, was recorded at increased incidences in both sexes of mid-and high dose groups. No differences in liver fatty changes could be observed between treated groups and control. A brownish foreign pigment was recorded within the bile ducts in animals from both sexes of high dose group. Peribiliar chronic inflammation was observed at a minimal severity in F1 animals of high dose group only. Relative thyroid weights were statistically significantly increased in high and mid-dose dose females and high dose males. The incidence of follicular hypertrophy and hyperplasia in the thyroid gland of both sexes of mid- and high dose group was increased. Several other changes attaining statistical significance (brain, kidney, testis, prostate and epididymidis weight in high dose males and brain, pituitary, spleen, kidney, adrenals and ovaries in high dose females) were attributed to the bodyweight difference between control and high-dose animals.

F2 pups: Relative body, brain, thymus and spleen weight were significantly decreased in male and female pups of high dose group (additionally relative uterus weight in females). Liver and thyroid weights were not measured in F2 pups. Increased incidence of hepatocellular hypertrophy was only recorded in pups of high dose group. In F2 pups, as in F1 parent animals, a brownish foreign pigment was recorded within the bile ducts in animals from both sexes of high dose group.

Organ weight data and histological findings of F1 parental animals and F2 pups are given in table below.

Table 58: Organ weights and histopathological findings (F1 parent generation and F2 pups)

Diet concentration (ppm)	**Males**				**Females**			
	0	**400**	**1000**	**3000**	**0**	**400**	**1000**	**3000**
Organ weights (F1 parental animals)								
Necropsy bodyweight (g)	489	481	465	**392****	279	289	280	**228****
Absolute liver weight (g)	13.4	14.2	14.6	**14.8***	13.6	14.7	14.2	13.1
Relative liver weight (%)	2.8	**2.9****	**3.1****	**3.8****	4.9	5.1	5.1	**5.8****
Absolute thyroid weight (mg) R/L[a)]	14/13	14/14	15/14	14/14	19/19	18/18	**24*/22**	**28**/26***
Relative thyroid weight (%) R/L[a)]	0.003/ 0.003	0.003/ 0.003	0.003/ 0.003	**0.004**/ 0.004****	0.007/ 0.007	0.006/ 0.006	0.008/ 0.008	**0.012**/ 0.012****
Histopathology (incidence) - F1 parental animals and F2 pups								
Liver, hepatocellular hypertrophy								
F1 Parents	0/24	0/24	**5/24**	**10/24**	0/24	0/24	**6/24**	**20/24**
F2 Pups	0/24	0/22	0/21	**2/23**	0/24	0/22	0/21	**5/22**
Liver, fatty change								
F1 Parents	9/24	11/24	16/24	12/24	2/24	0/24	2/24	2/24
Liver, bile duct pigmentation								

F1 Parents	0/24	0/24	0/24	**16/24**	0/24	0/24	0/24	**14/24**
F2 Pups	0/24	0/22	0/21	**8/23**	0/24	0/22	0/21	**7/22**
Liver, peribiliar inflammation								
F1 Parents	0/24	0/24	0/24	**7/24**	0/24	0/24	0/24	**3/24**
Thyroid, follicular cell hypertrophy								
F1 Parents	1/24	0/24	1/24	**7/24**	3/24	0/24	**13/24**	**22/24**
Thyroid, follicular cell hyperplasia								
F1 Parents	0/24	0/24	0/24	**6/24**	0/24	0/24	0/24	**13/24**

p < 0.05 in comparison to controls, ** p<0.01 in comparison to controls; No statistical analysis on histological findings reported

a) R = right lobe; L = left lobe

Sperm evaluation

No differences were detected between control and high dose P parental sperm parameters after examination of sperm motility, morphology, or homogenisation-resistant spermatid count. Similarly, no alteration of sperm quality was detected in the high dose F1 parental male animals. In the absence of a detectable effect, no further investigations were undertaken.

Ovary staging

Detailed histological assessment of the ovaries revealed no treatment-related disturbance. The number of primordial, growing and antral follicles, as well as corpora lutea was comparable between control and F1 high dose females.

Conclusion:

In the 2-generation study, fenpyrazamine was administered continuously in the feed to rats at dietary concentrations of 0, 400, 1000, and 3000 ppm. The toxicological response of the treated animals was principally characterised by increase in relative liver and thyroid weight and histological findings in these organs (cell hypertrophy) and reduced food consumption and decreased body weight gain throughout the whole study period. Significantly lower number of implantations, significantly increased post implantation loss and significantly increased post natal loss in the high dose group of F1 generation is considered to be the effect of systemic toxicity in maternal animals (body weight gain loss, body weight reduction, thyroid enlargement, hepatocellular and thyroid cell hypertrophy). Fenpyrazamine showed no effects on fertility. Sperm evaluation and ovary staging did not reveal any test substance related findings.

A No-Observed-Adverse-Effect Level (NOAEL) for parental effects in P and F1 generation is proposed at the dietary level of 400 ppm (20.3 mg/kg bw/d; mean substance intake of males of F1 generation during after-mating period), based on occurrence of increased relative liver and thyroid weights and corresponding histological findings (liver and thyroid cell hypertrophy) at 3000 ppm and 1000 ppm. Statistically significant decrease of relative liver weight of males at 400 ppm was not considered adverse because of absence of histopathological findings in liver at this dose. A No-Observed-Adverse-Effect Level (NOAEL) for offspring effects in F1 and F2 pups is

proposed at 400 ppm (28.5 mg/kg bw/d; mean substance intake of females of F1 generation during gestation), based on significantly lower body weights of F1 and F2 pups during lactation at 3000 ppm and 1000 ppm. Statistically significant increase of post implantation loss and decreased viability index for pups of all F1 parents are within the historical control data are therefore not regarded as relevant for setting the NOAEL. A No-Observed-Adverse-Effect Level (NOAEL) for reproductive effects is proposed at the dietary level of 1000 ppm (73.7 mg/kg bw/d; mean substance intake of females of F1 generation during gestation), based on significantly decreased number of implantations observed in F1 females at 3000 ppm.

4.11.1.2 Human information

A formal statement from the manufacturer is presented (Ohtsubo, T (2009)), that members of staff involved in the synthesis and development of fenpyrazamine are routinely monitored and that no indication of fenpyrazamine-related ill-health have been detected by, or reported to, medical staff. No poisoning incidents or clinical cases have been reported. Fenpyrazamine is a new chemical currently in development. There has been no exposure of the general population or epidemiology study.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Rats:

Reference:	S-2188: Dose range-finding prenatal development toxicity study in the Han Wistar Rat
Author(s), year:	Pössnecker, A.; 2006
Report/Doc. number:	Sumitomo Chemical Co. Ltd. Report No. QNT-0005
Guideline(s):	Japanese MAFF 12 NohSan 8147 Teratology (2-1-18), EPA OPPTS 870.3700, OECD 414
GLP:	Yes (laboratory certified by National Authority)
Deviations:	No
Validity:	Yes (supplementary study)

Material and methods:

Test Material: S-2188
- Lot/Batch: 030-050914-1G
- Purity: 94.7%

Test animals:
- Species: Rat
- Strain: Wistar, HanRcc:WIST(SPF)
- Age at pairing: 12 weeks
- Weight at start: 192 – 228 g
- Source: RCC Ltd, Laboratory Animal Services, Füllinsdorf, Switzerland
- Diet: Pelleted standard Kliba-Nafag 3433 maintenance diet; *ad libitum*

The purpose of the study was to detect adverse effects of fenpyrazamine on the pregnant rats and on the development of the embryo and the foetus consequent to exposure of the females to the test substance (0, 150, 300 and 500 mg/kg bw/d) from implantation through day 20 post coitum in order to establish suitable dose levels for a subsequent developmental study. Investigations were limited to maternal growth and food consumption, and pregnancy parameters to ensure that dose selection in future study would result in sufficient pups for a full evaluation of developmental toxicity.

Animal assignment and treatment
Groups of young adult female rats were paired with male rats of proven fertility in special automatic mating cages with synchronised timing to initiate the nightly mating period until evidence of copulation was observed. Once evidence of mating was seen (presence of copulation plug or sperm positive vaginal smear), 5 presumed-pregnant females were allocated to each dosage group (0, 150, 300 and 500 mg/kg bw/d).

Diet preparation, analysis and administration:

The test substance was weighed and then ground in an agate stone mortar. The vehicle (1% aqueous CMC solution) was added (w/v) and a homogenous mixture was prepared. The mixture was continuously stirred up to the surface during the application procedure.
Separate formulations were prepared for each concentration. Accuracy of mixing, stability and homogeneity of dose formulations were confirmed by analysis. Samples for determination of concentration, homogeneity and stability (4 hours and 7 days) of the dose formulations were taken during the first week of the administration period.
Presumed-pregnant female rats were administered fenpyrazamine by gavage at a dose volume of 10 mL/kg bw once daily during days 6 to 20 of pregnancy, and sacrificed on day 21 of pregnancy for examination of litters. Dose volumes were determined on the basis of the most recent bodyweight, determined daily.

Clinical observations:

All animals were checked at least twice daily for any mortality. Any female sacrificed or found dead during the study was subjected to macroscopic examination with emphasis on the uterus and its contents. Specimens of abnormal tissue were fixed in neutral phosphate buffered 4% formaldehyde solution.
All animals were observed at least twice daily for signs of reaction to treatment and/or symptoms of ill health.

Food consumption and body weight:

Food consumption was recorded on 3-day intervals (day 0–3, 3–6, 6–9, 9–12, 12–15, 15–18, and 18–21) throughout pregnancy. Body weights were recorded daily from day 0 until day 21 of pregnancy.

Sacrifice and pathology

At the scheduled necropsy on day 21 post coitum, females were sacrificed and the foetuses removed by Caesarean section.

Post mortem examination, including gross macroscopic examination of all internal organs, with emphasis on the uterus, uterine contents, position of foetuses in the uterus and number of corpora lutea, was performed and the data recorded. All parental organs or tissues showing grossly visible

abnormalities were preserved in neutral phosphate buffered 4% formaldehyde solution. The uteri (and contents) of all females with live foetuses were weighed at necropsy on day 21 post coitum to enable the calculation of the corrected body weight gain. Placental weights were measured separately.

Foetal examination

The foetuses were sexed, weighted individually examined for gross external abnormalities and stored in 70% alcohol. One foetus with an obvious skeletal defect was stained for skeletal examination.

Findings:
Mortality and clinical signs:
One female of the high dose group was found dead on pregnancy day 11. There were no preceding clinical signs. Necropsy of this animal showed signs of discharge on the snout; the stomach was distended with gas and contained red particles; the fore stomach was reported to have a red surface. Lungs were partly emphysematous with red discolouration. The uterus contained 13 implantations, and 14 corpora lutea were counted in this animal. It could not be excluded that the findings were not treatment-related. One female of the mid-dose group was found to have started parturition on day 21, and was sacrificed within the terminal sacrifice procedure before completion of parturition.

No clinical signs and no changed behaviour were noted in any other female during the whole study.

Food consumption, body weight and body weight gain:

Food consumption of the high dose females was lower (-8.8%) than that of the controls, but no statistical significance was achieved. There was no effect on food consumption at the mid- or low-dose. Bodyweight gain of females in the mid- and high dose groups showed dose-related impairment (-15.5% and -19.8% respectively, compared to control) without statistical significance. Females of high dose group had significantly lower corrected body weight gain (excluding uterus) (1.8% of starting weight) comparing to control (12% of starting weight), while uterus weight was comparable between all groups. Between days 19 and 21 post coitus, the body weight of mid-dose females was statistically significantly decreased when compared to control. There were no statistically significant effects at the low and high dose.

Reproduction data

All mated females were pregnant. Mean number of corpora lutea per dam was comparable in all groups.

Necropsy findings

One dam of the high dose group was found dead at day 11 post coitum. Necropsy of this animal showed signs of discharge on the snout; the stomach was distended with gas and contained red particles; the fore stomach was reported to have a red surface. Lungs were partly emphysematous with red discolouration. It can not be excluded that the death was not treatment-related. One dam of the mid-dose group was found to have started parturition on day 21, and was sacrificed within the terminal sacrifice procedure before completion of parturition. No abnormal necropsy findings were noted in any other animal.

Foetal data

The post implantation loss was markedly higher in the mid-dose group than in the control and the litter size of the mid-dose group was smaller (11.6 foetuses comparing to 13.4 in control). These findings are mainly contributed to 1 dam of mid-dose group which had 5 resorptions. No similar findings were observed in high dose animals, therefore, it was considered incidental.

The mean foetal weight of high dose group was statistically significantly decreased. The mean placental weight of animals in all treated groups was significantly increased (26.7%, 23.1% and 46.3% compared to control). No histopathological examinations of placenta or hormone level measurements were conducted. The information provided by the Notifier, that anaemia and restricted food consumption are common reasons for increased placental weight can not be truly followed since the female rats in 90 days study did not show any sign of anaemia at comparable dose levels and a restriction in food consumption could be observed in the current study only for animals of highest dose. Therefore, the reason of the increased placental weight stays unclear and could be, e.g. also a result of hormonal imbalance or prompted induction of placental metabolic activity. The sex ratio was not significantly shifted by the treatment (males/females: 35/32, 25/42, 30/28, 23/34).

During necropsy, malformations were noted in 1 foetus of each low (hind limb flexure of both legs) and mid-dose group (umbilical hernia with exposed short length of intestine, subcutaneous oedema in cervical region). The malformations showed no consistency between groups and were not considered treatment-related (no effected foetuses in high dose group). No findings were observed in other foetuses.

Results of preliminary developmental study in rats are given in table below.

Table 59: Results of the preliminary rat developmental study

Dose (mg/kg bw/day)	**0**	**150**	**300**	**500**
Number of females mated	5	5	5	5
Non-pregnant	0	0	0	0
Mortality	0	0	0	1
Number of females with live foetuses	5	5	5	4
Food consumption, days 6-21 - g/rat/day - % of control	23.8	23.6 (-0.8%)	24.0 (+0.8%)	21.7 (-8.8%)
Maternal weight gain, days 6-21 (g) - % of control	116	111 (-4.3%)	98 (-15.5%)	93 (-19.8%)
Corrected body weight gain (excluding gravid uterus), days 6-21 - total (g) - % of starting weight	27.6 12.0	23.5 10.3	19.6 8.6	**3.9*** 1.8
Maternal body weight, day 21 (g)	347	339	**323***	331
Corpora lutea (mean/dam)	16.2	14.8	16.2	16.0
Post implantation loss (total)	2	2	10	0
Mean litter size (live foetuses)	13.4	13.2	11.6	14.3

Mean placental weight (g) - % of control	0.438	**0.555*** (+26.7%)	**0.539*** (+23.1%)	**0.641**** (+46.3%)
Mean foetal weight (g)	4.9	4.5	4.7	**4.2****
Litters with external malformations	0/5	1/5	1/5	0/5

* p<0.05 in comparison to controls, ** p<0.01 in comparison to controls

Conclusion:
In the preliminary developmental study in rat, the doses of 150, 300 and 500 mg/kg bw/d were administered. Except for death of one dam, no excess toxicity was observed in any treated group. In the high dose group, corrected body weight gain of dams and mean weight of foetuses were significantly decreased. Mean placental weight was significantly increased in all treated groups but no histopathological examinations of placenta or hormone level measurements were conducted. Therefore, it can not be excluded, that increased placental weight is an adverse effect and not only an adaptive response. Observed malformations in two foetuses (1 per litter) from low and mid-dose were not considered treatment-related.
Based on the results of this study, a high dose of 500 mg/kg bw/day was recommended for main developmental study and dose lower than 150 mg/kg bw/d was chosen as a low dose, in order to be able to establish NOAEL.

Reference:	Prenatal developmental toxicity study in the Han Wistar Rat
Author(s), year:	Gerspach, R., and Flade, D.; 2009
Report/Doc. number:	Sumitomo Chemical Co. Ltd. Report No. QNA-0039
Guideline(s):	Japanese MAFF 12 Nousan 8147 Teratology (2-1-18), EPA OPPTS 870.3700, OECD 414, EC 2004/73 B31
GLP:	Yes (laboratory certified by National Authority)
Deviations:	No
Validity:	Yes

Material and methods:

Test Material: S-2188
- Lot/Batch: 030-050914-1G
- Purity: 94.7%

Test animals:
- Species: Rat
- Strain: Wistar, HanRcc:WIST(SPF)
- Age at mating: Minimum 11 weeks
- Weight: 191 – 236 g (day 0 post coitum)
- Source: Harlan, Laboratory Animal Services, Füllinsdorf, Switzerland
- Diet: Pelleted standard Kliba-Nafag 3433 maintenance diet; *ad libitum*

The purpose of the study was to detect adverse effects of fenpyrazamine on the pregnant rats and on the development of the embryo and the foetus consequent to exposure of the females to the test substance (0, 30, 125 and 500 mg/kg bw/d) from implantation (day 6 post coitum) through day 20 p.c.

Animal assignment and treatment:
Groups of young adult female rats were paired with male rats of proven fertility in special automatic mating cages with synchronised timing to initiate the nightly mating period until evidence of copulation was observed. Once evidence of mating was seen (presence of copulation plug or sperm positive vaginal smear), 22 presumed-pregnant females were allocated to each dosage group (0, 30, 125 and 500 mg/kg bw/d).

Diet preparation, analysis and administration:

The test item was weighed and then ground. The vehicle (1% aqueous CMC (carboxymethylcellulose) solution) was added (w/v) and a homogenous mixture was prepared. The mixture was continuously stirred up to the surface during the application procedure. Separate formulations were prepared for each concentration. Accuracy of mixing, stability and homogeneity of dose formulations were confirmed by analysis. Samples for determination of concentration, homogeneity and stability (4 hours and 7 days) of the dose formulations were taken during the first week of the administration period. Additionally, samples for determination of concentration and homogeneity were taken during the last week of the administration period.

On each occasion three samples of approximately 2 g were taken from the top, middle and bottom of each formulation. Stability samples were taken from the middle only. The samples were frozen (-25 °C to -15 °C) prior to analysis.
Dams were administered fenpyrazamine by gavage at a dose volume of 10 mL/kg bw once daily during days 6 to 20 of pregnancy, and sacrificed on day 21 of pregnancy for examination of litters. Dose volumes were determined on the basis of the latest bodyweight, determined daily.

Clinical observations:

All animals were checked at least twice daily for any mortality. Any female sacrificed or found dead during the study was subjected to macroscopic examination with emphasis on the uterus and its contents. Specimens of abnormal tissue were fixed in neutral phosphate buffered 4% formaldehyde solution.
All animals were observed at least twice daily for signs of reaction to treatment and/or symptoms of ill health.

Food consumption and body weight:

Food consumption was recorded on 3-day intervals (days 0-3, 3-6, 6-9, 9-12, 12-15, 15-18 and 18-21 post coitum) throughout pregnancy. Body weights were recorded daily from day 0 until day 21 of pregnancy.

Sacrifice and pathology:

At the scheduled necropsy on day 21 post coitum, females were sacrificed and the foetuses removed by Caesarean section. Post mortem examination, including gross macroscopic examination of all internal organs, with emphasis on the uterus, uterine contents, position of foetuses in the uterus and number of corpora lutea, was performed and the data recorded. All organs or tissues showing grossly visible abnormalities were preserved in neutral phosphate buffered 4% formaldehyde solution. The uteri (and contents) of all females with live foetuses were weighed at necropsy on day 21 post coitum to enable the calculation of the corrected body weight gain. Placental weights were measured separately. The placentae were fixed in neutral phosphate buffered 4% formaldehyde solution for possible microscopic examination.

Foetal examination:

Fetuses were removed from the uterus, sexed, weighed individually, examined for gross external abnormalities, and allocated either to microdissection technique or skeletal examination.

1. Visceral examination (microdissection technique)
At least one half of the foetuses from each litter were fixed in Bouin's fixative (one foetus per container). They were examined by a combination of serial sections of the head and microdissection of the thorax and abdomen. This included detailed examination of the major blood vessels and sectioning of the heart and kidneys. After examination, the tissue was preserved in a solution of glycerine/ethanol. Descriptions of any abnormalities and variations were recorded.

2. Skeletal examination
The remaining foetuses were prepared for skeletal examination, whereby with the exception of paws, the skin was removed and discarded. Foetuses were processed through solutions of ethanol, glacial acetic acid with Alcian blue (for cartilage staining), potassium hydroxide with Alizarin red S (for clearing and staining ossified bone) and aqueous glycerin for preservation and storage. The skeletons were examined and all abnormal findings and variations were recorded. Non-removal of skin on the paws precluded examination of the cartilaginous regions of all forepaw and hindpaw bones. This included the carpals, tarsals, metacarpals, metatarsals and phalanges. The specimens were preserved individually in plastic bags.

If no implantation sites were evident, the uterus was placed in an aqueous solution of ammonium sulfide to accentuate possible hemorrhagic areas of implantation sites. When considered appropriate, macroscopic changes in the dams were photographed and samples of tissue fixed in neutral phosphate buffered 4% formaldehyde solution for possible microscopic examination. Foetuses with abnormalities were photographed, when considered appropriate.

Findings:
Mortality and clinical signs:

There were no mortalities. All animals survived to terminal sacrifice. Occurrence of clinical signs (hair los on legs, abdomen and chest), which were considered incidental, was restricted to one animal of mid-dose group and 4 animals of high dose group. No clinical signs were observed in low dose animals.

Food consumption, body weight and body weight gain:

Food consumption of rats in the high dose level was lower (-7.3%) than that in control. In the last three days of pregnancy (18–21) this finding achieved statistical significance.There was no disturbance of food consumption in females of mid- and low dose.
Bodyweight of females in the high dose group was statistically significantly lower than in the control. This was achieved towards the end of the gestation period (day 20 and 21). No effects on body weight were observed in the low and mid-dose animals. Bodyweight gains (% of starting weight) of females in the mid- and high dose animals were statistically significantly lower than those of the controls (38% of starting weight and 32% of starting weight, respectively). This effect started at day 13 of pregnancy in the high dose females and at day 19 of pregnancy in the mid-dose females. Females of high dose group had significantly lower corrected body weight gain (excluding uterus) (3.3% of starting weight comparing to 13% in control animals).There were no effects on body weight gain at the low dose.

Reproduction data

Of 22 mated females in each group, 22, 21, 22 and 21 were pregnant in control, low, mid- and high dose, respectively. No effects on mean number of corpora lutea/dam was observed (this parameter shows only the biological variability, since the treatment began on day 6 p.c).

Foetal data

No effects on post implantation loss and mean litter size could be observed in any treated group. One foetus of the high dose was found with whole body oedema (anasarca). As a single finding, this was considered incidental. The mean foetal weight of high dose foetuses was statistically significantly decreased. The mean placental weight of animals in mid-dose group was increased comparing to control (+8.9%); in high dose group placental weight was significantly higher compared to the control (+42.7%). Comparable increase of placental weight was observed also in the preliminary rat developmental study. No histopathological examinations of placenta or hormone level measurements were conducted. Therefore, the reason of the increased placental weight stays unclear and could be, e.g. also a result of hormonal imbalance or prompted induction of placental metabolic activity. The sex ratio (male% / female%) was 47.5/52.5, 54.1/45.9, 48.7/51.3, 52.1/47.9 and thus close to equal distribution.

Maternal, reproduction and foetal data are given in table below

Table 60: Maternal, reproductive and foetal findings of the rat developmental study

Dose (mg/kg bw/d)	**0**	**30**	**125**	**500**
Number of females mated	22	22	22	22
Non-pregnant	0	1	0	1
Premature delivery	0	0	0	1
Number of females with live foetuses	22	21	22	20
Food consumption, days 6-21 - g/rat/day - % of control	23.2	23.5 (+1.3%)	23.8 (+2.6%)	21.5 (-7.3%)
Maternal bodyweight (g) day 21 - % of control	351	348 (-0.9%)	348 (-0.9%)	**324**** (-7.7%)
Maternal weight gain, days 6-21 (g) - % of control	117	115 a) (-1.7%)	110 a) (-5.9%)	90 a) (-23.1%)
Maternal weight gain (% starting weight), days 0-20	42	41	**38****	**32****
Corrected body weight gain (excluding gravid uterus), days 6-21 - total (g) - % of starting weight	30.4 (13.0%)	27.6 (11.8%)	22.5 (9.3%)	**7.8**** **(3.3%**)**
Mean number of corpora lutea/dam	14.6	14.4	14.9	15.0
Post-implantation loss (%)	6.0	5.1	3.8	8.3
Mean litter size (live foetuses)	13.5	13.3	13.7	13.3
Mean foetal weight (g)	4.9	4.9	4.7	**4.2****
Mean placenta weight (mg) - % of control	501	499 (-0.4%)	546 (+8.9%)	**715**** (42.7%)

** p<0.01 in comparison with control (Dunnett's test)

# p<0.05 in comparison with control (Fisher's exact test), ## p<0.01 in comparison with control (Fisher's exact test)
a) Statistical analysis for maternal weight gain not reported

Visceral examination

Incidence of abnormal liver lobation (including supernumerary lobes and lateral lobe supernumerary cleft) was significantly higher in high dose foetuses/litters (10% of foetuses and 50% of litters affected). Incidence of dilated renal pelvis was also significantly increased in foetuses of the high dose group (7% of foetuses and 25% of litters). Dilated ureter and enlarged urinary bladder were observed only in high dose foetuses/litters (in 3 pups of 3 litters). Regarding historical control data for these findings, only the incidences in high dose group were above historical controls. There were no significant, dose dependant alterations in litters of low and mid-dose.

No visceral malformations were recorded in any treated group. The visceral findings in low and mid-dose groups were neither statistically significant nor above historical control data.

Visceral foetal findings are given in table below.

Table 61: Visceral foetal findings of the rat developmental study

Dose (mg/kg bw/d)	**0**	**30**	**125**	**500**
Liver abnormal lobation - total foetuses/total litter - (%litter)	4/4 (18)	6/4 (19)	8/8 (36)	**14##/10#** (50)[a)]
Liver abnormal lobation (% litter) – historical control data	5 - 41 (2002 – 2007)			
Renal pelvis dilated - total foetuses/total litter - (%litter)	1/1 (5)	0/0 (0)	2/2 (9)	**7#**/5 (25)[a)]
Renal pelvis dilated (%litter) – historical control data	5 - 14 (2002 – 2007)			
Ureter dilated - total foetuses/total litter - (%litter)	0/0	0/0	0/0	3/3 (15%)[a)]
Ureter dilated (%litter) – historical control data	0 (2002 – 2007)			
Urinary bladder distended - total foetuses/total litter - (%litter)	0/0	0/0	0/0	3/3 (15%)[a)]
Urinary bladder distended (%litter) - historical control data	0 - 5 (2002 – 2007)			

# p<0.05 in comparison with control (Fisher's exact test), ## p<0.01 in comparison with control (Fisher's exact test)
a) Above historical control data

Skeletal examination

A statistically significant zygomatic arch fusion incidence was apparent in litters of high dose group. Since the incidence of zygomatic arch fusion in concurrent control was above historical control data and close to incidence of mid-dose group, only the statistically significant increase in high dose group was regarded as relevant. The significant increase in the incidence of sternabral offset and/or misshapen sternebral ossification centres were above historical control data in high

dose litters. Incidence in low and mid-dose was within the range of historical controls and showed no statistical significance. Increased incidence of costal cartilages being misaligned at the sternum was significant and above historical control data in high dose only. Statistically significant increase in incompletely ossified left and right os frontale in 25% of litters of high dose was above historical control data and was most likely related to the delayed development of high dose litter.

No skeletal malformations were recorded in any treated group. The skeletal findings in low and mid-dose groups were neither statistically significant nor above historical control data.

Skeletal foetal findings are given in table below.

Table 62: Skeletal foetal findings of the rat developmental study

Dose (mg/kg bw/d)	**0**	**30**	**125**	**500**
Skull: zygomatic arch fusion - total foetuses/total litter - (%litter)	6/5 (23)[a)]	3/2 (10)	13/7 (32)[a)]	**29##/17##** (85)[a)]
Skull: zygomatic arch fusion (%litter) - historical control data	5 – 18 (2002 – 2007)			
Sternebrae offset/misshapen ossification site - total foetuses/total litter - (%litter)	2/1 (5)	0/0 (0)	3/3 (14)	**7/7#** (35)[a)]
Sternebrae offset/misshapen ossification site (%litter) - historical control data	0 – 14 (2002 – 2007)			
Costal cartilage asymmetrically aligned at sternum - total foetuses/total litter - (%litter)	1/1 (5)	2/2 (10)	4/4 (18)	**8#/7#** (35)[a)]
Costal cartilage asymmetrically aligned at sternum (%litter) - historical control data	0 – 23 (2002 – 2007)			
Os frontal: incompletely ossified (L) - (%litter)	0	0	0	**25##** [a)]
Os frontal: incompletely ossified (L) (%litter) - historical control data	0 (2002 – 2007)			
Os frontal: incompletely ossified (R) - (%litter)	0	0	0	**25##** [a)]
Os frontal: incompletely ossified (R) (%litter) - historical control data	0 (2002 – 2007)			

# p<0.05 in comparison with control (Fisher's exact test), ## p<0.01 in comparison with control (Fisher's exact test)
a) Above historical control data

Conclusion:

In the main developmental study in rats fenpyrazamine at dose levels of 0, 30, 125, and 500 mg/kg bw/day was well tolerated. All animals survived to the scheduled sacrifice and did not show any adverse clinical signs. No malformations were observed in the study.
At 125 and 500 mg/kg dose level, mean body weight gains of dams were statistically significantly lower when compared to the control group, thus indicating the occurrence of maternal toxicity.

At 500 mg/kg dose level, placenta weights were statistically significantly increased, as already observed in preliminary developmental study. Since no histolopathological examinations or wet/dry weight measurements for estimation of hyperplasia or hypertrophy and no hormone level measurement were conducted, it can not be excluded that the increase in placental weight is an adverse effect, and not only an adaptive response.
No visceral or skeletal malformations were observed in the study.
The increased incidences of visceral (dilated kidney pelvis, dilated ureters, distended urinary bladder, and of additional liver lobes in foetuses) and skeletal (zygomatic arch fusions, sternebra offset or sternebra with misshapen ossification site, incomplete ossification of the frontal bone and costal cartilages asymmetrically aligned at sternum) foetal findings in high dose animals were considered related to observed maternal toxicity in this dose group.
In conclusion, the No Observed Adverse Effect Level (NOAEL) for maternal animals is proposed at 30 mg/kg bw/day (lowest tested dose), based on statistically significantly lower body weight gain in mid-and high dose dose animals during pregnancy. The developmental NOAEL is proposed at 125 mg/kg bw/day (mid-dose), based on significant decrease of mean foetal weight and significant increase of placental weight, visceral and skeletal variations and delayed ossifications (above historical control data) in foetuses of high dose group (500 mg/kg bw/d).

Rabbits:

Reference:	Dose range-finding teratology study in rabbits with S-2188
Author(s), year:	Inawaka, K.; 2008a
Report/Doc. number:	Sumitomo Chemical Co. Ltd. Report No. QNT-0030
Guideline(s):	None stated (range finding study)
GLP:	No
Deviations:	Supplementary study
Validity:	Yes

Material and methods:

Test Material: S-2188
- Lot/Batch: 030-050914-1G
- Purity: 94.7%

Test animals:
- Species: Rabbit
- Strain: New Zealand White (Kbl:NZW SPF)
- Age at receipt: 19 weeks (male) or 17 weeks (female)
- Weight: 3.44-3.66 kg (males), 3.34-3.78 kg (females)
- Source: Kitayama Labes Co., Ltd.
- Diet: LRC4, Oriental Yeast Co., Ltd.; available *ad libitum*

The purpose of the study was to select proper dose levels of fenpyrazamine for main developmental study in rabbits.

Animal assignment and treatment:
Groups of female rabbits were artificially inseminated using semen collected from the males. The day of insemination was designated Day 0 of gestation. Five (presumed pregnant) females were

allocated to each dosage group (control, 60, 90, 120 and 150 mg/kg bw/d). Rabbits were dosed once each day during days 6 to 27 of gestation.

Diet preparation, analysis and administration:

The highest concentration was prepared by suspending test material in CMC solution; lower doses were prepared by serial dilution of top dose material. Dose suspensions were prepared at least once each two weeks. Test material stability in dose suspension was confirmed prior to the study. During the study, concentration and homogeneity of dose preparations were confirmed by analysis.

Fenpyrazamine technical was administered orally (using disposable syringe and silicon catheter) as a suspension in a 1% aqueous carboxymethylcellulose (CMC) solution, at a dose volume of 5 ml/kg bw.

Clinical observations:

Animals were observed at least once daily for mortality and clinical signs. During the dosing period (days 6 – 27 of gestation), animals were observed at least twice (prior to dosing, and 1-3 hours after dosing) each day.

Food consumption and body weight:

Food consumption was measured between days 3-6, 6-9, 9-12, 12-15, 15-18, 18-21, 21-24, 24-27 and 28 of gestation. Bodyweights were measured on days 0, 6, 9, 12, 15, 18, 21, 24, 27 and 28 of gestation.

Sacrifice and pathology:

From females that gave birth prematurely or aborted, numbers of foetuses or placentas was counted on discovery; animals were then sacrificed and necropsied.

All surviving females were sacrificed on gestation day 28, by i.v. injection of sodium pentobarbital.

A gross examination was performed. Gravid uterine weight was measured. Foetuses were removed by caesarean section, and number of corpora lutea, number of implantations, number of live foetuses, number and intrauterine position of embryonic/foetal deaths, and time of death were recorded (also from animals that aborted or gave birth prematurely).

Foetal examination:

Each live foetus was weighed and examined externally (including the oral cavity). Sex was determined from observation of internal genitalia. After observation, all foetuses were fixed in dehydration alcohol.

Findings:

Clinical signs and mortality:

Two animals (one each in 90 and 150 mg/kg bw group) did not become pregnant; the number of pregnant females was therefore 5, 5, 4, 5 and 4.

One pregnant doe in 120 mg/kg bw group died on day 26 of gestation. Additionally, abortion and/or premature delivery were seen in 1, 2 and 3 does in 90, 120 and 150 mg/kg bw group, respectively. These events occurred between days 21 and 28 of gestation. In one of these animals (high dose) it was not possible to determine the day of abortion.

The number of live litters available for examination at termination was 5, 5, 3, 2 and 1 for the control, 60, 90, 120 and 150 mg/kg bw/day groups, respectively.

Orange discoloured urine, red fluid under the cage grid, or red fluid around the vagina was seen in several animals from the 120 and 150 mg/kg bw/day groups.

Food consumption, body weight and body weight gain

Between the three high dose groups (90, 120 and 150 mg/kg bw/d), a total of 6 animals were observed to have ceased eating as the study progressed. These animals either died, aborted or delivered prematurely.

Food consumption among rabbits of the 150 mg/kg/day group was lower almost throughout the study. Animals given 120 or 150 mg/kg bw/day generally lost weight during gestation; the majority of these animals aborted or delivered early.

No marked difference in bodyweight, adjusted body weight or bodyweight gain was seen in animals which survived until termination.

Sacrifice and pathology:

At terminal necropsy on day 28 of pregnancy no notable abnormalities among surviving animals of any group were found.
Among rabbits that aborted or delivered prematurely, findings of abnormal caecal contents (dark brown and muddy in appearance), pale liver, distension of the gallbladder and/or red fluid around the vagina, were considered treatment-related.

Foetal data:

Foetal bodyweight in the single surviving litter of the top dose group was marginally lower than that of controls at terminal sacrifice. Post implantation losses, sex distribution and foetus weights were comparable across the groups. Foetal examination was limited to observation for external defects. No abnormalities or malformations were found.

Results of preliminary developmental study in rabbit are given in table below.

Table 63: Results of the preliminary developmental study in rabbit

Dose (mg/kg bw/day)	**0**	**60**	**90**	**120**	**150**
Number of females inseminated	5	5	5	5	5
Non-pregnant	0	0	1	0	1
Reduced/no food consumption	0	0	1	3	3
Pregnant does dead or moribund	0	0	0	1	0
Abortion/ premature delivery	0	0	1	2	3
Total litter resorptions	0	0	0	0	0

Number of litters for evaluation	5	5	3	2	1
Maternal bodyweight (kg) - day 6 - day 28 (surviving does)	 3.98 4.23	 4.06 4.28	 4.00 4.12	 4.11 4.45	 4.12 4.34
Adjusted (without gravid uterus) body weight (kg) on day 28	3.82	3.94	3.68	4.02	4.04
Number of implantations/doe	9.0	7.8	10.7	8.0	7.0
Mean litter size (live foetuses)	8.8	7.0	9.0	6.5	7.0
Mean foetus weight (g)	33.7	34.7	30.4	37.8	30.6
Clinical signs					
Orange urine	0	0	0	3	1
Orange urine, red fluid under grid floor, pale eyes (non-pregnant animal)	0	0	0	0	1
Red fluid under grid floor	0	0	0	3	2
Red fluid around vagina	0	0	0	1	1

No statistical significance reported

Conclusion:

In the range finding rabbit developmental study, five presumed-pregnant female rabbits per group were administered fenpyrazamine at doses of 0, 60, 90, 120 or 150 mg/kg bw/day as a suspension in 1% aqueous CMC from days 6 to 27 of pregnancy.

Mortality was observed at 120 mg/kg bw/d (one doe). Reduced food consumption/refusal of food was observed in three high dose groups (90, 120 and 150 mg/kg bw/day), followed by abortion and/or premature deliveries. As a result, there was a reduced litter number for further examination. Clinical findings among affected does were largely restricted to orange discoloured urine and/ or a red fluid beneath the cage, mainly observed in 120 and 150 mg/kg bw group. No effect on development of the foetuses was detected. Although the study was only a range-finding study, maternal and foetal NOAELs are proposed at 60 mg/kg bw/d, based on food refusal of does, and subsequent abortions/premature deliveries at next higher dose.

Reference:	Teratology study in rabbits with S-2188 Technical Grade
Author(s), year:	Inawaka, K.; 2008b
Report/Doc. number:	Sumitomo Chemical Co. Ltd. Report No. QNT-0032
Guideline(s):	Japanese MAFF 12 Nousan 8147, EPA OPPTS 870.3700, OECD 414
GLP:	Yes (self-certification)
Deviations:	- One animal of the control group was excluded from the study on day 16 since it bit and swallowed the catheter - According to OECD 414, the study may not be appropriate if there are less than 16 litters left in one treated group. Although in the current study there were only 15 litters left, it is considered acceptable, since there are enough litters for evaluation in the next lower dose, which is considered to be LOAEL for foetal and maternal effects.
Validity:	Yes

Material and methods:

Test Material:	S-2188
Lot/Batch:	030-050914-1G
Purity:	94.7%

Test animals:

Species:	Rabbit
Strain:	New Zealand White (Kbl:NZW SPF)
Age at receipt:	19 weeks (male) or 17 weeks (female)
Weight:	3.40-3.82 kg (males), 3.10-3.80 kg (females)
Source:	Kitayama Labes Co., Ltd.
Diet:	LRC4, Oriental Yeast Co., Ltd.; available *ad libitum*

The purpose of the study was to detect adverse effects of fenpyrazamine on pregnant rabbits and on the development of embryos and the foetuses consequent to exposure of the females to the test substance (0, 30, 50 and 90 mg/kg bw/d) from implantation (day 6 post coitum) until day 28 p.c.

Animal assignment and treatment:
Groups of 24 female rabbits were artificially inseminated using semen collected from the males. The day of insemination was designated Day 0 of gestation. 24 (presumed pregnant) females were allocated to each dosage group (control, 30, 50 and 90 mg/kg bw/d). Rabbits were dosed once each day during days 6 to 27 of gestation.

Diet preparation, analysis and administration:

The highest concentration was prepared by suspending test material in CMC solution; lower doses were prepared by serial dilution of top dose material. Dose suspensions were prepared at least once each two weeks. Test material stability in dose suspension was confirmed prior to the study. During the study, concentration and homogeneity of dose preparations were confirmed by analysis.

Fenpyrazamine technical was administered orally (using disposable syringe and silicon catheter) as a suspension in a 1% aqueous carboxymethylcellulose (CMC) solution, at a dose volume of 5 ml/kg bw.

Clinical observations:

Animals were observed at least once daily for mortality and clinical signs. During the dosing period (days 6 – 27 of gestation), animals were observed at least twice (prior to dosing, and 1-3 hours after dosing) each day.

Food consumption and body weight:

Food consumption was measured between days 3-6, 6-9, 9-12, 12-15, 15-18, 18-21, 21-24, 24-27 and 28 of gestation. Bodyweights were measured on days 0, 6, 9, 12, 15, 18, 21, 24, 27 and 28 of gestation.

Sacrifice and pathology:

From females that gave birth prematurely or aborted, number of foetuses was counted on discovery, the animals were then sacrificed and necropsied. All remaining females were sacrificed on gestation day 28, by i.v. injection of sodium pentobarbital.

A gross examination was performed. Gravid uterine weight was measured. Foetuses were removed by caesarean section, and number of corpora lutea, number of implantations, number of live foetuses, number and intrauterine position of embryonic/foetal deaths (including time of resorptions) were recorded.

Foetal examination:

Each live foetus was weighed and examined externally (including the oral cavity). Sex was determined from observation of internal genitalia. Foetuses with external abnormalities were fixed in buffered 10% formalin and stored; these were not subject to visceral or skeletal examination. The cervical, thoracic and abdominal viscera of all foetuses were examined for visceral alterations.
The heads of approximately one-half of the live foetuses were examined by cross-section of the coronal suture. The heads of the remaining foetuses, and hearts of all foetuses, were fixed in Bouin's solution and examined.
The remaining skeletons were fixed in dehydrating alcohol, stained with Alizarin Red S using Kawamura's method, then examined for skeletal abnormalities. After examination, skeletons were stored in glycerol. Skeletal examination of heads fixed in Bouin's solution was not performed.

Findings:
Clinical signs and mortality:

A small proportion of females in each group did not become pregnant; the number of pregnant females was 20, 19, 21 and 22. One further animal of the control group was sacrificed on day 12 of gestation after a technical malfunction during dosing, and excluded from evaluation.

No animal died as a result of maternal toxicity. As a result of abortion/premature delivery, one mid-dose and seven top-dose animals failed to complete the study; animals were killed prior to scheduled kill. The number of animals reaching scheduled necropsy was therefore 19, 19, 20, and 15 in the control, 30, 50, and 90 mg/kg groups, respectively.

Seven does in the high dose, and one in the mid dose, aborted or gave birth prematurely; these events occurred between days 24 and 27 of gestation. In one of these animals (high dose) it was not possible to determine the day of abortion. Orange urine was seen in one and three animals of the mid- and high dose groups, respectively. Additionally, red fluid was seen under the grid floor of a mid-dose animal on day 23 and 24 of gestation. These findings were made in animals that aborted or delivered prematurely.

Food consumption, body weight and body weight gain

Among all groups including control, a total of 11 animals showed almost no food consumption (less than 10 g/day) on at least one measuring period during the study. In eight of these animals (2 in the mid- and 6 in the high dose does) marked suppression of food consumption was continuous and marked weight loss was seen. Between days 21 and 24 (prior to abortions/premature deliveries) food consumption of mid-dose females was 12.6% and of high dose females 33.1%

lower than for control animals and mean weight of animals who later aborted/prematurely delivered was 11.3% (mid-dose) and 19.6% (high dose) lower compared to animals of same dose without premature deliveries. In the remaining three animals (one each in control, low and mid-dose groups), low food consumption was concluded to be incidental since it was transient and/or there was no corresponding effect on bodyweight on day 28 of pregnancy.

Animals of mid- and high dose group without abortions/premature deliveries had comparable food consumption to control animals throughout the study and comparable body weight to control animals at the end of treatment period.

Table 64: Results of the developmental study in rabbit

Dose (mg/kg bw/day)	**0**	**30**	**50**	**90**
Number of females inseminated	24	24	24	24
Non-pregnant	4	5	3	2
Accidental death	1	0	0	0
Abortion/ premature delivery	0	0	1	7
Total litter resorptions	0	0	0	0
Number of litters for evaluation	19	19	20	15
No/reduced food consumption in at least one observation period	1	1	3	6
Persistent suppression of food consumption	0	0	2	6
Mean food consumption (all animals) - (g/day) (days 21 and 24 – prior to abortions/premature deliveries) - (% compared to control)	127	130 (+2.4%)	111 (-12.6%)	85 (-33.1%)
Maternal bodyweight (kg) - day 6 (all animals) - day 24 (animals without future abortions) - day 24 (animals prior to abortion) (% compared to animals without abortions) - day 28 (surviving does)	3.84 4.04 - - 4.05	3.85 4.03 - - 4.09	3.90 3.98 3.53 (-11.3%) 4.03	3.85 3.93 3.16 (-19.6%) 4.07
Adjusted (without gravid uterus) body weight (kg) on day 28	3.66	3.66	3.67	3.73
Clinical signs				
Orange urine	0	0	1	3
Red fluid under grid floor	0	0	1	0

Sacrifice and pathology:

At terminal necropsy on day 28 of pregnancy one animal (mid-dose group) was found with a pale liver. Since the same finding was made in some animals killed prematurely, this finding was considered treatment-related.
Caecal contents of one animal (mid-dose group) was noted to be dark brown and muddy in appearance. Among animals which aborted or delivered prematurely, distension of the

gallbladder, pale liver, white focus in the liver, abnormal caecal contents (dark brown and muddy in appearance), and/or pale heart were observed. There were no other treatment-related macroscopic findings.

Table 65: Necropsy findings in the developmental study in rabbit

Dose (mg/kg bw/day)	**0**	**30**	**50**	**90**
Number of litters for evaluation	19	19	20	15
Necropsy findings				
Animals that aborted or delivered prematurely - muddy and dark brown content in the caecum - pale heart - pale liver - white focus in the liver - distension of the gallblader	 0 0 0 0 0	 0 0 0 0 0	 1 0 0 0 0	 6 2 3 2 3
Animals at necropsy day 28 - muddy and dark brow content in the caecum - pale liver	 1 0	 0 0	 1 1	 0 0

Foetal data:

Post implantation losses, sex distribution, mean litter size and foetuses weights were comparable across the groups. Detailed examination of the foetuses identified no treatment-related effects. There was no increase in the incidence of malformation or variations. The incidence of findings within the study was considered in range of those seen at the testing laboratory. There was no effect on the incidence or degree of skeletal ossification.

Table 66: Foetal findings in the developmental study in rabbit

Dose (mg/kg bw/day)	**0**	**30**	**50**	**90**
Number of implantations/doe	8.4	9.3	7.9	6.8
Resorption & foetal death (%)	11.3	5.6	8.9	5.9
Mean litter size (live foetuses)	7.4	8.8	7.2	6.4
Mean foetal weight (g): - males - females	 36.8 36.3	 34.9 34.3	 36.7 33.3	 37.6 36.9
Sex ratio (% of male)	59	53	52	50
Foetuses with external malformations - total foetuses affected /total examined -% of examined foetuses	 1/141 (0.7)	 0/167 (0.0)	 1/143 (0.7)	 1/96 (1.0)
Foetuses with skeletal malformation - total foetuses affected /total examined -% of examined foetuses	 5/140 (3.6)	 2/167 (1.2)	 3/142 (2.1)	 0/95 (0.0)

Dose (mg/kg bw/day)	0	30	50	90
Foetuses with skeletal variations - total foetuses affected /total examined -% of examined foetuses	 120/140 (85.7)	 131/167 (78.4)	 108/142 (76.1)	 70/95 (73.7)
Foetuses with visceral malformations - total foetuses affected /total examined - % of examined foetuses	 3/140 (2.1)	 4/167 (2.4)	 1/142 (0.7)	 1/95 (1.1)

Conclusion:
In the developmental study in rabbit presumed-pregnant female rabbits were administered fenpyrazamine at doses of 0, 30, 50, or 90 mg/kg bw/day as a suspension in 1% aqueous CMC from days 6 to 27 of pregnancy, sacrificed shortly before term, and foetuses examined for indications of effects on development.
Seven females of the high dose, and one of the mid-dose either aborted or gave birth prematurely and as a result, were sacrificed before the end of the study. The abortions/early deliveries were attributed to severely reduced food consumption and markedly reduced body weight which was seen in each affected doe. The number of litters available for detailed examination at the high dose was consequently lower.
No effect on development of examined foetuses was detected. Incidence of variations and malformations observed in the treated groups was either comparable or lower than in the control animals. Regarding observed effects, it is proposed to set maternal NOAEL at 30 mg/kg bw/d, based on severely reduced food consumption, markedly reduced body weight and consequent premature delivery at 50 mg/kg bw/d, joint by clinical signs like orange urine and red fluid under grid floor of cages. Since abortion/premature birth is an offspring effect, the offspring NOAEL is proposed to be set at same dose of 30 mg/kg bw/, based on abortions/premature deliveries at next higher dose.

4.11.2.2 Human information

A formal statement from the manufacturer is presented (Ohtsubo, T (2009)), that members of staff involved in the synthesis and development of fenpyrazamine are routinely monitored and that no indication of fenpyrazamine-related ill-health have been detected by, or reported to, medical staff. No poisoning incidents or clinical cases have been reported. Fenpyrazamine is a new chemical currently in development. There has been no exposure of the general population or epidemiology study.

4.11.3 Other relevant information

No other relevant information available.

4.11.4 Summary and discussion of reproductive toxicity

In the preliminary dose-finding study fenpyrazamine was well-tolerated at all doses, resulting only in weight gain loss in parental males of mid- and high dose group (during female gestation and lactation time) and in parental females of high dose group during gestation. Mating performance and fertility appeared unaffected at all dose levels. In the high dose females, an increase in post implantation loss and reduction in litter size were noted. Pups in the high dose

group showed statistically significant body weight loss from day 14 and 28 post-partum, but not at birth. At necropsy, an increased incidence of thyroid enlargement was reported in parental males and females of high dose. There were no notable necropsy findings among pups. Changes in organ weights of pups are considered secondary to body weight loss in high dose animals during lactation and weaning. Therefore, the NOAEL for parental systemic toxicity is set at 400 ppm for males (equivalent to 24.8 mg/kg bw/d) and 1000 ppm for females (equivalent to 75 mg/kg bw/d), based on decreased body weight gain of both sexes (> 10%) during gestation period. NOAEL for offspring systemic toxicity is proposed to be set at the dose level of 1000 ppm (equivalent to 75 mg/kg bw/day during gestation) due to increased post implantation loss, decreased number of live pups at birth and significantly lower body weight of pups from day 14 to 28 post partum. Additionally, absolute and relative organ weights alterations (statistically significant) were observed in high dose pups. Reproductive NOAEL of 3000 ppm (equivalent to 244.7 mg/kg bw/d (males) and 253.7 mg/kg bw/d (females)) was derived, due to no effects observed on reproduction at highest dose tested.

In the 2-generation study, fenpyrazamine was administered continuously in the feed to rats at dietary concentrations of 0, 400, 1000, and 3000 ppm. The toxicological response of the treated animals was principally characterised by increase in relative liver and thyroid weight and histological findings in these organs (cell hypertrophy) and reduced food consumption and decreased body weight gain throughout the whole study period. Significantly lower number of implantations, significantly increased post implantation loss and significantly increased post natal loss in the high dose group of F1 generation is considered to be the effect of systemic toxicity in maternal animals (body weight gain loss, body weight reduction, thyroid enlargement, hepatocellular and thyroid cell hypertrophy). Fenpyrazamine showed no effects on fertility. Sperm evaluation and ovary staging did not reveal any test substance related findings. A No-Observed-Adverse-Effect Level (NOAEL) for parental effects in P and F1 generation is proposed at the dietary level of 400 ppm (20.3 mg/kg bw/d; mean substance intake of males of F1 generation during after-mating period), based on occurrence of increased relative liver and thyroid weights and corresponding histological findings (liver and thyroid cell hypertrophy) at 3000 ppm and 1000 ppm. Statistically significant decrease of relative liver weight of males at 400 ppm was not considered adverse because of absence of histopathological findings in liver at this dose. A No-Observed-Adverse-Effect Level (NOAEL) for offspring effects in F1 and F2 pups is proposed at 400 ppm (28.5 mg/kg bw/d; mean substance intake of females of F1 generation during gestation), based on significantly lower body weights of F1 and F2 pups during lactation at 3000 ppm and 1000 ppm. Statistically significant increase of post implantation loss and decreased viability index for pups of all F1 parents are within the historical control data are therefore not regarded as relevant for setting the NOAEL. A No-Observed-Adverse-Effect Level (NOAEL) for reproductive effects is proposed at the dietary level of 1000 ppm (73.7 mg/kg bw/d; mean substance intake of females of F1 generation during gestation), based on significantly decreased number of implantations observed in F1 females at 3000 ppm.

In the preliminary developmental study in rat, the doses of 150, 300 and 500 mg/kg bw/d were administered. Except for death of one dam, no excess toxicity was observed in any treated group. In the high dose group, corrected body weight gain of dams and mean weight of foetuses were significantly decreased. Mean placental weight was significantly increased in all treated groups but no histopathological examinations of placenta or horomone level measurements were conducted. Therefore, it can not be excluded, that increased placental weight is an adverse effect and not only an adaptive response. Observed malformations in two foetuses (1 per litter) from low and mid-dose were not considered treatment-related.

Based on the results of this study, a high dose of 500 mg/kg bw/day was recommended for main

developmental study and dose lower than 150 mg/kg bw/d was chosen as a low dose, in order to be able to establish NOAEL.

In the main developmental study in rats fenpyrazamine at dose levels of 0, 30, 125, and 500 mg/kg bw/day was well tolerated. All animals survived to the scheduled sacrifice and did not show any adverse clinical signs. No malformations were observed in the study.

At 125 and 500 mg/kg dose level, mean body weight gains of dams were statistically significantly lower when compared to the control group, thus indicating the occurrence of maternal toxicity.

At 500 mg/kg dose level, placenta weights were statistically significantly increased, as already observed in the preliminary developmental study. Since no histolopathological examinations or wet/dry weight measurements for estimation of hyperplasia or hypertrophy and no hormone level measurements were conducted, it can not be excluded that the increase in placental weight is an adverse effect and not only an adaptive response.

No visceral or skeletal malformations were observed in the study.

The increased incidences of visceral (dilated kidney pelvis, dilated ureters, distended urinary bladder, and of additional liver lobes in foetuses) and skeletal (zygomatic arch fusions, sternebra offset or sternebra with misshapen ossification site, incomplete ossification of the frontal bone and costal cartilages asymmetrically aligned at sternum) foetal findings in high dose animals were considered related to observed maternal toxicity in this dose group.

In conclusion, the No Observed Adverse Effect Level (NOAEL) for maternal animals is proposed at 30 mg/kg bw/day (lowest tested dose), based on statistically significantly lower body weight gain in mid-and high dose dose animals during pregnancy. The developmental NOAEL is proposed at 125 mg/kg bw/day (mid-dose), based on significant decrease of mean foetal weight and significant increase of placental weight, visceral and skeletal variations and delayed ossifications (above historical control data) in foetuses of high dose group (500 mg/kg bw/d).

In the range finding rabbit developmental study, five presumed-pregnant female rabbits per group were administered fenpyrazamine at doses of 0, 60, 90, 120 or 150 mg/kg bw/day as a suspension in 1% aqueous CMC from days 6 to 27 of pregnancy.

Mortality was observed at 120 mg/kg bw/d (one doe). Reduced food consumption/refusal of food was observed in three high dose groups (90, 120 and 150 mg/kg bw/day), followed by abortion and/or premature deliveries. As a result, there was a reduced litter number for further examination. Clinical findings among affected does were largely restricted to orange discoloured urine and/ or a red fluid beneath the cage, mainly observed in 120 and 150 mg/kg bw group. No effect on development of the foetuses was detected. Although the study was only a range-finding study, maternal and foetal NOAELs are proposed at 60 mg/kg bw/d, based on food refusal of does, and subsequent abortions/premature deliveries at next higher dose.

In the developmental study in rabbit presumed-pregnant female rabbits were administered fenpyrazamine at doses of 0, 30, 50, or 90 mg/kg bw/day as a suspension in 1% aqueous CMC from days 6 to 27 of pregnancy, sacrificed shortly before term, and foetuses examined for indications of effects on development.

Seven females of the high dose, and one of the mid-dose either aborted or gave birth prematurely and as a result, were sacrificed before the end of the study. The abortions/early deliveries were attributed to severely reduced food consumption and markedly reduced body weight which was seen in each affected doe. The number of litters available for detailed examination at the high dose was consequently lower.

No effect on development of examined foetuses was detected. Incidence of variations and

malformations observed in the treated groups was either comparable or lower than in the control animals. Regarding observed effects, it is proposed to set maternal NOAEL at 30 mg/kg bw/d, based on severely reduced food consumption, markedly reduced body weight and consequent premature delivery at 50 mg/kg bw/d, joint by clinical signs like orange urine and red fluid under grid floor of cages. Since abortion/premature birth is an offspring effect, the offspring NOAEL is proposed to be set at same dose of 30 mg/kg bw/, based on abortions/premature deliveries at next higher dose.

4.11.5 Comparison with criteria

Regarding maternal and developmental findings from one 2-generation rat study and two developmental studies on each rat and rabbit, no classification and labelling is triggered for fenpyrazamine.

4.11.6 Conclusions on classification and labelling

There is no evidence of effects on reproduction and development caused by fenpyrazamine, therefore, no classification and labelling is proposed.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

With respect to the possible neurotoxicological potential of fenpyrazamine, two acute (preliminary and main study) and one subchronic neurotoxicity study have been performed. No studies on delayed neurotoxicity are available.

Table 67: Summary table of relevant neurotoxicity studies

Method	Dose range / NOAEL	Remarks	Reference
Acute oral neurotoxicity Peak-effect study in rats (preliminary study) (No appropriate guideline existing)	0, 100, 200, 2000 mg/kg bw NOAEL: 1000 mg/kg bw ♂ 2000 mg/kg bw ♀ Main effects: ↓ body weight gain ♂	HanRcc:WIST(SPF) rats Purity: 94.7%	Sommer, E.W, 2007
Acute oral neurotoxicity (gavage) study in rats (OECD 424)	0, 80, 400, 2000 mg/kg bw NOAEL: 80 mg/kg bw ♂ 400 mg/kg bw ♀ Main effects: ↓ total distance time ♂ ↓ total number of rearings ♂ ♀	HanRcc:WIST(SPF) rats Purity: 94.7%	Sommer, E.W, 2008a
90-day oral neurotoxicity (feeding) study in rats (OECD 424)	0, 500, 1200 and 3000 ppm equivalent to: 0, 36.8, 87.6, 223.6 mg/kg bw/d	HanRcc:WIST(SPF) rats	Sommer, E.W, 2008b

	in males and 0, 41.7, 100.2, 248.4 mg/kg bw/d in females NOAEL: 87.6 mg/kg bw/d ♂ 100.2 mg/kg bw/d ♀ Main effects: ↓ body weight ♂ ♀ ↓ body weight gain ♂ ♀	Purity: 94.7%	

Acute oral neurotoxicity Peak-effect study in rats

In the preliminary acute neurotoxicity study no neurotoxic effects could be determined due to the absence of relevant test substance-related clinical signs up to 2000 mg/kg bw. The NOAEL can be set at 1000 mg/kg bw for males, based on statistically significant decrease in body weight gain at 2000 mg/kg bw. For females, the NOAEL is > 2000 mg/kg bw since no effects were observed in any tested group.

Acute oral neurotoxicity study in rats

In the acute oral neurotoxicity study the NOAEL is set at 80 mg/kg bw for males, based on statistically significantly reduced total distance time and total number of rearings at day 1 of treatment in 400 mg/kg bw male group. For females, the NOAEL is set at 400 mg/kg bw, based on statistically significantly reduced total number of rearings at day 1 of treatment in 2000 mg/kg bw group.

90-day oral neurotoxicity (feeding) study in rats

In the subchronic (90 days) oral neurotoxicity study in rats no clinical signs or mortality, no significant changes during FOB or motor activity and no microscopic pathology indicative of neurotoxicity was found at necropsy. The only treatment-related finding was a decrease in body weight and bodyweight gain in both sexes at 3000 ppm. On the basis of the body weight and body weight gain decrease at 3000 ppm, the NOAEL in this study was determined to be 1200 ppm for both males and females (87.6 and 100.2 mg/kg bw/day, respectively).

4.12.1.2 Immunotoxicity

No data available.

4.12.1.3 Specific investigations: other studies

No other data available.

4.12.1.4 Human information

A formal statement from the manufacturer is presented (Ohtsubo, T (2009)), that members of staff involved in the synthesis and development of fenpyrazamine are routinely monitored and that no indication of fenpyrazamine-related ill-health have been detected by, or reported to, medical staff. No poisoning incidents or clinical cases have been reported. Fenpyrazamine is a new chemical currently in development. There has been no exposure of the general population or epidemiology study.

4.12.2 Summary and discussion

In the preliminary acute neurotoxicity study no neurotoxic effects could be determined due to the absence of relevant test substance-related clinical signs up to 2000 mg/kg bw. The NOAEL can be set at 1000 mg/kg bw for males, based on statistically significant decrease in body weight gain at 2000 mg/kg bw. For females, the NOAEL is > 2000 mg/kg bw since no effects were observed in any tested group.

In the acute oral neurotoxicity study the NOAEL is set at 80 mg/kg bw for males, based on statistically significantly reduced total distance time and total number of rearings at day 1 of treatment in 400 mg/kg bw male group. For females, the NOAEL is set at 400 mg/kg bw, based on statistically significantly reduced total number of rearings at day 1 of treatment in 2000 mg/kg bw group.

In the subchronic (90 days) oral neurotoxicity study in rats no clinical signs or mortality, no significant changes during FOB or motor activity and no microscopic pathology indicative of neurotoxicity was found at necropsy. The only treatment-related finding was a decrease in body weight and bodyweight gain in both sexes at 3000 ppm. On the basis of the body weight and body weight gain decrease at 3000 ppm, the NOAEL in this study was determined to be 1200 ppm for both males and females (87.6 and 100.2 mg/kg bw/day, respectively).

4.12.3 Comparison with criteria

According to the available studies, there was no indication of a neurotoxic potential of fenpyrazamine (neither neurobehavioural changes nor any morphological changes in the CNS or in the peripheral nerves).

Based on the available acute, subchronic and chronic studies, there was no indication of an immunotoxic potential.

4.12.4 Conclusions on classification and labelling

No classification and labelling is proposed for fenpyrazamine regarding neurotoxicity or immunotoxicity.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 68: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis Guideline: EEC Method C.7 , OPPTS 835-2110, J MAFF Nousan 8147, section 2-6-1 (2001)	Fenpyrazamine, [phenyl-14C] and [pyrazolyl-14C] labels: DT50 (pH 4, 20 °C): stable to hydrolysis DT50 (pH 7, 20 °C, extrapolated): > 1 year (SFO) DT50 (pH 9, 20 °C, extrapolated): 24 days (SFO)	radiochemical purity: > 99 % (HPLC)	Lewis, C.J. (2007) Report No.: 0333/257-D2149; QNM-0017
Photolysis Guideline: 95/36/EC, 94/37/EC, SETAC (1995), US-EPA N 161-2 (1982), J MAFF Nousan-8147 section 2-6-2 (2000)	Fenpyrazamine, [pyrazolyl-^{14}C] and [phenyl-^{14}C] labels: DT_{50} values (also equivalent to natural summer sunlight in UK/US) of 1.7 days S-2188-DC (max. 63.8% after 7 days) and MCNI (max 17.7% after 30 days).	radiochemical purity: ≥ 98 % (HPLC)	Lewis, C.J., Troth, K. (2007) Report No: 0333/258-D2149; QNM-0029
Biological degradation Guideline: Method C.4-C of Annex V of EU Directive 67/548/EEC, OECD 301 B	Not ready biodegradable	purity 94.7 %	Burwood, C.E., Scholey, A. (2006) Report No. 0333/261-D2149; QNM-0011
Water/Sediment Study Guideline: EC Directive 95/36/EC, OECD Guideline 308 (2002)	Water: DT50: 41 d DT90: 136 d Whole system: DT50: 68.1 d DT90: 226.3 d	radiochemical purity: > 99 % and > 98 % (HPLC)	Lewis, C.J., Troth, K. (2007) Report No: D2149-0333/260, QNM-0028 Jarvis, T., Callow, B., (2009) Report No: QNM-0040
Kinetic Evaluation of the Aerobic Aquatic metabolism (Menke, 2006c) Guideline: FOCUS Degradation Kinetics Report (FOCUS 2006)	Water: DT50: 25.5 d (geometric mean) DT90: 84.7 d (geometric mean) Whole system: DT50: 35.5 d (geometric mean) DT90: 117.9 d (geometric mean)		Lewis, C.J., Troth, K. (2007) Report No: D2149-0333/260, QNM-0028 Jarvis, T., Callow, B., (2009) Report No: QNM-0040

5.1.1 Stability

Hydrolysis:

Studies on the hydrolytic degradation were conducted with S-2188 at pH 4, 7 and 9, [phenyl-^{14}C] and [pyrazolyl-^{14}C] labels.

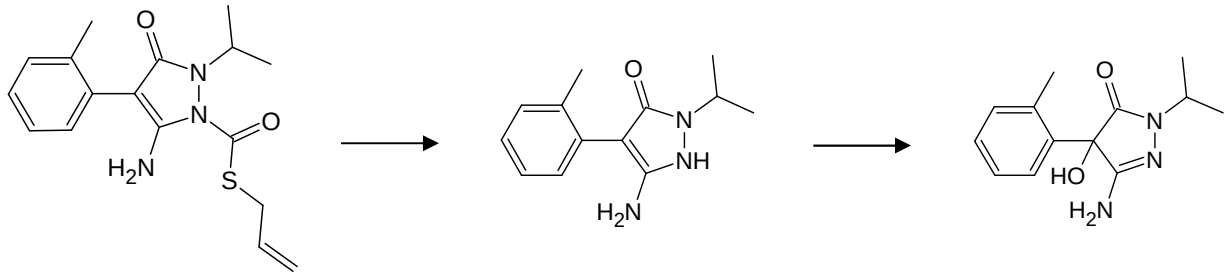
Reference:	**[14C]S-21988: Hydrolytic Stability**
Author(s), year:	Lewis, C.J., 2007
Study/report number:	0333/257-D2149; QNM-0017
Guideline(s):	EEC Method C.7 – Abiotic degradation. Hydrolysis as a function of pH (1992), OPPTS 835-2110 – Hydrolysis as a function of pH (1998), Japan MAFF New Test Guideline 12-Nousan 8147, section 2-6-1 (2001)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

Material and methods:

Test substance:	[Phenyl-14C]S-2188, 4.33 GBq mmol-1, > 99 % radiochemical purity (HPLC), batch CFQ14367 [Pyrazolyl-14C]S-2188, 2.04 GBq mmol-1, > 99 % radiochemical purity (HPLC), batch CFQ14368.
Reference substances:	S-2188 (unlabelled), S-2188-OH, S-2188-DC, S-2188-DTC, MCNI.
Test systems:	pH 4: 0.05 M citrate buffer (monopotatium citrate solution adjusted with sodium hydroxide) pH 7: 0.05 M phosphate buffer (potassium dihydrogen phosphate solution adjusted with sodium hydroxide) pH 9: 0.05 M borate buffer (sodium tetraborate solution adjusted with hydrochloric acid) All buffers sterilized by autoclaving. Oxygen content reduced by sonication and nitrogen bubbling.
Volatile traps:	No volatile traps (no volatiles expected – confirmed by complete material balance).
Test temperature:	Tier 1: 50 °C (pH 4 and pH 7), Tier 2: 25 °C (pH 9), 40 °C (pH 9) and 50 °C (pH 7 and pH 9), 60 °C (pH 7) and 70 °C (pH 7).
Test duration:	Up to 50 days in the dark.
Sample concentration:	1 mg L-1
Co-solvent:	Acetonitrile.
Analysis:	LSC, HPLC-UV/RAD, TLC LOQ < 1 % of AR (HPLC), < 0.1 % of AR (LSC).
Kinetic evaluation:	Simple first order (SFO) kinetics, Microsoft Excel.

Findings:

Mean material balances of all experiments were in a range of 93.9 – 101.7 % of AR. Owing to the complete mass balance $^{14}CO_2$ formation is considered to be negligible.
In Tier 1 test, S-2188 was hydrolytically stable at pH 4 and > 94 % of AR was recovered as the unchanged S-2188. At pH 7 more than 10 % hydrolysis occurred after 5-day application. Tier 1 test was not conducted at pH 9 because S-2188 is known to be unstable under alkaline conditions. The Tier 2 tests were subsequently conducted at pH 7 and pH 9. The product balances are presented in tables B.8.3.1-1 to B.8.3.1-3. Further incubations were undertaken at 60 and 70 °C (pH 7) and 40 and 50 °C (pH 9) but these were considered not to provide any further useful data and are not presented. No distinct differences between labels tested occurred.
Metabolite **S-2188-DC** was formed to a maximum occurrence of 59.4 % of AR (pH 7 at 50 °C, pyrazolyl label) and 49.0 % of AR for phenyl label at DAT 50. In trials at pH 9 and 25 °C metabolite S-2188-DC occurred at a maximum of 54.0 % of AR (pyrazolyl label) and 54.3 % of AR (phenyl label) at DAT 17. Metabolite **S-2188-OH** was found at a maximum occurrence of 10.0 % AR (pH 7 at 50 °C, phenyl label) and 7.4 % of AR with the pyrazolyl label at DAT 50. The maximum occurrence of S-2188-OH at pH 9 and 25 °C was 4.7 % of AR for the phenyl label and 5.1 % of AR for the pyrazolyl label at DAT 17. Unidentified radioactivity was below 10 % of AR.



S-2188 S-2188-DC S-2188-OH

Figure 1: Proposed hydrolysis degradation route of S-2188.

Conclusion:

S-2188 is stable at environmental relevant temperature at pH 4 and 7. S-2188 is rapidly degraded at alkaline pH of 9. The major hydrolysis product formed under sterile conditions and in the absence of air was S-2188-DC- This compound was almost hydrolytically stable but partially oxidised to S-2188-OH which never reached levels above 10 % of AR.

Comments (RMS):

None

Photolysis:
Studies on the photolytic degradation were conducted with S-2188 in sterile buffered water (pH 7.0), [pyrazolyl-^{14}C] and [phenyl-^{14}C] labels.
Quantum yield and half-life time under environmental conditions were determined for S-2188.

Reference:	**[14C]S-2188: Photodegradation and Quantum Yield in Sterile, Aqueous Solution.**
Author(s), year:	Lewis, C.J., Troth, K., 2007
Study/report number:	0333/258-D2149; QNM-0029
Guideline(s):	95/36/EC, 94/37/EC, SETAC (1995), US-EPA N 161-2 (1982), J MAFF Nousan-8147 section 2-6-2 (2000)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

Material and methods:

Test substances:	[Phenyl-14C]S-2188, 4.33 GBq mmol-1, ≥ 98 % radiochemical purity (HPLC), batch CFQ14367 [Pyrazolyl-14C]S-2188, 2.04 GBq mmol-1, ≥ 98 % radiochemical purity (HPLC), batch CFQ14368
Reference substances:	S-2188 (unlabelled), S-2188-OH, S-2188-DC, S-2188-DTC, MCNI, MPPZ
Test system:	Sterile pH 7.0 buffer (0.01 M phosphate buffer, autoclaved), adjusted with 2 M NaOH, sterility was checked throughout the experiment. A PNAP/PYR actinometer for determination of quantum yield.
Test temperature:	25 ± 1 °C
Test duration:	30 days continuous irradiation (1 day incubation equivalent to ca. 30 solar midsummer days in US and UK, ca. 3.3 days of natural Japanese spring sunlight) or dark incubation.
Sample concentration:	1.0 mg L^{-1}
Co-solvent:	Acetonitrile
Test system:	Xenon arc lamp (Suntest Accelerated Exposure machine), cut-off < 290 nm, ca. 25 watts m-2 (300 – 400 nm). Spectrum of experimental radiation is qualitatively similar to solar irradiation of Harrogate in summer. Three other Suntest machines were used and displayed similar radiation spectrum as the one presented.
Volatile traps:	Polyurethane bung, 1 x ethanediol trap and 2 x 2 M NaOH trap
Analysis:	LSC, HPLC-UV/RAD, TLC, HPLC-MS LOD < 0.1 % of AR (LSC), LOD < 0.5 % of AR (HPLC)
Kinetic evaluation:	Simple first order (SFO) kinetics, Microsoft Escel, ModelMker, curve fit based on mean values of both labels

Findings:

Mass balance was in a range of 94 to 100 % of AR for all experiments. Unit rinses contained ≤ 1.5 % of AR and hence confirmed no adsorption of radioactivity to the glass vessels. The ethanediol traps from the incubated samples contained no radioactivity during the entire incubation period whilst the polyurethane foam bungs contained only up to 1.4 % of AR. The

NaOH traps contained up to 1.5 % of AR from the [phenyl-^{14}C]S-2188 solution after 30 days and up to 10.3 % of AR from the [pyrazolyl-^{14}C] labelled S-2188 solution after 30 days (confirmed as CO_2 by barium hydroxide precipitation). Under irradiation S-2188 was subjected to extensive photolytic rearrangement procedures, resulting in two major metabolites: S-2188-DC (maximum 63.8 % of AR at DAT 7 for [pyrazolyl-^{14}C] label and 61.7 % of AR at DAT 7 for [phenyl-^{14}C] label) and MCNI (maximum 17.7 % of AR at DAT 30 for [pyrazolyl-^{14}C] label and 15.7 % of AR at DAT 30 for [phenyl-^{14}C] label). S-2188-DC degraded significantly until the end of the study. MCNI reached its maximum at the end of the study. Minor metabolites (S-2188-DTC and unk A) were detected but never reached levels above 10 % of AR. Unk A was characterised a dioxygenated compound of S-2188-DC. Without irradiation no degradation of S-2188 was observed and no metabolites were detected.

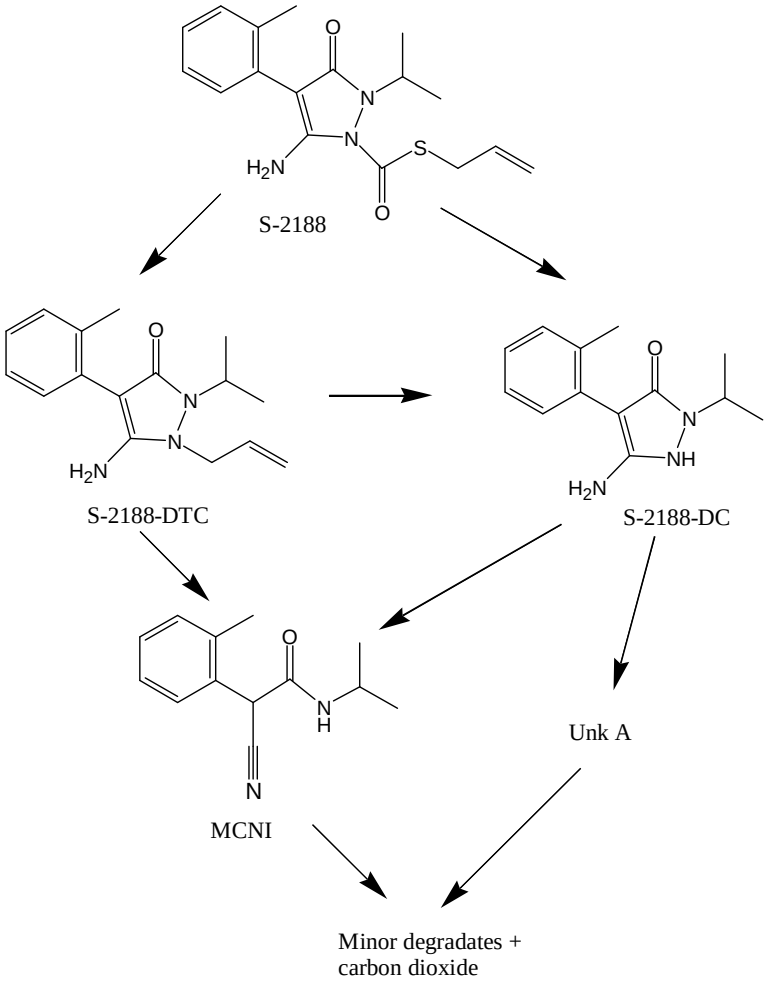


Figure 2: Proposed photolysis degradation route of S-2188

Table 69: Photo-transformation of S-2188 in sterile water buffered at pH 7 [% of AR]

Label	Conditions	DAT	S-2188	S-2188-DC	S-2188-DTC	MCNI	Unk A	Polar peak	Others	Undifferentiated/ unresolved	Total
[Pyrazolyl-^{14}C]	Irradiated	0	96.8	ND	ND	ND	ND	ND	ND	0.5	97.4
		1	63.4	29.7	1.2	ND	ND	ND	1.8	2.1	98.2
		2	38.5	47.7	2.1	0.4	2.1	ND	3.6	3.4	97.8
		3	29.5	55.3	2.2	0.6	1.3	ND	3.4	4.0	96.3
		7	7.1	63.8	4.2	2.6	3.8	ND	9.1	7.7	98.2
		20	2.0	48.1	4.4	9.0	2.8	4.2	10.7*	11.8	92.9
		30	1.1	9.5	4.2	17.7	5.0	13.9*	16.8*	18.7	86.8
	Dark	30	98.9	ND	ND	ND	ND	ND	ND	0.9	99.8
[Phenyl-^{14}C]	Irradiated	0	95.6	ND	ND	ND	ND	ND	ND	2.1	97.7
		1	62.3	23.7	1.1	0.3	2.0	ND	3.1	3.1	95.6
		2	40.8	36.4	2.1	1.0	3.0	ND	2.7	10.6	96.6
		3	26.5	54.7	3.0	1.0	3.8	ND	4.2	4.5	97.7
		7	4.4	61.7	4.8	4.0	3.5	ND	9.8	10.3	98.4
		20	1.0	37.1	4.7	9.9	8.5	3.4	15.9*	11.9	92.3
		30	1.6	7.4	6.3	15.7	4.7	4.6	30.1*	21.8	92.0
	Dark	30	93.2	ND	ND	ND	ND	ND	ND	3.1	96.4

* all individual peaks below 10 % of AR, ND: not detected

Table 70: Calculated aquatic photolytic DT50 and DT90 [days] of S-2188 and metabolites using SFO kinetics at pH 7

Compound	Label	DT_{50} (UK/US equivalent)	DT_{90} (UK/US equivalent)	r^2
S-2188	Pyrazolyl	1.7	5.5	0.985
	Phenyl	1.6	5.4	0.997
S-2188-DC	Pyrazolyl	13.2	43.9	n.s.
	Phenyl	11.8	39.3	n.s.
MCNI	Pyrazolyl	n.c.	n.c.	n.a.
	Phenyl	n.c.	n.c.	n.a.

n.s.: not stated; n.c.: not able to calculate; n.a.: not applicable.

Conclusion:

Under irradiated experimental conditions, S-2188 degraded with a half-life of 1.7 days at pH 7 and 25 °C (SFO kinetic) owing to photolysis. This experimental half-life corresponds to about 1.7 days under environmental conditions in US/UK summer (reference Harrogate 54 °N). Appropriate controls confirmed that there was no degradation in darkness. The main photolytic metabolites were S-2188-DC (max. 63.8% after 7 days) and MCNI (max 17.7% after 30 days). A mean SFO DT_{50} value of 12.5 days was calculated for S-2188-DC. The degradation of MCNI could not be calculated as the maximum occurrence was reached at the end of the study period (DAT 30). Extensive breakdown of the molecule structure was observed after 30 days incubation as evidenced by the large number of minor unknown peaks and undifferentiated/unresolved radioactivity.
The quantum yield for S-2188 was determined to be 0.021.

Comments (RMS):

No comments. The study is considered acceptable.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

As measured data are available estimation is not relevant for this dossier.

5.1.2.2 Screening tests

Readily biodegradability:

Reference:	**S-2188: Assessment of Ready Biodegradability by Measurement of Carbon Dioxide evolution.**
Author(s), year:	Burwood, C.E., Scholey, A., 2006
Study/Report number:	0333/261-D2149; QNM-0011
Guideline(s):	Method C.4-C of Annex V of EU Directive 67/548/EEC, OECD 301 B
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

Material and methods:

Test substance:	S-2188 (unlabelled), purity 94.7 %, batch 030-050914-1G
Reference substance:	Sodium benzoate
Inoculum:	Aeration tank of a waste water plant (Burley Menston) treating predominately domestic sewage (30 mg L-1)
Treatments:	Replicates (except for toxicity control): Blank control

	Reference substance: Na-benzoate (3 mL mg L-1) Test substance: S-2188 (3 mL L-1) Toxicity control: S-2188 (3 mL L-1) and Na-benzoate (3 mL mg L-1) pH of the test vessel at the end of test: 7.5 to 7.7
Analysis:	CO2 amount absorbed by each trap calculated from the reduction in the concentration of barium hydroxide solution (titration)
Incubation conditions:	21 ± 1 °C, 28 days

Findings:

Table 71: Biodegradation of S-2188 and reference compound [% of theoretically possible degradation]

DAT	Reference substance		Test substance	Toxicity control
	Replicate 1	Replicate 2		
2	34	10	0	29
3	45	19	0	39
6	56	30	0	51
8	62	37	0	56
10	66	47	0	59
13	70	44	0	61
14	72	47	0	63
17	75	50	0	65
20	78	54	0	69
24	83	58	0	73
28	90	64	1	79

Within 28 days almost no degradation (maximum 1 %) was determined for S-2188. The reference substance (Na-benzoate) has reached level for ready biodegradability by 8 and 28 days.

Conclusion:

S-2188 is considered to be not readily biodegradable.

Comments (RMS):

None

5.1.2.3 Simulation tests

Biodegradation in water/sediment systems:

One water sediment study was conducted:
- Aerobic water/sediment study with two test systems, [phenyl-^{14}C] and [pyrazolyl-^{14}C] label

Reference:	[14C]S-2188: Degradation and Retention in Water-Sediment Systems
Author(s), year:	Lewis, C.J., Troth, K., 2007
Study/report number:	D2149-0333/260, QNM-0028
Guideline(s):	EC Directive 95/36/EC, OECD Guideline 308 (2002)
GLP:	Yes
Deviations:	Minor deviations
Validity:	Study considered acceptable

Reference:	Determination of rates of degradation for S-2188 from a water sediment study incubated under laboratory conditions.
Author(s), year:	Jarvis, T., Callow, B., 2009
Report/Doc. number:	QNM-0040
Guideline(s):	FOCUS Degradation Kinetics Report (FOCUS 2006)
GLP:	Not applicable
Deviations:	None
Validity:	Yes

Material and methods:

Test substances:	[Phenyl-14C]S-2188,12.9 MBq mg-1, > 98 % radiochemical purity (HPLC), batch CFQ14367 [Pyrazolyl-14C]S-2188, 6.1 MBq mg-1, > 99 % radiochemical purity (HPLC), batch CFQ14368
Reference substances:	S-2188 (unlabelled), S-2188-OH, S-2188-DC, S-2188-DTC, MCNI
Application rate:	0.08 µg mL-1 (under the assumption of 750 g ai ha-1, 100 cm water depth)
Co-solvent:	Acetonitrile (0.005 %, v/v)
Incubation set-up:	3 cm depth of dry Calwich Abbey or Swiss Lake sediment (2 mm sieved) in individual borosilate glass cylinder (ca 4.5 cm in diameter), 9 cm depth of associated water (0.2 mm sieved). Sediment units were aerated, slightly agitated on an orbital shaker. Flow through system (moistured air at flow rate of ca. 20-60 mL min-1).
Acclimatization period:	Approx. 35 days
Test duration:	100 days
Incubation conditions:	20 ± 2 °C in darkness
Volatile traps:	Ethanediol (polar volatiles), 2 % paraffin in xylene (non-polar volatiles), sodium hydroxide (CO2), addition of barium chloride slution to confirm presence of 14CO2.
Analysis:	Water phase mixed with 130 mL acetonitrile and radio-assayed by LSC and analysed by chromatography. Sub-samples (ca 10 mL) collected and added to 2 M sodium hydroxide solution to determine presence of dissolved CO2.

	Sediment phase extracted 4 times with 100 mL of methanol:water (5:1 v/v, neutral extraction) and 3 times with 100 mL methanol:0.5 M hydrochloric acid (5:1 v/v, acidic extraction). Each extract type was combined and aliquots (200 µL) were analysed by LSC. Remaining sediment residues were dried and ground for combustion. 100-day sample: further extraction performed. One replicate was Soxhlet extracted for ca. 16 hours with 100 mL acetone:0.5 M hydrochloric acid (5:1 v/v), analysed by LSC. Non extractable residues: were separated into humic acids, fulvic acids and humins. Radioactivity from fulvic acid and humic acid was determined by LSC. Radioactivity from humin fraction was determined by combustion followed by LSC. Trapping solutions: radioactivity quantified by LSC.
Analytical techniques:	LSC (LOD ca 0.1 % of AR), HPLC-UV/RAD (LOD ca 0.5 % of AR), TLC, LC-MS (API)
Kinetic evaluation:	Simple first order (SFO) kinetics, first order multi-compartment (FOMC), KinGui v. 1.1 (BCS 2006), ModelMaker 4, FOCUS_DEGKIN v2.xls

Table 72: Physicochemical characteristics of the water/sediment matrices.

Name		**Calwich Abbey**	**Swiss Lake**
Geographic location		**Calwich Abbey lake, Calwich, Ashbourne, Derbyshire, UK**	**Swiss Lake, Chatsworth, Derbyshire, UK**
Sediment	Texture (USDA)	Silty clay loam or Clay loam	Sand
	Sand (USDA) [%]	41	91
	Silt (USDA) [%]	25	7
	Clay (USDA) [%]	34	2
	pH (1 M KCl)	7.3	6.0
	pH (water)	7.5	6.1
	Organic C [%]	4.6	0.6
	Redox [mV]	-236	-230
	CEC [mEq 100 g^{-1}]	19.8	5.4
	Microbial Biomass [µg C g^{-1}] – Start / End	1052 / 1666	157 / 197
Water	pH (sampling)	8.6	6.4
	Water hardness [mg L^{-1} as $CaCO_3$] – Start / End	145 / 149	57 / 52
	Oxygen content [mg L^{-1}]	7	8
	Conductivity [µS cm^{-1}]	99	92
	Redox [mV] – Start	160	178
	TOC [ppm] – Start / End	74 / 16	77 / 18
	Suspended solids [mg L^{-1}] – Start / End	25 / 42	2 / 63

Findings:

The oxygen content of the water phase of the samples ranged mostly between 7 and 10 mg L^{-1} in both systems. This indicates aerobic conditions throughout both experiments. The pH in the water phase decreased from pH 8.2 - 8.3 at the start of the study to pH 7.6 - 8.1 at the end in the Calwich Abbey water sediment system and from pH 7.3 - 8.0 at the start to pH 4.1 -5.6 at the end of the study in the Swiss lake system. The pH of the sediment of Calwich Abbey rose slightly from 0 DAT (pH 6.7 - 7.0) to pH 7.4 at the end of the study. In the Swiss Lake system the pH in

sediment decreased from pH 6.5 at the start to pH 4.6 - 5.7 at 100 DAT. The water redox potential evolved from values between +92 and +123 mV (Calwich Abbey) and between +88 and +102 mV (Swiss Lake) at 0 DAT to final values between +143 and +227 mV (Calwich Abbey) and between +256 and +411 mV (Swiss Lake). In the sediment of Calwich Abbey the redox potential was in the range of -130 and -155 mV at 0 DAT and between -70 and -128 mV at 100 DAT. In the sediment of Swiss Lake the redox potential increased from values between -118 and -125 mV at the start of the study to values between +37 and +90 mV at the end of the study.
Total mass balance was in a range of 92.2 to 100.0 % of AR for both systems and both labels. Formation of $^{14}CO_2$ using [pyrazolyl-^{14}C] label accounted for maximum 8.5 % of AR in the Calwich Abbey system and for maximum 3.3 % of AR in the Swiss lake system at study termination. Formation of $^{14}CO_2$ using [phenyl-^{14}C] label accounted for maximum 5.5 % of AR in the Calwich Abbey system and 3.1 % of AR in the Swiss lake system. Formation of NER increased up to maximum 47 % of AR with [pyrazolyl-^{14}C] label and to 47.4 % of AR with the [phenyl-^{14}C] label in the Calwich Abbey system, and to 19.5 % of AR [pyrazolyl-^{14}C] label and to 17.2 % of AR with the [phenyl-^{14}C] label in the Swiss lake system.
Distribution and recovery of radioactivity in both water/sediment systems are presented in tables B.8.3.4.3-2 and B.8.3.4.3-3. Only data on major fractions are shown. Therefore, the mass balance values presented in the tables below do not fit the presented data. In all incubations there was no radioactivity in the traps for volatile organic compounds. MCNI reached a maximum of 0.7 % of AR in sediment in the Calwich Abbey system and was never detected in the water. One unknown compound, Unk E (postulated MW=172.16 g mol^{-1}) reached a maximum of 7.4 % of AR in water and 1.7 % of AR in sediment (9.1 % of AR in the total system). Up to six other unidentified metabolites were quantified but all were below 10%.

Table 73: Distribution and recovery of radioactivity [% of AR] after application of [pyrazolyl-14C] labelled S-2188 (750 g ai ha-1) to the aerobic water/sediment systems 'Calwich abbey' and 'Swiss lake'.

System	Time	Water			Sediment			Unextractable	CO_2	Mass balance	Total water/sediment		
	(day)	S-2188	S-2188-DC	S-2188-OH	S-2188	S-2188-DC	S-2188-OH				S-2188	S-2188-DC	S-2188-OH
Calwich abbey	0	90.5	0.8	ND	NA	NA	NA	0.1	NA	98.7	90.5	0.8	0.0
	7	46.7	10.2	2.4	24.7	6.1	0.9	2.3	0.5	98.1	52.8	11.1	4.7
	14	38.4	3.7	7.0	19.7	6.1	1.1	7.9	1.3	92.2	44.5	4.8	14.9
	30	7.8	2.6	6.8	18.7	6.3	3.8	24.2	2.5	94.9	14.1	6.4	31.0
	61	1.6	4.3	12.2	4.1	4.4	3.7	42.3	3.9	93.6	6.0	8.0	54.5
	100	3.1	3.8	6.1	2.0	3.1	4.4	47.0	8.5	95.7	6.2	8.2	53.1
Swiss lake	0	94.5	1.5	ND	NA	NA	NA	ND	NA	100.0	94.5	1.5	0.0
	7	53.1	2.6	2.5	30.3	0.3	0.2	0.8	0.7	96.3	53.4	2.8	3.3
	14	41.4	1.4	5.0	32.8	0.7	0.8	2.6	1.4	97.0	42.1	2.2	7.6
	30	34.2	1.5	9.1	23.0	2.5	2.5	7.1	1.4	97.7	36.7	4.0	16.2
	61	21.4	0.6	8.2	22.2	1.2	1.9	13.3	3.1	93.2	22.6	2.5	21.5
	100	13.5	0.9	7.3	27.6	3.2	3.2	19.5	3.3	98.6	16.7	4.1	26.8

Table 74: Distribution and recovery of radioactivity [% of AR] after application of [phenyl-14C] labelled S-2188 (750 g ai ha-1) to the aerobic water/sediment systems 'Calwich abbey' and 'Swiss lake'.

System	Time (day)	Water			Sediment			Unextractable	CO_2	Mass balance	Total water/sediment		
		S-2188	S-2188-DC	S-2188-OH	S-2188	S-2188-DC	S-2188-OH				S-2188	S-2188-DC	S-2188-OH
Calwich abbey	0	93.6	0.4	0.3	NA	NA	NA	ND	NA	99.6	93.6	0.4	0.3
	7	48.3	11.9	2.6	21.4	8.6	1.0	1.3	0.3	98.1	69.7	20.5	3.6
	14	40.7	2.6	5.8	20.0	10.5	1.8	7.0	0.7	95.2	60.7	13.1	7.6
	19	24.3	2.8	7.2	24.2	7.8	1.8	12.4	1.4	98.2	48.5	10.6	9.0
	30	14.5	2.4	7.3	17.1	6.6	2.8	19.3	1.7	93.3	31.6	9.0	10.1
	61	2.2	3.0	7.8	9.4	3.7	2.6	37.6	4.4	95.6	11.6	6.7	10.4
	100	0.3	5.5	9.2	2.1	3.8	3.6	47.4	5.5	93.1	2.4	9.3	12.8
Swiss lake	0	95.8	0.7	0.1	NA	NA	NA	ND	NA	99.4	95.8	0.7	0.1
	7	67.0	0.8	3.0	22.4	0.5	0.2	0.6	0.4	98.1	89.4	1.3	3.2
	14	54.0	1.3	5.4	29.2	0.9	0.6	1.3	0.6	99.4	83.2	2.2	6.0
	30	39.9	1.4	12.5	20.1	1.7	1.8	4.6	1.9	97.5	60.0	3.1	14.3
	61	18.6	0.7	10.3	29.0	0.3	3.0	16.4	2.9	99.4	47.6	1.0	13.3
	100	11.8	1.5	9.3	25.9	2.5	2.6	17.2	3.1	96.8	37.7	4.0	11.9

S-2188 degraded in both water/sediment systems with a faster degradation rate in the Calwich Abbey system than in the Swiss Lake system. Transfer of the test substance into sediment was relatively fast with occurrences of S-2188 between 21.4 and 30.3 % of AR after 7 days. Maximum occurrence of [pyrazolyl-^{14}C] labelled S-2188 in sediment was 24.7 (DAT 7) and 32.8 % of AR (DAT 14) in Calwich Abbey and Swiss Lake respectively. For [phenyl-^{14}C] labelled S-2188 the maximum occurrence in sediment was 24.2 % of AR (DAT 19) and 29.2 % of AR (DAT 14) in Calwich Abbey and Swiss Lake respectively. In the Calwich Abbey system the degradation of S-2188 led to the formation of the major metabolite **S-2188-DC** at concentrations in water between 10.2 (DAT 7) and 11.9 % (DAT 7) of AR with the [pyrazolyl-^{14}C] and [phenyl-^{14}C] labelling respectively. In sediment the metabolite reached maximum levels of 6.3 % of AR (DAT 30) for the [pyrazolyl-^{14}C] label and 10.5 % of AR (DAT 14) for the [phenyl-^{14}C] label. Metabolite S-2188-DC did not reach levels above 10 % of AR in the Swiss Lake neither in water nor in the sediment phase. Metabolite S-2188-DC further degraded into metabolite **S-2188-OH** which reached 12.2 % of AR ([pyrazolyl-^{14}C] label) after 61 days in the water phase of the Calwich Abbey system and 12.5 % of AR ([phenyl-^{14}C] label) after 30 days in the water phase of the Swiss Lake system. S-2188-OH was not detected at concentrations above 5 % of AR in sediment in both systems with both labelling.
The proposed degradation pathway of S-2188 in water/sediment system is shown in the figure below. The degradation rates were fitted with Single First Order (SFO) kinetic. For comparative purposes First Order Multi-Compartment (FOMC) kinetic was also presented. SFO kinetics was considered acceptable for determining simulation endpoints allowing good visual fit and χ^2 error values below 8 %. Too few data values were available to calculate degradation rates of metabolites S-2188-DC and S-2188-OH.

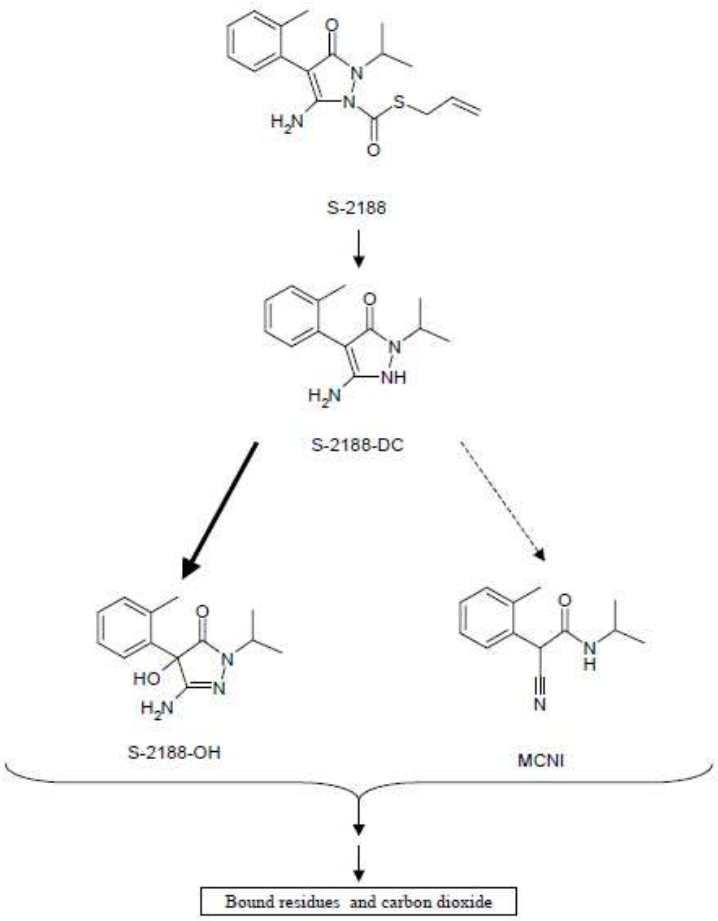


Figure 3: Proposed metabolic pathway for S-2188 in water-sediment systems

Table 75: Degradation rates of S-2188 in water sediment systems (whole system) following Single First Order (SFO) kinetics and First Order Multi-Compartment (FOMC) kinetics for comparison purposes.

Parameter	Calwich Abbey		Swiss Lake	
	[Pyrazolyl-^{14}C]	[Phenyl-^{14}C]	[Pyrazolyl-^{14}C]	[Phenyl-^{14}C]
Model	SFO	SFO	SFO	SFO
Chi^2 error [%]	5.3	3.4	7.1	5.0
k [day^{-1}] *	0.0385 (1.3 x 10^{-4})	0.0345 (2.2 x 10^{-6})	0.0102 (0.0026)	0.0108 (6.2 x 10^{-4})
DT_{50} [Day]	18.0	20.1	68.1	64.4
DT_{90} [Day]	59.9	66.7	226.3	214.0

*P value from the t-test is given in brackets.

The geometric mean DT_{50} for S-2188 in the water sediment system (whole system) was calculated to 35.5 days. Half-lives of S-2188 in the water phase and in the sediment phase of the water sediment systems are presented in following table .

Table 76: Calculated half-lives [days] of S-2188 in the water and sediment phase in the aerobic water/sediment systems Calwich Abbey and Swiss Lake based on SFO kinetics.

System	Label	Water	Sediment	Kinetics
		Dissipation	Dissipation	

		DT_{50} [days]	**X^2 error [%]**	**T-test [p value]**	**DT_{50} [days]**	**X^2 error [%]**	**T-test [p value]**	
Calwich Abbey	[Pyrazolyl-^{14}C]	28.8	11.3	0.141	11.5	17.1	0.071	SFO
Calwich Abbey	[Phenyl-^{14}C]	19.8	9.4	0.012	21.3	10.6	0.089	SFO
Swiss Lake	[Pyrazolyl-^{14}C]	18.0	5.3	0.062	n.c.	17.4	n.c.	SFO
Swiss Lake	[Phenyl-^{14}C]	41.0	5.8	0.093	363	20.9	0.449	SFO

n.c.: not calculable, k value negative.

Conclusion:

S-2188 degraded via S-2188-DC and S-2188-OH, with both metabolites exceeding 10% in the water phase and the overall system (maximum in whole system 20.5 % of AR and 54.5 % of AR, respectively). In addition, S-2188-DC exceeded 10% in the sediment phase alone. No other compounds were detected at levels above 10 % of AR in the overall system. Mineralisation over 100 days was relatively small (3.1 - 8.5 % of AR) and not greatly different between the [phenyl-^{14}C] and [pyrazolyl-^{14}C] labelled forms of S-2188. Amounts of bound residues remaining after 100 days were also very similar between the two radiolabelled forms, but different between the two water/sediment systems being about 47 % of AR for Calwich Abbey (high organic carbon) and about 18 % of AR for Swiss Lake (low organic carbon). S-2188 dissipated from the water and reached maximum amounts of 32.8 % of AR in the sediment phase (DAT 14). Degradation in the whole system was determined using SFO (following guidance document FOCUS, 2006) with first order DT_{50} values of 18.0 – 68.1 days (geomean 35.5 days) and DT_{90} values of 59.9 - 226.3 days.

Comments (RMS):

The study is considered correct.

5.1.3 Summary and discussion of degradation

Aquatic hydrolysis

One hydrolysis study was carried out in dark sterile buffer solutions at pH 4, 7 and 9 at 50 °C for pH 4 and pH 7 (Tier 1), 25 °C and 40 °C (pH 9), 50 °C for pH 7 and pH 9, 60 °C and 70 °C for pH 7 using [pyrazolyl-^{14}C] and [phenyl-^{14}C] labelled S-2188. S-2188 was hydrolytically stable at environmental temperature at pH 4 and 7 but at pH 9, degradation to S-2188-DC and subsequently to S-2188-OH occurred. At 20 ºC and pH 9 the DT_{50} for hydrolysis was 24 days. As the degradation rate of fenpyrazamine by hydrolysis at 20 °C takes longer than 16 days the active substance can not be considered to be rapidly hydrolysed.

Aquatic photolysis

Aquatic photolysis of S-2188 was investigated in sterile buffer solutions at pH 7.0 using [pyrazolyl-^{14}C] and [phenyl-^{14}C] labelled parent. The test systems were continuously irradiated with a xenon arc lamp (> 290 nm) for 30 at 25 °C to simulate the impact of natural light. Under irradiation in sterile buffer solutions at pH 7.0, S-2188 degraded with a half-life of 1.7 days (following SFO kinetics). The experimental half-life corresponds to 1.7 days under environmental summer sunlight in UK/US. Appropriate controls confirmed that there was no degradation in darkness. The main photolytic products were S-2188-DC (maximum occurrence of 63.8 % of AR at DAT 7) and MCNI (maximum occurrence of 17.7 of AR at DAT 30). A mean SFO DT_{50} value of 12.5 days was calculated for S-2188-DC. MCNI reached its maximum at the end of the study and therefore no degradation rate could be calculated. Extensive breakdown of the molecule

structure was observed after 30 days incubation as evidenced by the large number of minor unknown peaks and undifferentiated/unresolved radioactivity. The quantum yield for S-2188 was determined to be 0.021.

Biological degradation

Results of a **readily biodegradability study** indicate that S-2188 is not readily biodegradable.
Dark aerobic **water/sediment studies** were conducted with two contrasting (pH, texture) natural systems, Calwich Abbey and Swiss Lake, using [pyrazolyl-^{14}C] and [phenyl-^{14}C] labelled S-2188. The Calwich Abbey test system represents a silty clay loam or clay loam sediment with an organic carbon content of 4.6 %, a microbial biomass of 1052 µg C g^{-1} and a pH of 7.3 (KCl). The Swiss Lake test system is characterized by a sand sediment with a pH of 6.0 (KCl), with an organic carbon content of 0.6 % and a lower microbial biomass (157 µg C g^{-1}). In both systems, water and sediment stayed aerobically throughout the test period.
Mineralisation of S-2188 using [phenyl-^{14}C] label accounted by study termination for maximum 5.5 % of AR in the Calwich Abbey system and 3.1 % of AR in the Swiss lake system. Formation of NER increased up to maximum 47 % of AR with [pyrazolyl-^{14}C] label and to 47.4 % of AR with the [phenyl-^{14}C] label in the Calwich Abbey system, and to 19.5 % of AR [pyrazolyl-^{14}C] label and to 17.2 % of AR with the [phenyl-^{14}C] label in the Swiss lake system.
In the total system, decline of S-2188 was observed with $DegT_{50}$ of 19 and 66 days in the Calwich Abbey respectively the Swiss Lake test system (following SFO kinetics), respective $DegT_{90}$ values were 63 and 220 days. Geomean was calculated to 35.5 days. Dissipation in the water phase was calculated to be 24 and 30 in the Calwich Abbey and Swiss Lake test systems respectively. No distinct differences between labels used were observed.
Major metabolites S-2188-DC and S-2188-OH both exceeded 10% in the water phase and the overall system (maximum in whole system 20.5 % of AR and 15.9 % of AR, respectively). In addition, S-2188-DC exceeded 10% in the sediment phase alone. No degradation rates were calculated for the major metabolites S-2188-DC and S-2188-OH.

Table 77: Summary on DT50 and DT90 [days] for the dissipation and degradation of S-2188, S-2188-DC and S-2188-OH in aerobic water/sediment studies.

Compound	Test system	Water				Sediment		Total system	
		Degradation		Dissipation		Dissipation		Degradation	
		$DegT_{50}$	$DegT_{90}$	DT_{50}	DT_{90}	$DegT_{50}$	$DegT_{90}$	$DegT_{50}$	$DegT_{90}$
S-2188	Calwich Abbey	nc	nc	23.9	79.3	15.7	52.1	19.0	63.2
	Swiss Lake	nc	nc	27.2	90.3	nc	nc	66.2	220.1
	Geometric mean	nc	nc	25.5	84.7	nc	nc	35.5	117.9
S-2188-DC	Calwich Abbey	nc	nc	nc	nc	nc	nc	nc	nc
	Swiss Lake	nc	nc	nc	nc	nc	nc	nc	nc
	Geometric mean	nc	nc	nc	nc	nc	nc	nc	nc
S-2188-OH	Calwich Abbey	nc	nc	nc	nc	nc	nc	nc	nc
	Swiss Lake	nc	nc	nc	nc	nc	nc	nc	nc
	Geometric mean	nc	nc	nc	nc	nc	nc	nc	nc

nc denotes not calculated

Table 62: Summary on maximum occurrence [% of AR] of S-2188 and metabolites in aerobic water/sediment studies (mean of labels used, data stated in brackets give day of maximum occurrence).

Compound	Water	Sediment	Total
S-2188	-	31.0 (14)	-
S-2188-DC	11.1 (7)	8.3 (14)	15.8 (7)
S-2188-OH	10.8 (30)	4.0 (100)	32.5 (61)

5.2 Environmental distribution

Route of degradation in soil

The route of degradation of the active substance fenpyrazamine (S-2188) was established on 4 EU soils in one study S-2188. The aerobic laboratory soil degradation study was conducted under flow-through conditions (moistured air at a flow rate of ca 20-60 mL min^{-1}). Under standard **aerobic conditions** (20 °C), S-2188 ([phenyl-^{14}C] and [pyrazolyl-^{14}C] labels) degraded to CO_2 and bound residues. No volatile organics could be detected. The CO_2 concentrations were in the range of 5.2 to 8.5 % of AR at the end of the study (120 DAT). The NER concentrations increased steadily during the study period to reach at the end of the study values between 38.9 and 69.9 % of AR. Two metabolites were identified, S-2188-DC and S-2188-OH, but were always < 5 % of AR in the four soils. No **anaerobic degradation** study was presented as the substance is to be applied to grapes during the summer months (in North and South Europe) and to fruiting vebgetables under glasshouse conditions. S-2188 is considered to be stable to **photolysis** on soil.

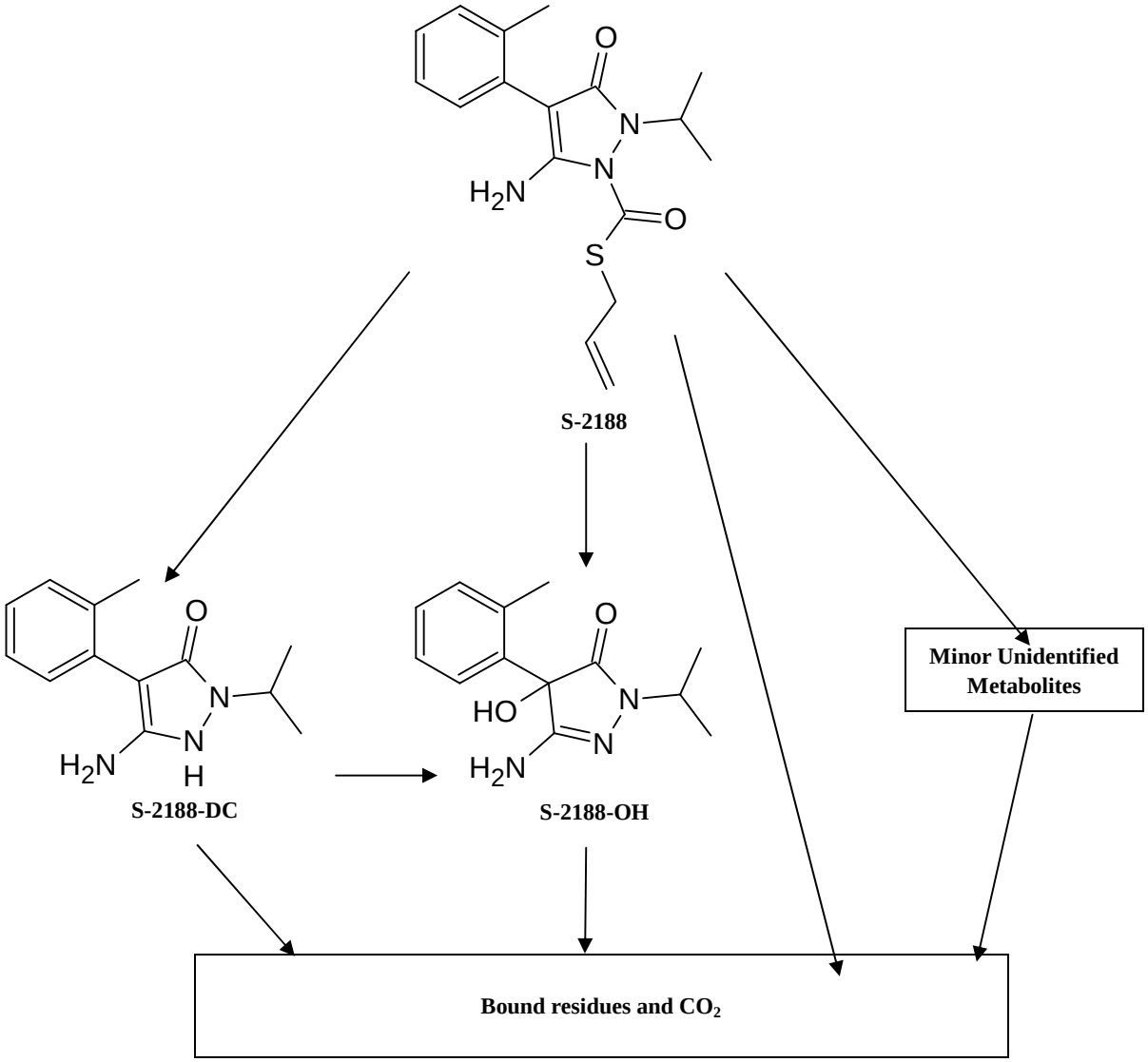


Figure 4: Proposed metabolic pathway for fenpyrazamine (S-2188) in soil

Rate of degradation in laboratory studies

The laboratory soil degradation rate of S-2188 was investigated in 4 EU soils with the following range of soil properties (pH, organic C, clay content) using [phenyl-^{14}C] and [pyrazolyl-^{14}C] labelled S-2188 as parent.

- pH ($CaCl_2$) 4.3 – 6.8
- organic C 1.7 – 4.2 %
- clay content 10 – 26 %

Under **aerobic conditions**, S-2188 was found to degrade following biphasic degradation in two soils and SFO kinetic in two other soils. Following best fit kinetics in soils PT102 and SK920191 (i.e. single first order, SFO) non-normalised DT_{50} values were between 23.6 and 40.0 days (pyrazolyl-label) and 33.5 days for the phenyl label. In soils PT103 and SK15556090 following the best fit kinetics (i.e. double first order in parallel kinetic, DFOP), non-normalized degradation half-life ($DegT_{50}$) values of S-2188 were 39.0 and 28.7 days respectively (chi^2 error ≤ 3.0 %); respective $DegT_{90}$ values were 1846.8 and 515.5 days. Since most environmental models are not capable to handle DFOP kinetics, a conservative SFO-$DegT_{50}$ for modelling may be derived from

the DFOP-slow rate k values. This procedure results in non-normalised $DegT_{50recalc}$ values of 888.0 and 230.1 days respectively. These values are extrapolated past the incubation time.
Two aerobic soil metabolites were identified (S-2188-DC and S-2188-OH) but did not reach values above 5 % of applied radioactivity.
No rate of degradation for **photolysis** on soil could be calculated since S-2188 is stable to photolysis.

Field dissipation studies

One field dissipation study (bare soils) was conducted with S-2188 on four European soils. The substance was applied at 1200 g a.s. ha^{-1} (single application) as a 50 % WG formulation.
Dissipation of S-2188 in field trials was following multi-compartment kinetics and dissipation half-lifes (DT_{50}) ranged between 7.7 and 39.8 days (pseudo-DT_{50} from FOMC kinetics or slow phase DT_{50} from DFOP kinetics). Since almost no transfer of S-2188 into soil layers below 10 cm was observed and volatilization is considered to be minimal (vapour pressure < 10^{-5} Pa), dissipation of S-2188 is considered more or less consistent with degradation.
No metabolites were observed in the field trials.

Table 78: Summary of the results of the kinetic determinations for S-2188 at the field dissipation sites following normalisation of data to 20°C and pF2.

	UK site (Site 0333/266/1)	**German site (Site 0333/266/2)**	**Italian site (Site 0333/266/3)**	**French site (Site 0333/266/4)**
Model	SFO	SFO	SFO	SFO
χ^2 error [%]	24.2	29.1	40.2	28.2
k [day^{-1}] *	0.156	0.5082	0.0985	0.0547
	(0.0019)	(0.0035)	(0.0129)	(0.0046)
DT50 [day]	4.4	1.4	7.0	12.7
DT90 [day]	14.8	4.5	23.4	42.1
Model	FOMC	FOMC	FOMC	FOMC
χ2 error [%]	11.4	10.0	20.0	19.9
α*	0.6249	0.5526	0.4855	0.7555
	(0.0011)	($6.7x\ 10^{-5}$)	(0.0045)	(0.0093)
β*	1.7278	0.4030	0.9022	4.197
	(0.0344)	(0.0117)	(0.1378)	(0.0959)
DT_{50} [day]	3.5	1.0	2.9	6.3
DT_{90} [day]	67.1	25.6	102.6	84.2
Model	DFOP	DFOP	DFOP	DFOP
χ^2 error [%]	Not required	Not required	8.1	10.0
k fast*	-	-	1.7297	3.0350
	-	-	(0.4330)	(0.4990)
k slow*	-	-	0.0174 (2.2×10^{-4})	0.0243 (5.8×10^{-4})
g*	-	-	0.5800 (1.2×10^{-6})	0.4339 (1.2×10^{-4})
DT_{50} [day]	-	-	1.1	5.1
DT_{90} [day]	-	-	82.4	71.3

*P value from the t-test is given in brackets. Selected kinetic fit for each soil highlighted in light grey.

5.2.1 Adsorption/Desorption

Reliable adsorption constants according to Freundlich isotherms (equilibrium batch experiments) could be achieved for **S-2188** using 5 soils from the EU and Japan with a representative set of soil properties:

- Soil pH ($CaCl_2$): 4.2 – 7.5
- Organic carbon: 0.8 – 4.8 %
- Clay: 9 – 28 %

No dependency of the adsorption behaviour onto the soil pH could be stated in the pH range tested. Freundlich adsorption constants (K_F) of S-2188 have been determined in batch equilibrium experiments with five different soils using the [phenyl-^{14}C] labelled test substance. Based on organic carbon content, K_{FOC} values for the different soils were in a range of 112 – 731 L kg^{-1} (arithmetic mean 1/n = 0.911). Based on these values, S-2188 is classified as medium mobile according to the classification scheme of McCall et al. (1981).

Table 79: Adsorption/desorption characteristics of [14C]S-2188 on five soils

Soil	Organic carbon [%]	pH ($CaCl_2$)	Adsorption			Desorption		
			K_F^{ads}	K_{Foc}	1/n	K_F^{des}	$K_{Foc-des}$	1/n
SK961089	4.8	7.5	9.36	195	0.8801	10.82	225	0.8592
SK104691	2.7	6.1	7.87	292	0.9055	9.11	338	0.8918
SK179618	3.8	5.5	4.27	112	0.9321	5.07	133	0.9293
SK566696	0.8	4.2	5.85	731	0.9525	7.63	954	0.9507
Saitama	3.2	5.3	6.99	218	0.8855	8.62	269	0.8950
Mean (n=5)				310	0.9111		384	0.9052

5.2.2 Summary of behaviour in soil

The route of degradation of S-2188 was studied in four soils (pH 4.3 - 7.4, %OC 1.7 - 4.2, sandy loam to clay loam) under aerobic conditions using compound radiolabelled ([phenyl-^{14}C] or [pyrazolyl-^{14}C] rings). Levels of CO_2 after 120 days incubation were similar for either radiolabelling position, being 5.2 - 8.5 % of AR. Levels of bound residues showed greater variation between soils types than between labelling positions and were 38.9-69.9%, at the completion of the 120 day incubation. Two metabolites, S-2188-OH and S-2188-DC were identified but did not reach levels above 5 % of AR in any soil. There was no evidence of any cleavage between the phenyl and pyrazolyl rings. A proposed degradation pathway is shown in Figure B.8.1.1-2. Soil photolysis studies showed greater degradation under non-irradiated conditions than irradiated conditions (and the same metabolites were present as in the aerobic soil degradation study), hence it was concluded that S-2188 is stable to photolysis. S-2188 will be applied to grapes in North and South Europe during the summer months, and to fruiting vegetables under glasshouse conditions. Therefore it is unlikely to be present in soil during waterlogged (anaerobic) conditions in winter and hence an anaerobic soil degradation study was not considered relevant.

The rate of degradation in four soils under laboratory conditions was studied. In two soils degradation was clearly seen to follow SFO kinetics and DT_{50} values of 23.6 - 39.9 days were obtained. In the other two soils, a more biphasic degradation was seen to follow DFOP kinetics and DT_{50} values of 28.8 – 39.2 days were obtained (although the chi^2 error was still acceptable for SFO kinetics). Following the FOCUS kinetics group guidance, the best fit kinetics was used as persistence endpoints to trigger further studies. Hence DT_{50} values were 23.6 - 39.9 days and DT_{90} values were 78.3 - >1000 days. Based on these values a field dissipation study is not triggered since DT_{50} is below 60 days. However, a study was undertaken at four sites (UK, Germany, Italy, Southern France) and the data were normalised following the time step normalisation. Best fit for the UK and German sites were obtained with FOMC kinetic, and for the Italian and French sites with DFOP kinetic. The range of pseudo-DT_{50} (FOMC kinetic) and DT_{50} values calculated from the slow rate k value (DFOP kinetic) ranged between 7.7 and 39.8 days.

For use as simulation input values, the FOCUS (2006) document recommends in the first instance using the slow phase from the DFOP kinetics as a first order value to be input into the models if the fit to SFO kinetics is not acceptable. However, this leads to unrealistic degradation rates in the PT103 and SK15556090 soils (equivalent SFO DT_{50} values would be 231-936 days based on the

slow rate from DFOP kinetics) since the DFOP fit is clearly strongly influenced by minor variation at the later timepoints. For simulation purposes the Notifier proposed to use SFO kinetics data for PT103 and SK15556090 soils. The values obtained (DT_{50} 67.9 and 56.4 days, respectively (DT_{90}s 225.7 and 187.5 days)) were consistent with the other two laboratory degradation studies and the field dissipation study. The use of SFO was not considered adequate by RMS since even if the χ^2 error values were below 15 % the visual fit was not satisfactory as supported by the biased residual plots. The consistency to the other two degradation studies and the field study was not considered as a valid argument. Therefore the field degradation studies were normalised and the obtained geometric mean DT_{50} value of 20.5 days was used for modelling purposes.
The sorption of S-2188 was studied in four European soils and one Japanese soil (pH range 4.2 to 7.5, % OC 0.8 – 4.8 loamy sand to clay loam). Adsorption K_{Foc} values were between 112 and 731 ml g^{-1} (mean 310 ml g^{-1}, mean 1/n = 0.911) and desorption $K_{Foc\text{-}des}$ values were between 133 and 954 ml g^{-1} (mean 384 ml/g, mean 1/n= 0.905) indicating that S-2188 is slightly to moderately mobile. There was no evidence of any pH dependence. No metabolites reached levels above 3 % of AR in soil and hence there was no need for further sorption or column leaching studies.

5.2.3 Volatilisation

S-2188 has a very low vapour pressure of below 10^{-5} Pa at 25 ºC (see Annex IIA point 2.3.1) and is predicted to degrade rapidly in air through reaction with hydroxyl radicals (DT_{50} of 1.221 hr assuming 1.5 x 10^6 hydroxyl radicals cm^{-3}, see Annex IIA point 2.10). Therefore it is considered that there is no risk of exposure to air and no further data are required.

5.2.4 Distribution modelling

No information available.

5.3 Aquatic Bioaccumulation

Table 80: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient n-octanol/water Japanese MAFF (12-Nousan-No. 8147, Part 2-9-11, 2000)	log P_{ow} = 3.52 at 25 ± 1 ºC pH: 7.2	Test substance: PGAI, Batch: R-4CM03G Purity: 99.3%	Lentz, N.R., 2005b (QNP-0002)

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

No estimations are available.

5.3.1.2 Measured bioaccumulation data

Reference:	**Bioconcentration of [^{14}C]S-2188 by Bluegill Sunfish (*Lepomis macrochirus*)**
Author(s), year:	Panthani, A.M., Herczog, K.J.S., 2007
Report/Doc. number:	Report No. QNM-0018, Study No. 019492
Guideline(s):	OECD Guideline 305, US EPA FIFRA 165-4, US EPA OPPTS 850.1730
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	[pyrazolyl-5-^{14}C] S-2188: radiochemical purity: 98.6 - 99.6 %, batch: CFQ14368 Reference substances: unlabelled S-2188: purity 99.3 %, batch: R-4CM03G S-2188-OH: purity: 98.3 %, batch: CTS05014 S-2188-DC: purity: 99.9 %, batch: CTS04019 S-2188-CH_2OH-DC: purity: 100 %, batch: CTS05018 MCNI: purity: 99.9 %, batch: CTS05012 MPPZ: purity: 100 %, batch: CTS04003
Test species:	Bluegill Sunfish (*Lepomis macrochirus*)
Number of organisms:	125 fish per test concentration and solvent control, 0.43 g fish/L/day (at initiation)
Weight, length:	2.1 (1.9 – 2.3) g, 56 (52 - 63) mm, n = 30
Type of test, duration:	Flow-through test, 28 d exposure period and 14 d depuration period
Applied concentrations:	
Nominal:	0 (solvent control), 0.005 and 0.05 mg a.s./L
Measured (mean):	- (solvent control), 0.00525 and 0.0479 mg a.s./L
Solvent:	Acetone (CAS No. 67-64-1)

Test conditions:

Water quality:	Well water, total hardness: 42 – 56 mg/L as $CaCO_3$
Temperature:	23 - 25 °C
pH:	6.8 – 7.6
O_2 content:	Exposure phase: 5.4 mg O_2/L (64 – 101% saturation) Depuration phase: 7.5 – 8.5 mg O_2/L (90 – 101% saturation)
Light regime:	16 hours light / 8 hours darkness
Feeding:	Pelleted food: 1 % of biomass daily
Test parameters:	Samples were taken at day 0, 1, 3, 7, 14, 21 and 28 (exposure phase) and 1, 3, 7 and 14 (depuration phase). Concentration of [^{14}C] S-2188 equivalents in fish tissues were determined by LSC-method. Five fish for tissue analysis were removed from each test concentration and control at each sampling time. Additionally a lipid analysis (by chloroform/methanol extraction) was carried out on fish sampled at day 1, 3, 7, 14, 21 and 28 (exposure phase) and at day 1, 3, 7 and 14 (depuration phase). For chemical analysis (LSC, HPLC/RAM) of S-2188 in test solutions samples were taken at -2 and -1 d (pre-exposure phase), 0, 1, 3, 7, 14, 21 and 28 d (exposure phase) and 1, 3, 7 and 14 d (depuration phase). Daily observations were made of the appearance and behaviour of the fish. Other parameters like temperature, pH and dissolved oxygen concentrations were measured daily in each vessel.
Calculations/statistics:	BCF was calculated as ratio of [^{14}C] S-2188 equivalents concentration in water and [^{14}C] S-2188 equivalents concentration in fish tissues and as ratio of K_d (depuration constant) and K_u (uptake constant), rate constant K was determined by SigmaPlot™.
Findings:	
Analytical data – water:	The mean measured concentrations of [^{14}C] S-2188 equivalents were 94.3% (low concentration) and 89.5 % (high concentration) of nominal. HPLC/RAM analysis confirmed that S-2188 was stable in both test concentrations. In the high test concentration (42 µg/L), 82.4 – 95.5 % was determined as active substance. In the low test concentration (4.5 µg/L), 89.7 – 99.9 % was determined as active substance.
Lipid content:	No differences between male and female fish were noted. The steady state average lipid content of day 14, 21 and 28 was 1.96% (w/w, low concentration) and 1.94% (w/w, high concentration), respectively.
Analytical data – fish tissues (LSC):	See Table
BCF:	See Table

Table 81: Uptake, bioconcentration and depuration of [^{14}C] residues in the bluegill sunfish

Day	Mean concentration of [^{14}C] residues [ppm] (% of TRR)					
	Edible		Non-edible		Whole fish	
	0.005 mg/L	0.05 mg/L	0.005 mg/L	0.05 mg/L	0.005 mg/L	0.05 mg/L
Exposure phase						
1	0.311 (98.9)	3.047 (98.8)	0.685 (96.3)	6.408 (97.3)	0.501	4.824
3	0.541 (96.9)	4.607 (98.4)	1.574 (93.8)	13.28 (96.5)	1.101	9.071
7	0.708 (97.8)	5.698 (98.1)	2.214 (94.0)	18.04 (96.1)	1.470	12.18
14	0.692 (97.3)	8.057 (98.0)	2.197 (94.4)	22.27 (96.0)	1.505	15.84
21	0.683 (96.8)	6.749 (97.5)	2.064 (94.1)	19.26 (95.5)	1.437	13.40
28	0.755 (96.3)	6.641 (97.1)	2.271 (93.6)	18.95 (94.0)	1.562	13.28

Day	Mean concentration of [^{14}C] residues [ppm] (% of TRR)					
	Edible		Non-edible		Whole fish	
	0.005 mg/L	0.05 mg/L	0.005 mg/L	0.05 mg/L	0.005 mg/L	0.05 mg/L
Depuration phase						
1	0.524 (94.4)	4.0838 (96.2)	1.990 (90.8)	14.18 (93.9)	1.315	9.456
3	0.171 (87.9)	1.514 (91.8)	0.637 (88.1)	6.214 (90.4)	0.446	4.042
7	0.043 (71.5)	0.277 (74.6)	0.207 (75.1)	1.237 (77.1)	0.164	0.961
14	0.013 (51.1)	0.117 (61.0)	0.056 (57.4)	0.522 (67.5)	0.060	0.467

n.d…not detectable

Table 82: Uptake, bioconcentration and depuration of [^{14}C] S-2188 in the bluegill sunfish

Day	Mean concentration of [^{14}C] S-2188 [ppm] (% of TRR)							
	Edible		Non-edible		Whole body			
	0.005 mg/L	0.05 mg/L	0.005 mg/L	0.05 mg/L	0.005 mg/L		0.05 mg/L	
					ppm	BCF	ppm	BCF
Exposure phase								
1	0.042 (13.5)	0.29 (9.4)	0.041 (5.7)	0.366 (5.6)	0.042 (8.3)	9.1	0.328 (6.8)	0.0076
3	0.024 (4.3)	0.3 (6.4)	0.042 (2.5)	0.571 (4.2)	0.033 (3.0)	7.2	0.431 (4.8)	9.6
7	0.03 (4.1)	0.33 (5.7)	0.063 (2.7)	0.629 (3.3)	0.045 (3.1)	9.4	0.477 (3.9)	10.4
14	0.031 (4.4)	0.466 (5.7)	0.052 (2.2)	0.354 (1.5)	0.041 (2.8)	8.2	0.409 (2.6)	8.5
21	0.029 (4.1)	0.281 (4.1)	0.048 (2.2)	0.452 (2.2)	0.038 (2.7)	7.8	0.365 (2.7)	7.9
28	0.045 (5.8)	0.252 (3.7)	0.041 (1.0)	0.401 (2.0)	0.043 (2.8)	9.6	0.324 (2.4)	7.9
Depuration phase								
1	0.009 (1.6)	0.052 (1.2)	0.006 (0.3)	0.055 (0.4)	0.007 (0.6)	-	0.053 (0.6)	-
3	0.001 (0.7)	0.02 (1.2)	n.d.	0.031 (0.4)	0.001 (0.2)	-	0.025 (0.6)	-
7	0.001 (1.8)	0.006 (1.7)	0.001 (0.4)	0.011 (0.7)	0.001 (0.7)	-	0.008 (0.9)	-
14	0.001 (2.8)	0.006 (3.0)	0.001 (0.6)	0.008 (1.1)	0.001 (1.1)	-	0.007 (1.5)	-

n.d…not detectable

Table 83: Distribution of ^{14}C residues in whole fish samples

Concentration of ^{14}C residues [% TRR] in whole body										
	0.005 mg/L					0.05 mg/L				
Day	1	7	14	21	28	1	7	14	21	28
Exposure phase										
Extractable	97.1	95.1	95.1	94.8	94.3	97.8	96.6	96.5	96.0	94.8
Unextractable	2.9	4.9	4.9	5.2	5.7	2.2	3.4	3.5	4.0	5.2
S-2188	8.3	3.1	2.8	2.7	2.8	6.8	3.8	2.6	2.7	2.4
S-2188-OH	4.4	2.8	4.0	3.7	4.6	2.9	4.9	5.5	5.0	4.1
S-2188-DC	16.1	8.5	9.8	13.1	9.1	18.8	9.4	10.5	8.0	11.5
S-2188-DC conjugate	16.1	33.3	29.8	32.7	28.6	19.4	31.6	28.8	32.7	27.3
65 – 72 min [a]	27.8	14.8	15.9	9.1	12.4	22.3	15.4	14.2	16.0	18.6
Minor unknowns [b]	3.5	12.5	10.9	13.3	14.2	5.4	10.7	15.1	13.0	12.0
Others	20.8	19.0	19.6	16.8	19.8	22.0	19.5	15.9	16.6	17.0
Depuration phase										
Extractable	91.6	74.4	56.0	-	-	94.4	76.6	66.1	-	-
Unextractable	8.4	25.6	44.0	-	-	5.6	23.4	33.9	-	-
S-2188	0.6	0.7	1.1	-	-	0.6	0.9	1.5	-	-
S-2188-OH	0.9	n.d.	n.d.	-	-	2.8	n.d.	n.d.	-	-
S-2188-DC	7.4	n.d.	n.d.	-	-	7.6	2.1	n.d.	-	-
S-2188-DC conjugate	26.5	22.3	16.9	-	-	37.0	30.5	16.0	-	-

Concentration of ^{14}C residues [% TRR] in whole body										
	0.005 mg/L					0.05 mg/L				
Day	1	7	14	21	28	1	7	14	21	28
65 – 72 min [a]	13.5	7.9	10.4	-	-	16.2	9.8	12.3	-	-
Minor unknowns [b]	23.4	7.1	1.8	-	-	12.8	7.4	5.7	-	-
Others	16.7	12.6	2.2	-	-	14.3	11.1	0.6	-	-

n.d…not detectable
[a] A broad region of radioactivity containing multiple minor components. The retention times are characteristics of lipids and fatty acid esters.
[b] Minor unknowns consist of several components each less than 4% of the TRR, except for one component eluting at 19.4 minutes present in the depuration day 1 fish which consisted of 15.3% TRR (336 µg/kg) in the non-edible fraction and 11.9% TRR (156 µg/kg) in the whole fish.

Table 84: Summary of bioconcentration factors

	Bioconcentration factors (BCFs)					
	TRR			S-2188		
BCF	Edible	Non-edible	Whole body	Edible	Non-edible	Whole body
	0.005 mg/L (low concentration)					
Steady-state BCF	139	437	283	7	10	9
Uptake rate constant (Ku)	62.915	156.528	106.497	22.6	18.452	22.212
Depuration rate constant (Kd)	0.447	0.345	0.367	3.222	1.799	2.614
Kinetic BCF (BCFK) a	141	453	290	7	10	8
Lipid BCF b	102	168	144	5	4	4
	0.05 mg/L (high concentration)					
Steady-state BCF	149	431	289	7	9	8
Uptake rate constant (Ku)	62.663	149.356	104.849	13.658	17.92	15.638
Depuration rate constant (Kd)	0.439	0.347	0.369	1.86	1.687	1.755
Kinetic BCF (BCFK) a	143	430	284	7	11	9
Lipid BCF b	113	166	149	6	3	4

[a] Ratio Ku/Kd
[b] Steady-state BCF/average of steady-state lipid content [% tissue wet weight]

Conclusion: S-2188 was stable under the test conditions and reached the steady-state plateau at day 28 of exposure.
The active substance S-2188 accumulated in whole fish with steady-state BCF values in whole fish tissues of 8 and 9. In non-edible portions the BCF of 9 and 10 were determined. BCF values for the total ^{14}C residues (TRR) were determined to be 283 and 289 for whole fish, and 437 and 431 for non-edible portions.
The modelled uptake rate constants (K_u) for S-2188 in whole fish tissues ranged from 15.6 to 22.2 per day, depuration constants (K_d) for S-2188 in whole fish tissues ranged from 1.76 to 2.61 per day. Greater than 95% of the ^{14}C residues were eliminated during the depuration phase (within 14 d). The depuration half-life (CT_{50}) was < 1 day.
S-2188 was extensively metabolized in fish. The major residues were a glucuronic acid conjugate of the metabolite S-2188-DC and the metabolite S-2188-DC itself. The concentrations of S-2188-DC conjugate and S-2188-DC were determined to be between 16.1 to 33.3 % TRR and 8.0 to 18.8 % TRR in whole fish during the exposure phase, respectively.

5.3.2 Summary and discussion of aquatic bioaccumulation

Fenpyrazamine has a log P_{OW} of 3.52 and therefore a fish bioconcentration study is triggered. Based on the fish bioaccumulation study (Panthani, A.M., Herczog, K.J.S., 2007) with *L.*

macrochirus a BCF (whole fish) of 9 was determined, which indicate a low potential to bioaccumulate in the aquatic food chain.
In DAR BCF was determined only for viscera and fillet, but was not corrected by lipid content.

The active substance was extensively metabolized in fish and the residues were eliminated quickly (CT_{50} < 1 d).

The major residues were a glucuronic acid conjugate of the metabolite S-2188-DC and the metabolite S-2188-DC itself. The concentrations of S-2188-DC conjugate and S-2188-DC were determined to be between 16.1 to 33.3 % TRR and 8.0 to 18.8 % TRR in whole fish during the exposure phase, respectively.

The bioaccumulation potential of all major metabolites in water and sediment (S-2188-OH, S-2188-DC and MCNI) is also assumed to be low, due to log P_{OW} values clearly lower than 3. Thus, it can be concluded that the risk of bioaccumulation of the major metabolites in the aquatic ecosystem is acceptable.

5.4 Aquatic toxicity

Standard toxicity studies on fish, aquatic invertebrates and algae with Fenpyrazamine were performed. Fenpyrazamine is toxic (LC_{50}/EC_{50} is ≥ 1 mg and < 10 mg/L) to the used standard fresh water test species. The most sensitive species is the algae *Pseudokirchneriella subcapitata* with an E_bC_{50} of 0.42 mg a.s./L.

Table 85: Summary of relevant information on aquatic toxicity

Method	Test organism	Test condition	Exp. time	Test conc.	Results			Reference
					Endpoint	NOEC [mg a.s./L]	EC_{50}/LC_{50} [mg a.s./L]	
OECD 203, OPPTS 850.1075, EU Directive 92/69/EEC C.1	*Oncorhynchus mykiss* Rainbow trout	flow-through	96 hr	mm	Mortality	1.1	5.2	Cafarella, M.A., 2006a Report No.: QNW-0002 Study No. 13048.6504
OECD 203, OPPTS 850.1075, EU Directive 92/69/EEC C.1	*Lepomis macrochirus* Bluegill sunfish	flow-through	96 hr	mm	Mortality	3.4	5.4	Cafarella, M.A., 2006b Report No.: QNW-0006 Study No. 13048.6505
OECD 210, OPPTS 850.1400	*Oncorhynchus mykiss* Rainbow trout	flow-through	90 d	mm	Fry survival Growth	0.37	> 0.75	Cafarella, M.A., 2006c Report No.: QNW-0011 Study No. 13048.6506
OECD 202, OPPTS 850.1010, JMAFF No.12-Nousan-8147, Daphnia Acute Immobilisation Test (2-7-2-1), EU Directive 92/69/EEC C.2	*Daphnia magna* Water flea	flow-through	48 hr	mm	Immobility	< 0.61	5.5	Putt, A.E., 2006a Report No.: QNW-0007 Study No. 13048.6507
OECD 211, FIFRA 72-4, OPPTS 850.1300	*Daphnia magna* Water flea	flow-through	21 d	mm	Mortality adults Reproduction Growth	1.4 0.34 0.34	> 1.4 1.1 n.d.	Putt, A.E., 2006b Report No.: QNW-0012 Study No. 13048.6508
OECD 201, JMAFF No.12-Nousan-8147, Alga Growth Inhibition Test (2-7-7), OPPTS 850.5400, EU Directive 92/69/EEC C.3	*Pseudokirchneriella subcapitata* Freshwater green alga	static	96 hr	mm	Biomass Growth rate Cell density	0.22 0.22 0.053	0.42 > 0.9 0.19	Hoberg, J.R., 2006a Report No.: QNW-0004 Study No. 13048.6509

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Reference:	**S-2188 Technical Grade – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions**
Author(s), year:	Cafarella, M. A., 2006a
Report/Doc. number:	Report No. QNW-0002, Study No. 13048.6504
Guideline(s):	OECD Guideline 203, US EPA OPPTS 850.1075, EC Guideline Annex V - Method C.1
GLP:	Yes
Deviations:	None relevant
Validity:	Acceptable
Material and methods:	
Test substance:	Fenpyrazamine (S-2188) technical grade, purity: 94.7%, batch: 030-050914-1G
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms:	10 fish per replicate, 2 replicates per treatment, control and solvent control
Weight, length:	1.7 g (range 0.98 – 2.4 g) and 5.7 cm (range 4.7 – 6.8 cm), n = 30
Loading:	0.18 g fish/L solution
Type of test, duration:	Flow-through test, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.5, 1.0, 2.0, 4.0 and 8.0 mg a.s./L
Measured (mean):	- (control and solvent control), 0.51,1.1, 2.1, 3.8 and 7.8 mg a.s./L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2), 0.1 mL DMF/L
Test conditions:	
Water quality:	Well water, total hardness: 50 mg/L as $CaCO_3$
Temperature:	12 – 13 °C
pH:	7.0 (0 h, new solution), 7.2 (96 h, aged solution)
O_2 content:	8.5 – 10.8 mg O_2/L (80 – 103% saturation)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Mortality and sublethal effects were assessed after 0, 3, 6, 24, 48, 72 and 96 hours. For chemical analysis (LC/MS/MS) of S-2188 in test solutions samples were taken at test initiation (0 h) and test termination (96 h) from all treatment groups and the control. Measurement of pH, temperature and dissolved oxygen concentrations were made at initiation and once daily in both vessels of each treatment.
Statistics:	LC_{50}: Binominal probability, NOEC: Directly from raw data
Findings:	
Analytical data:	Over the whole test period the mean measured concentrations were in the range from 94 to 106% of nominal.
Behavioural effects:	Controls and concentration levels up to 1.1 mg a.s./L: No sublethal effects

were reported over the whole test period. At test concentration 2.1 mg a.s./L following symptoms were noted after 48 hours: Lethargic behaviour remained at water surface. At test concentration 3.8 mg a.s./L following symptoms were noted after 3 hours: Lethargic behaviour, loss of equilibrium, remained at water surface. At test concentration 7.8 mg a.s./L following symptoms were noted after 3 hours: Complete loss of equilibrium.
Thus the NOEC was 1.1 mg a.s./L based on sublethal effects.

Mortality: See Table

Table 86: Effects on rainbow trout (O. mykiss) exposed to technical fenpyrazamine

Fenpyrazamine [mg a.s./L] (mean measured)	**Cumulative mean mortality [%]**					
	3 hours	**6 hours**	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Control	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0
0.51	0	0	0	0	0	0
1.1	0	0	0	0	5	5
2.1	0	0	0	0 [ae]	0	0 [ae]
3.8	0 [ace]	0 [abc]	0 [abcd]	0 [abce]	0 [abc]	5 [abc]
7.8	0 [c]	100	100	100	100	100
NOEC = 1.1 mg a.s./L						
LC_{50} (96 h) = 5.2 mg a.s./L (95 % C.I. 3.8 – 7.8 mg a.s./L)						

[a] lethargic behaviour
[b] partial loss of equilibrium
[c] complete loss of equilibrium
[d] dark in colour
[e] fish on surface of test solution

Conclusion: 96 h LC_{50} = 5.2 mg a.s./L
96 h NOEC = 1.1 mg a.s./L
based on mean measured concentrations

Comment RMS: The observed mortality of 5% at the test concentration of 1.1 mg a.s./L was not considered for the NOEC determination because the effect did not establish a dose-response relationship. Hence, the NOEC was determined to be 1.1 mg a.s./ha, based on behavioural effects (lethargy and loss of equilibrium) on fish at application rates of 2.1 mg a.s./L and higher.

Reference:	**S-2188 Technical Grade – Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-through Conditions**
Author(s), year:	Cafarella, M. A., 2006b
Report/Doc. number:	Report No. QNW-0006, Study No. 13048.6505
Guideline(s):	OECD Guideline 203, US EPA OPPTS 850.1075, EC Guideline Annex V - Method C.1
GLP:	Yes
Deviations:	None relevant
Validity:	Acceptable

Material and

methods:

Test substance:	Fenpyrazamine (S-2188) technical grade, purity: 94.7%, batch: 030-050914-1G
Test species:	Bluegill Sunfish (*Lepomis macrochirus*)
Number of organisms:	10 fish per replicate, 2 replicates per treatment, control and solvent control
Weight, length:	1.7 g (range 1.1 – 2.9 g) and 4.7 mm (range 4.0 – 5.6 mm), n = 30
Loading:	0.17 g fish/L solution
Type of test, duration:	Flow-through test, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 1.0, 2.0, 4.0, 8.0 and 16.0 mg a.s./L
Measured (mean):	- (control and solvent control), 0.88, 1.8, 3.4, 6.9 and 12.0 mg a.s./L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2), 0.1 mL DMF/L
Test conditions:	
Water quality:	Well water, total hardness: 48 mg/L as $CaCO_3$
Temperature:	22 ± 1 °C
pH:	7.2 ± 0.1 (0 h, new solution), 7.3 ± 0.1 (96 h, aged solution)
O_2 content:	7.6 – 9.5 mg O_2/L (94 – 108% saturation)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Mortality and sublethal effects were assessed after 0, 3, 6, 24, 48, 72 and 96 hours. For chemical analysis (LC/MS/MS) of S-2188 in test solutions samples were taken at test initiation (0 h) and test termination (96 h) from all treatment groups and the control. Measurement of pH, temperature and dissolved oxygen concentrations were made at initiation and once daily in both vessels of each treatment.
Statistics:	LC_{50}: Binominal probability, NOEC: Directly from raw data
Findings:	
Analytical data:	Over the whole test period the mean measured concentrations were in the range from 77 to 91% of nominal concentrations.
Behavioural effects:	Controls and concentration levels up to 3.4 mg a.s./L: No sublethal effects were reported over the whole test period. At test concentration 6.9 mg a.s./L following symptoms were noted after 3 hours: Partial and complete loss of equilibrium. At test concentration 12.0 mg a.s./L following symptoms were noted after 3 hours: Complete loss of equilibrium. Thus the NOEC was 3.4 mg a.s./L based on sublethal effects.
Mortality:	See Table

Table 87: Effects on bluegill sunfish (L. macrochirus) exposed to technical fenpyrazamine

Fenpyrazamine [mg a.s./L] (mean measured)	**Cumulative mortality [%]**					
	3 hours	**6 hours**	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Control	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0
0.88	0	0	0	0	0	0
1.8	0	0	0	0	0	0
3.4	0	0	0	0	0	0

Fenpyrazamine [mg a.s./L] (mean measured)	Cumulative mortality [%]					
	3 hours	6 hours	24 hours	48 hours	72 hours	96 hours
6.9	0 [ab]	0 [bc]	25 [c]	30 [c]	70 [c]	85 [c]
12.0	0 [c]	100	100	100	100	100
NOEC = 3.4 mg a.s./L						
LC_{50} (96 h) = 5.4 mg a.s./L (95 % C.I. 3.4 – 6.9 mg a.s./L)						

[a] lethargic behaviour
[b] partial loss of equilibrium
[c] complete loss of equilibrium

Conclusion: 96 h LC_{50} = 5.4 mg a.s./L
96 h NOEC = 3.4 mg a.s./L
based on mean measured concentration

5.4.1.2 Long-term toxicity to fish

Chronic toxicity to fish (IIA 8.2.2)

Prolonged toxicity (21 day exposure) to fish (IIA 8.2.2.1)

No study submitted. The requirement for data on the chronic effects of fenpyrazamine on juvenile fish has been addressed by the submission of an early life stage toxicity test (ELS-test) with rainbow trout (*O. mykiss*).

Fish early life stage toxicity test (IIA 8.2.2.2)

Reference:	**S-2188 Technical Grade – Early Life Stage Toxicity Test with Rainbow Trout (*Oncorhynchus mykiss*)**
Author(s), year:	Cafarella, M. A., 2006c
Report/Doc. number:	Report No. QNW-0011, Study No. 13048.6506
Guideline(s):	OECD Guideline 210, US EPA OPPTS 850.1400
GLP:	Yes
Deviations:	None relevant
Validity:	Acceptable
Material and methods:	
Test substance:	Fenpyrazamine (S-2188) technical grade, purity: 94.7%, batch: 030-050914-1G
Test species:	Rainbow Trout (*Oncorhynchus mykiss*)
Number of organisms:	2 replicates per test concentration, control and solvent control. 50 eggs per egg incubation cup, after completion of hatch larvae were thinned to 20 individuals per aquarium. At the highest nominal treatment level, an additional of 10 eggs were selected from each incubation cup and suspended in each exposure aquarium. For the recovery/reversibility test group 20 (high dose recovery group – HDR) larvae per aquarium from the highest test concentration were transferred to clean dilution water containing no S-2188.
Age:	Freshly fertilized eggs, 2.5 hours old
Type of test, duration:	Flow-through test, 90 days (60 days post hatch)

Applied concentrations:	
Nominal:	0 (control and solvent control), 50, 100, 200, 400 and 800 µg/L
Measured (mean):	- (control and solvent control), 50, 100, 190, 370 and 750 µg/L
Solvent	Dimethylformamide (DMF, CAS No. 68-12-2), 0.01 mL/L
Test conditions:	
Water quality:	Well water, total hardness: 32 – 58 mg/L as $CaCO_3$
Temperature:	11 – 13 °C
pH:	6.9 – 7.8 during the total test period
O_2 content:	8.2 – 11.1 mg O_2/L (76 – 103 % saturation)
Light regime:	Continuous darkness during incubation phase and prior to the larval development to the swim-up stage. From swim-up phase onwards: 16 hours light / 8 hours darkness, sudden transitions were avoided
Feeding	Larvae were fed of live brine shrimp nauplii (*Artemia salina*) 3 times daily beginning on day 11 post-hatch. Larvae were not fed during the final 48 hours of the test. Residual food and fecal matter were brushed and siphoned when necessary in order to minimise microbiological growth.
Test parameters:	Abnormal appearance and behaviour of larvae were assessed daily. Number of surviving larvae was estimated at least twice a week. At test termination the length and the weight were determined. Determined endpoints were: Hatching success, overall fry survival (fry survival before and after thinning), mean length, wet and dry weight. Temperature, pH and dissolved oxygen concentration were measured daily. Total hardness, alkalinity and specific conductance were measured weekly. Analytical measurements (LC/MS/MS) of S-2188 in test solutions samples were taken at 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 73, 77, 84 and 90 days.
Statistics:	If control and solvent control can be pooled: t-Test Testing for normal distribution: Shapiro Wilks' Test Homogeneity of variance, data for embryo viability: Bartlett's Test Larval survival: Bonferroni's t-Test, Kruskal-Wallis Test and ANOVA Time to hatch, hatching success, mean length, mean dry and wet weight: Williams test with previous acrisine transformation
Findings:	
Analytical data:	Overall mean measured concentrations in test media were 93 – 100 % of nominal.
Biological observation:	Total length of larvae: No significant differences were observed in the mean total length of the larvae from the treatment groups (57.3 – 57.6 mm) and the pooled control groups (57.6 mm). Time to hatch: In control and all treatment levels hatching began on day 4 and continued until day 5. Morphological and behavioural effects: Over the total test period no morphological and behavioural effects were observed. High dose recovery group: During the first few weeks of clean water exposure sublethal effects like lethargy, darkened pigmentations and haemorrhage were observed in several larval fish. These were consistent with observation made at the highest treatment level tested. As the exposure in clean water continued these sublethal effects diminished and were no longer evident by test day 57. Based on the observations it can be assumed that larval fish exposed to concentrations of S-2188 at ≤ 0.75 mg

a.s./L during embryonic development will recover.

Effects: See Table

Table 88: Hatching success and fry survival

Fenpyrazamine [mg a.s./L] (mean measured)	**Mean embryo viability [%] [a] phd -11**	**Mean hatching survival [%] [b] phd 0**	**Mean larvae survival [%] [c] phd 60**
Control	62	99	98
Solvent control	67	100	98
Pooled control [d]	64	100	98
0.05	70	100	98
0.1	69	99	100
0.19	70	99	98
0.37	61	98	100
0.75	68	96 *	45 *
HDR	n.a.	n.a.	95
NOEC = 0.37 mg a.s./L			
LOEC = 0.75 mg a.s./L			
MATC = 0.53 mg a.s./L			

phd…post hatch day, HDR…high dose recovery group (0.75 mg a.s./L)
[a] Mean viability of embryos assessed on test day 19 (phd -11).
[b] Mean survival at complete hatch was observed on test day 30 (phd 0).
[c] Survival of larvae on test day 90 (phd 60).
[d] No statistically significant difference between dilution control and solvent control (t-Test)
* Significantly different compared to the pooled control (alpha = 0.05)

Table 89: Length and Weight

S-2188 [mg a.s/L] (mm)	**Mean length [mm] (SD) phd 60**	**Mean dry weight [mg] (SD) phd 60**
Control	57.7 (2.74)	0.337 (0.0637)
Solvent control	57.5 (4.76)	0.338 (0.102)
Pooled control [a]	57.6 (3.86)	0.337 (0.0842)
0.05	57.3 (4.24)	0.332 (0.0831)
0.1	57.6 (3.14)	0.335 (0.0714)
0.19	57.3 (2.93)	0.333 (0.0674)
0.37	57.3 (2.64)	0.335 (0.0568)
0.75	55.8 (3.33) [b]	0.336 (0.068) [b]
HDR	57.6 (3.35)	0.345 (0.0868)
NOEC = 0.37 mg a.s./L		
LOEC = 0.75 mg a.s./L		
EC_{50} > 0.75 mg a.s./L		

mm...mean measured, phd…post hatch day, HDR…high dose recovery group (0.75 mg a.s./L)
[a] No statistically significant difference between dilution control and solvent control.
[b] Excluded from further statistical analyses due to a significant effect on larval survival.

Conclusion: 90 d NOEC = 0.37 mg a.s./L (fry survival)
90 d LOEC = 0.75 mg a.s./L
90 d EC_{50} > 0.75 mg a.s./L
based on mean measured concentrations

Fish life cycle test (IIA 8.2.2.3)

No study was submitted. The BCF of fenpyrazamine is 9 and thus clearly less than 1000. In addition there was more than 95 % elimination of fenpyrazamine residues in fish in 14 days during the depuration phase of the fish bioaccumulation study.
The acute toxicity to fish was determined to be greater than 0.1 mg/L (LC_{50} = 5.2 mg a.s./L) and the persistence in water and sediment was observed to be below the trigger of DT_{90} > 100 d.
On this basis, a fish life-cycle test is not required and therefore was not conducted.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Reference:	**S-2188 Technical Grade – Acute Toxicity to Water Fleas, (*Daphnia magna*) Under Flow-Through Conditions**
Author(s), year:	Putt, A. E., 2006a
Report/Doc. number:	Report No. QNW-0007, Study No. 13048.6507
Guideline(s):	OECD Guideline 202, US EPA OPPTS 850.1010, JMAFF No.12-Nousan-8147, Daphnia Acute Immobilisation Test (2-7-2-1), EC Guideline Annex V - Method C.2
GLP:	Yes
Deviations:	- The temperature measured on test day 2 was between 19 and 22 °C instead of 20 ± 1 °C as stated in the protocol. The performance of the control organisms was satisfactory according to the given criteria. Therefore, the deviation was considered not to have an adverse effect on the results of the study.
Validity:	Acceptable

Material and methods:	
Test substance:	Fenpyrazamine (S-2188) technical grade, purity: 94.7%, batch: 030-050914-1G
Test species:	Water flea (*Daphnia magna*)
Number of organisms:	2 replicates each with 10 daphnids per treatment, control and solvent control
Age:	First instar, ≤ 24 hours old
Type of test, duration:	Flow-through test, 48 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.75, 1.5, 3.0, 6.0 and 12 mg a.s./L
Measured (mean):	- (control and solvent control), 0.61, 1.2, 2.2, 3.8 and 8.0 mg a.s./L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2), 0.1 mL DMF/L
Test conditions:	
Water quality:	Fortified well water, total hardness: 160 - 170 mg/L as $CaCO_3$
Temperature:	19 - 22 °C
pH:	8.3 (0 - 48 h)
O_2 content:	8.2 – 9.1 mg O_2/L (90 – 100 % saturation)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Immobility and sublethal effects were assessed after 0, 24 and 48 hours.

For chemical analysis (LC/MS/MS) of S-2188 in the test media samples were taken at test initiation (0 h) and termination (48 h). The water samples were analysed by automated injection into the LC/MS/MS instrument without centrifugation.
Measurements of pH, temperature and dissolved oxygen concentrations were made at initiation and once daily. Total hardness, total alkalinity and specific conductance were measured at test initiation.

Statistics: EC_{50}: Probit analysis, NOEC: Directly from the raw data

Findings:

Analytical data: The overall mean measured concentration ranged from 63 – 82 % of nominal concentrations.

Effects: After 48 hours no immobilisation was observed in the control, solvent control and in test concentrations up to 2.2 mg/L. At 3.8 and 8.0 mg/L the immobilisation was between 5 and 95 %. Sublethal effects (lethargy) were observed at all treatment groups.
Thus the NOEC was determined to be < 0.61 mg/L and the EC_{50} was 5.5 mg/L.

Table 90: Effects on daphnids (D. magna) exposed to technical fenpyrazamine

Fenpyrazamine [mg a.s./L] (mean measured)	**Mean cumulative immobilized organisms [%]**	
	24 hours	**48 hours**
Control	0	0
Solvent control	0	0
0.61	0	0
1.2	0	0
2.2	0	0
3.8	0	5
8.0	45	95
NOEC < 0.61 mg a.s./L		
EC_{50} (48 h) = 5.5 mg a.s./L (95 % C.I. 4.7 – 6.5 mg a.s./L)		

Conclusion: 48 h EC_{50} = 5.5 mg a.s./L
48 h NOEC < 0.61 mg a.s./L
based on mean measured concentrations

5.4.2.2 Long-term toxicity to aquatic invertebrates

Reference:	**S-2188 Technical Grade – Full Life-Cycle Toxicity Test with Water Fleas, *Daphnia magna* Under Flow-Through Conditions**
Author(s), year:	Putt, A.E., 2006b
Report/Doc. number:	Report No. QNW-0012, Study No. 13048.6508
Guideline(s):	OECD Guideline 211, FIFRA 72-4, OPPTS 850.1300
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and

methods:	
Test substance:	Fenpyrazamine (S-2188) technical grade, purity: 94.7 %, batch: 030-050914-1G
Test species:	Water flea (*Daphnia magna*)
Number of organisms:	4 replicates per treatment group and controls, each with 10 daphnids
Age:	First instar, < 24 hours old
Type of test, duration:	Flow-through test, 21 d
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.1, 0.2, 0.4, 0.8 and 1.6 mg a.s./L
Measured (mean):	- (control and solvent control), 0.085, 0.18, 0.34, 0.76 and 1.4 mg a.s./L
Solvent:	Acetone (CAS No. 67-64-1)
Test conditions:	
Water quality:	Fortified well water, hardness: 170 – 180 mg/L as $CaCO_3$
Temperature	19 – 21 °C
pH	7.9 – 8.3
O_2 content:	5.6 – 8.7 mg O_2/L (> 60 % saturation)
Light regime:	16 hours light / 8 hours darkness
Feeding	Daphnids were fed with *Ankistrodesmus falcatus* suspension (4 x 10^7 cells/mL), and 1 mL of a yeast, cereal leaves and digested flaked fish food suspension, three times daily.
Test parameters:	Parent mobility, mortality and abnormal behaviour were observed daily. Reproduction (mean time to first brood, age at first brood, offspring per surviving parental) were observed on day 7 and three times per week through day 21. At test termination body length and parental body mass (dry weight) were reported. For chemical analysis (LC/MS/MS) of S-2188 in test media duplicate samples were taken on days 0, 7, 14 and 21 from each test concentration. Measurements of pH, dissolved oxygen and temperature were made at initiation and once weekly in all vessels of each treatment and once daily on a rotating basis in a single representative vessel of each treatment.
Statistics:	In general data for parent mobility were acrisine transformed before further evaluation. Variance homogeneity and normal distribution were analysed by Bartletts test. If control groups can be pooled a t-Test was performed. All NOEC were derived by comparing each treatment group with pooled controls: Reproduction and mean dry weight: Williams's Test Parent mortality and mean total body length: Wilcoxon's Rank Sum Test and Bonferroni's Test Statistical analyses considering nominal concentrations are based on data of pooled controls. Statistical analyses considering mean measured concentrations are using only solvent control.
Findings:	
Analytical data:	The mean measured concentrations ranged from 85 – 95% of nominal concentrations.
Biological	First brood release by daphnids exposed to the control, 0.085, 0.18, 0.34,

observation: 0.76 and 1.4 mg a.s./L treatment levels occurred on test day 7, 7, 7, 7, 9 and 10.
Effects: See Table

Table 91: Summary of effects of long-term exposure of fenpyrazamine on *Daphnia magna*

Fenpyrazamine [mg a.s./L] (nom)	**S-2188 techn. [mg a.s./L] (mm)**	**Mean parent mortality at day 21 [%]**	**Offspring per surviving female at day 21**	**Mean dry weight of parent after 21 d [mg]**	**Mean body length of parent after 21 d [mm]**
Control	Control	10 ± 8	201 ± 14	1.60 ± 0.14	5.2 ± 0.05
Solvent control	Solvent control	7 ± 10	210 ± 29	1.74 ± 0.13	5.3 ± 0.08
Pooled control [a]	Pooled control [a]	9 ± 8	205 ± 22	1.67 ± 0.14	5.3 ± 0.07
0.1	0.085	5 ± 10	216 ± 22	1.57 ± 0.18	5.3 ± 0.03
0.2	0.18	10 ± 8	200 ± 16	0.67 ± 0.06	5.2 ± 0.04
0.4	0.34	2 ± 5	205 ± 13	1.66 ± 0.07	5.2 ± 0.02
0.8	0.76	0	156 ± 3*	1.49 ± 0.09*	4.8 ± 0.03**
1.6	1.4	7 ± 10	58 ± 24*	0.82 ± 0.22*	4.0 ± 0.31**
NOEC (based on mean measured)		1.4 mg a.s./L	0.34 mg a.s./L	0.34 mg a.s./L	0.34 mg a.s./L
LOEC (based on mean measured)		> 1.4 mg a.s./L	0.76 mg a.s./L	0.76 mg a.s./L	0.76 mg a.s./L
EC_{50} (based on mean measured)		> 1.4 mg a.s./L	1.1 mg a.s./L	n.d.	n.d.
MATC (based on mean measured)		0.51 mg a.s./L			

n.d…not determined, mm…mean measured, nom…nominal
[a] No statistically significant difference between control and solvent control.
* Significantly reduced compared to the pooled control, based on Williams' Test
** Significantly reduced compared to the pooled control, based on Wilcoxon's Rank Sum Test

Conclusion: NOEC = 1.4 mg a.s./L (adult mortality)
LOEC > 1.4 mg a.s./L
NOEC = 0.34 mg a.s./L (reproduction)
LOEC = 0.76 mg a.s./L
NOEC = 0.34 mg a.s./L (growth, weight and length)
LOEC = 0.76 mg a.s./L
based on mean measured concentrations

5.4.3 Algae and aquatic plants

Reference: **S-2188 Technical Grade – Acute Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata***
Author(s), year: Hoberg, J.R., 2006a
Report/Doc. number: Report No. QNW-0004, Study No. 13048.6509
Guideline(s): OECD Guideline 201, JMAFF No.12-Nousan-8147, Alga Growth Inhibition Test (2-7-7), US EPA OPPTS 850.5400, EC Guideline Annex V - Method C.3
GLP: Yes
Deviations: - During the definitive test, the initial control solution pH was 6.8. The 72 h control pH was 8.6 and exceeded the initial value by 1.8 units instead of acceptable 1.5 units as stated in the protocol. This increase in solution pH is due to photosynthesis by the algae and cannot be controlled. The 72 h mean control cell density (95.33 * 10^4 cells/mL) exceeds the required 16 times increase form the initial density (1.0 * 10^4 cells/mL). Therefore, the growth

	of the algal population was not affected by the increase solution pH.
Validity:	Acceptable
Material and methods:	
Test substance:	Fenpyrazamine (S-2188) technical grade, purity: 94.7%, batch: 030-050914-1G
Test species:	Green alga (*Pseudokirchneriella subcapitata)*
Number of organisms:	1 x 10^4 cells/mL; 4 replicates per treatment group, medium control and solvent control
Type of test, duration:	Static test, 96 hours
Applied concentrations:	
Nominal:	0 (medium control and solvent control), 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L
Measured (mean):	- (medium control and solvent control), 0.053, 0.11, 0.22, 0.43 and 0.90 mg a.s./L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2), 0.1 mL/L
Test conditions:	
Water quality:	Algal Assay Procedure (AAP) medium (according to OECD guideline), total hardness: 15 mg/L as $NaHCO_3$
Temperature:	22 – 24 °C
pH:	6.7 – 7.0 (0 h), 6.9 – 9.2 (96 h)
Incubation:	Continuous illumination at 3900 to 4700 lux
Test parameters:	Cell counts were estimated using a haemocytometer and microscope. Observations of the health and morphology of the algal cells were made under the microscope on each study day. For chemical analysis (LC/MS/MS method) of test the substance, samples of test solution were taken at test initiation, after 72 h and at test termination. Measurements of pH and conductivity were made at initiation, after 72 h and at termination, light intensity was measured ad daily intervals and temperature was monitored continuously.
Statistics:	Comparison of medium and solvent control: t-Test No significant differences, both sets of control data were pooled for all parameter. Determination of EC_{50}: TOXSTAT® software Normal distribution and homogeneity of variance: Shapiro Wilks' Test and Bartlett's Test Determination of NOEC: Williams' Test and Kruskal-Wallis' Test
Findings:	
Analytical data:	Mean measured concentrations were in the range of 83 – 90% of nominal concentrations over the whole test duration.
Morphological effects:	After 96 h of exposure cells were observed to be bloated in the highest test concentration.
Biomass, growth rate and cell density:	See Table

Table 92: Effects of technical fenpyrazamine on the green alga P. subcapitata

Fenpyrazamine [mg/L] (mean measured)	**Percent inhibition relative to the pooled control [%]**		
	Biomass (0 – 72 h)	**Growth rate (0 – 72)**	**Cell density (0 – 96 h)**
0 (pooled control) [a]	-	-	-
0.053	- 10	- 4	18
0.11	- 10	4	24 *
0.22	- 7	- 2	59 *
0.43	49*	20*	82 *
0.90	83*	39*	95 *
NOEC	0.22 mg a.s./L	0.22 mg a.s./L	0.053 mg a.s./L
EC_{50} (95 % C.l.)	0.42 mg a.s./L (0.40 – 0.46 mg a.s./L)	> 0.90 mg a.s./L	0.19 mg a.s./L (0.15 – 0.22 mg a.s./L)

[a] Results of statistical analyses of control groups indicated no significant differences, thus pooled control are used for statistical analysis.
* Significantly different compared to the pooled control, based on Williams' Test

.

Conclusion: 72 h E_bC_{50} = 0.42 mg a.s./L
72 h E_rC_{50} > 0.90 mg a.s./L
96 h EC_{50} = 0.19 mg a.s./L (cell density)
72 h NOEC = 0.22 mg a.s./L (biomass and growth rate)
96 h NOEC = 0.053 mg a.s./L (cell density)
based on mean measured concentrations

5.4.4 Other aquatic organisms (including sediment)

Not relevant

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

Endpoint	Classification Criteria (criteria in bold)		Evidence for fenpyrazamine
	CLP (2nd ATP)	**DSD**	
Degradation Fenpyrazamine	Hydrolotic degradataion of Fenpyrazamine pH 4: stable at 50°C pH 7: 32.5 d at 50 °C (a.s.) pH 9: 11 d at 25 °C Photodegradation of fenpyrazamine was fast with an experimental half-life of 1.6 days under the test conditions. Fenpyrazamine is not readily biodegradable, and does not meet the criterion for rapid degradation in a water/sediment study with a DT50 whole system of 35.5 days. Based on available data a non rapid degradation is proposed for fenpyrazamine.		The classification as **R53** according to Directive 67/548/EEC. is based on the fact that the active substance is **not considered as ready biodegradable/rapid degradable.**
Bioaccumulation Fenpyrazamine	**Log K_{ow} is < 4** Fenpyrazamine Log K_{ow} =3.52 at pH 7.2 and 25 °C	**Log K_{ow} is > 3** Fenpyrazamine Log K_{ow} = 3.52 at pH 7.2 and 25 °C	The measured log P_{OW} is 3.52 (at pH 7.2 and 25 °C) and is below the classification criteria of 4 (CLP) but above the classification criteria of 3 (DSD), therefore fenpyrazamine is considered to have **a moderate bioaccumulation potential.**
Acute aquatic toxicity Fenpyrazamine	**E_rC_{50} > 0.9 mg/L (algae)** **LC_{50} > 1 mg/L (fish, aquatic invertebrates)**		Fenpyrazamine is of moderate toxicity to green algae (E_rC_{50} > 0.9 mg/L) and of low toxicity to fish and aquatic invertebrates (LC_{50} > 1 mg/L) and fulfills the criteria for the proposed classification as **R51** according to Directive 67/548/EEC. The criteria for the proposed classification as **H400** according to Regulation EC 1272/2008 are not met.
Chronic aquatic toxicity Fenpyrazamine	**For not rapidly degradable substances: NOEC ≤ 1 mg/L**		Fenpyrazamine is of high chronic toxicity to algae with a $NOEC_{GROWTH\ RATE}$ = 0.22 mg/L. Therefore fenpyrazamine fulfills the criteria for the proposed classification as **H411** according to Regulation EC 1272/2008.
	P. subcapitata	NOEC = 0.22 mg/L	
SUMMARY	**H411**	**R51/53**	**PROPOSED CLASSIFICATION**

Conclusion of environmental classification according to Regulation EC 1272/2008

Pictogram: GHS 09

Aquatic Chronic 2

H411 'Toxic to aquatic life with long lasting effects'

Justification for the proposal

H411 follows from the toxicity of the active substance Fenpyrazamine to algae (*P. subcapitata*, NOEC = 0.22 mg/L, Hoberg, 2006a) and the fact that the active substance is not readily biodegradable (Burwood, C. & Scholey, A., 2006) and not rapidly biodegradable (Lewis, C.J. & Troth, K., 2007f). In the water-sediment study a DT_{50} of 35.5 days (geomean) was determined for the whole system. Also Fenpyrazamine does not meet the criterion of rapid degradation > 70 % within a 28-day period the aquatic environment.

Based on the fish bioaccumulation study (Panthani, A.M., Herczog, K.J.S., 2007) with *L. macrochirus* a BCF (whole fish) of 9 was determined, which indicate a low potential to bioaccumulate in the aquatic food chain. The substance Fenpyrazamine does not meet the CLP criteria (BCF ≥ 500) based on the measured fish BCF.

Fenpyrazamine fulfils the criteria for classification as aquatic environmental hazard based on the CLP Regulation and should be classified.

The statements **P273**, **P391** and **P501** follow a general precautionary approach for dangerous substances.

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

Hazard symbol(s)	 Dead fish, dead tree
Indications of danger	N Dangerous for the environment
Risk phrases	R51/53 Toxic to aquatic organisms, may cause long-term adverse effect in the aquatic environment.
Safety phrases	S61 Avoid release to the environment. Refer to special instructions/safety data sheets.

Justification for the proposal

R51/53 follows from the toxicity of the active substance Fenpyrazamine to fish (*Oncorhynchus mykiss*, LC_{50} = 5.2 mg/L, Cafarella, 2006a) and the fact that the active substance is not ready biodegradable (Burwood, C. & Scholey, A., 2006). The log P_{ow} of fenpyrazamine is 3.52 (Lentz, N.R., 2005b) and the measured fish BCF was 9 (Panthani, A.M., Herczog, K.J.S., 2007). The substance Fenpyrazamine does not meet the DSD criteria (BCF ≥ 500) based on the measured fish BCF.

The safety phrase S61 has to be applied based on the proposed R51/53.

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

Fenpyrazamine was hydrolytically stable at environmental temperature at pH 4 and 7 but at pH 9, degradation to S-2188-DC and subsequently to S-2188-OH occurred. At 20ºC and pH 9 the DT_{50} for hydrolysis was 24 days which is longer than the 16 days trigger for rapidly hydrolysed compounds. In contrast S-2188 was rapidly photolysed in aqueous solution, with experimental DT_{50} values of 1.7 days. The main photolytic products were S-2188-DC (max. 63.8% after 7 days) and MCNI (max 17.7% after 30 days).
Fenpyrazamine is not readily biodegradable and can not be classified as rapidly degraded in water sediment systems since less than 70 % is degraded within 28 days ($DT_{50whole\ system}$ of 35.5 days). Furthermore, mineralisation of the active substance is below 10 % of AR after 100 days after application.
Fenpyrazamine has a low potential of bioaccumulation in aquatic system because of a measured fish BCF of 9 (Panthani, A.M., Herczog, K.J.S., 2007).

Fenpyrazamine is chronic toxic to algae (*P. subcapitata*) with a NOEC value of 0.22 mg/L (Hoberg, 2006a).

Hazard pictogram		Environment
Hazard class and category:	Hazardous to the aquatic environment, Chronic Hazard Category 2	
Signal word	-	
Hazard statement:	H411	Toxic to aquatic life with long lasting effects
Precautionary statements - Prevention	P273	Avoid release to the environment
Precautionary statements - Response	P391	Collect spillage
Precautionary Statement Disposal	P501	Proper disposal of contents/container

6 OTHER INFORMATION

7 REFERENCES

7.1 Physico-chemical properties

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Asada, Y	2010	Explosive properties of S-2188 (Sumitomo QNP-0019) Not GLP, Unpublished	Y	SUM
Beckwith, R.C. & DiFrancesco, D.	2005	Determination of Dissociation Constant (pKa) - S-2188 Ricerca Biosciences LLC, Report No. 018410-1 (Sumitomo QNP-0001) GLP, Unpublished	Y	SUM
DiFrancesco, D.	2006	Determination of vapour pressure - S-2188 Ricerca Biosciences LLC, Report No. 018435-1 (Sumitomo QNP-0004) GLP, Unpublished	Y	SUM
Lentz, N.R.	2005a	Determination of water solubility - S-2188 Ricerca Biosciences LLC, Report No. 018315-1 (Sumitomo QNP-0003) GLP, Unpublished	Y	SUM
Lenz, N.R.	2005b	Determination of n-Octanol/Water Partition Coefficient - S-2188 Ricerca Biosciences LLC, Report No. 018434-1 (Sumitomo QNP-0002) GLP, Unpublished	Y	SUM
Lewis C.J.	2007	[14C]S-2188: Hydrolytic Stability Covance Laboratories Ltd, Report No. 0333/257-D2149 (Sumitomo QNM-0017) GLP, Unpublished.	Y	SUM
Lewis, C.J. & Troth, K.	2007d	[14C]S-2188: Photodegradation and Quantum Yield in Sterile, Aqueous Solution Covance Laboratories Ltd, Report No. 0333/258-D2149 (Sumitomo QNM-0029) GLP, Unpublished.	Y	SUM
Liney, P. & Jarvis, T.	2009	S-2188 – Stability in Air Exponent International Ltd., (Sumitomo QNM-0032) Not GLP, Unpublished	Y	SUM

Liney, P. & Jarvis, T.	2009	S-2188 – Oxidising Properties Assessment of Structure Exponent International Ltd., (Sumitomo QNP-0008) Not GLP, Unpublished	Y	SUM
Sweetapple, G.G. & Lentz, N.R.	2006a	Determination of Physical-Chemical Properties of S-2188PAI (amended report) Ricerca Biosciences LLC, Report no. 019388-1-1 (Sumitomo QNP-0006) GLP, Unpublished	Y	SUM
Sweetapple, G.G. & Lentz, N.R.	2006b	Determination of Physical-Chemical Properties of S-2188TGAI (amended report) Ricerca Biosciences LLC, Report no. 019387-1-1 (Sumitomo QNP-0007) GLP, Unpublished	Y	SUM
Weissenfeld, M	2009	S-2188 Technical Grade: Determination of the Relative Self-Ignition Temperature Harlan Laboratories Ltd, Report no. C40706 (Sumitomo QNP-0014) GLP, Unpublished	Y	SUM

7.2 Human health hazard assessment

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Deguchi, Y.	2007a	Acute Oral Toxicity Study of S-2188 Technical Grade in Rats Sumitomo, Report No. 4053 (Sumitomo QNT-0013) GLP, Unpublished.	Y	SUM
Deguchi, Y.	2007b	Acute Dermal Toxicity Study of S-2188 Technical Grade in Rats Sumitomo, Report No. 4054 (Sumitomo QNT-0012) GLP, Unpublished.	Y	SUM
Deguchi, Y.	2007c	Acute Inhalation Toxicity Study of S-2188 Technical Grade in Rats Sumitomo, Report No. 4057 (Sumitomo QNT-0016) GLP, Unpublished.	Y	SUM
Dohn, D.R.	2007	The Pharmacokinetics of [^{14}C]S-2188 in the Rat Upon Administration of Single Oral High and Low Doses PTRL West, Inc., Report No. 1434W-1 (Sumitomo QNM-0022) GLP, Unpublished.	Y	SUM

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Dohn, D.R., Kovatchev, A. & Estigoy, L.E.	2007	The Metabolism and Excretion of [^{14}C]S-2188 in the Rat Upon Administration of Single Oral High and Low Doses PTRL West, Inc., Report No. 1440W-1 (Sumitomo QNM-0027) GLP, Unpublished.	Y	SUM
Gerspach, R & Flade, D.	2009	Prenatal Developmental toxicity Study in the Han Wistar Rat Harlan Laboratories Ltd, report No. 77466 (Sumitomo QNT-0039) GLP, Unpublished.	Y	SUM
Gerspach, R., Weber, K. & Flade, D.	2009	S-2188: Two-generation Reproduction Study in the Han Wistar Rat Harlan Laboratories Ltd, report No. A08954 (Sumitomo QNT-0041) GLP, Unpublished.	Y	SUM
Hadfield N.	2006	S-2188 50WG – *In vitro* absorption of S-2188 through human and rat epidermal membranes. Central Toxicology Laboratory, Cheshire, UK. Report No. JV1932-REG Sumitomo Chemical Co., Ltd. Report No QNM-0015 GLP, Unpublished	Y	SUM
Inawaka, K.	2008a	Dose range finding Teratology Study in Rabbits with S-2188 Sumitomo, Report No. D0279 (Sumitomo QNT-0030) Non-GLP, Unpublished.	Y	SUM
Inawaka, K.	2008b	Teratology Study in Rabbits with S-2188 technical grade Sumitomo, Report No. 4073 (Sumitomo QNT-0032) GLP, Unpublished.	Y	SUM
Kitamoto, S.	2006a	Reverse Mutation Test of S-2188 Technical Grade in bacterial systems Sumitomo, Report No. 4032. (Sumitomo QNT-0004) GLP, Unpublished.	Y	SUM
Kitamoto, S.	2006b	*In vitro* chromosomal aberration test on S-2188 Technical Grade in Chinese hamster lung cells (CHL/IU) Sumitomo, Report No. 4029 (Sumitomo QNT-0006) GLP, Unpublished.	Y	SUM

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Kitamoto, S.	2007	Micronucleus Test on S-2188 Technical Grade in CD-1 Mice. Sumitomo, Report No. 4030 (Sumitomo QNT-0014) GLP, Unpublished.	Y	SUM
Kondo, M.	2010	Study for Mode of Action Analysis for Rat Liver and Thyroid Tumors by S-2188: Evaluation for time course alteration mainly focusing on hepatocellular proliferation, liver enzyme induction and thyroid hormone Environmental Health Science Laboratory, Study No. S1346 (Sumitomo QNT-0048) Not GLP, Unpublished	Y	SUM
Odawara, K.	2007a	Primary skin irritation test of S-2188 Technical Grade in rabbits Sumitomo, Report No. 4022 (Sumitomo QNT-0008) GLP, Unpublished.	Y	SUM
Odawara, K.	2007b	Primary eye irritation test of S-2188 Technical Grade in rabbits Sumitomo, Report No. 4021 (Sumitomo QNT-0007) GLP, Unpublished.	Y	SUM
Odawara, K.	2007c	Skin sensitization test of S-2188 Technical Grade in guinea pigs (Maximization Test) Sumitomo, Report No. 4024 (Sumitomo QNT-0010) GLP, Unpublished.	Y	SUM
Ogata, H.	2008	A 28-day Repeated Dose Dermal Toxicity Study of S-2188 Technical Grade in Rats Mitsubishi Chemical Safety Institute Ltd, Report No.: P070345 (Sumitomo QNT-0027) GLP, Unpublished.	Y	SUM
Ohtsubo, T.	2009	Statement from S-2188 Manufacturer (Sumitomo QNT-0040) Non-GLP, Unpublished	Y	SUM
Pössnecker, A.	2006	S-2188: Dose Range-Finding Prenatal Development Toxicity Study in the Han Wistar Rat RCC Ltd, report No. A08908 (Sumitomo QNT-0005) GLP, Unpublished.	Y	SUM

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Pössnecker, A. & Flade, D.	2008	S-2188: Preliminary Reproduction Toxicity Study in the Han Wister Rat RCC Ltd, Report No. A58948 (Sumitomo QNT-0020) GLP, Unpublished.	Y	SUM
Quistad, G.B. & Kovatchev, A.	2007a	The Tissue Distribution of [^{14}C]S-2188 in the Rat Upon Administration of Single Oral High and Low Doses PTRL West, Inc., Report No. 1441W (Sumitomo QNM-0023) GLP, Unpublished.	Y	SUM
Quistad, G.B. & Kovatchev, A.	2007b	The Metabolism, Excretion and Tissue Distribution of [^{14}C]S-2188 in the Rat Upon Administration of Repeated Oral Doses PTRL West, Inc., Report No. 1555W (Sumitomo QNM-0026) GLP, Unpublished.	Y	SUM
Sato, S.	2008	A 3-Month Oral Dose Toxicity Study of S-2188 Active ingredient in Dogs Ina Research Inc, Study No. ST06255 (Sumitomo QNT-0034) GLP, Unpublished.	Y	SUM
Sato, S.	2009	A 1-Year Oral Dose Toxicity Study of S-2188 Active ingredient in Dogs Ina Research Inc, Study No. ST06263 (Sumitomo QNT-0035) GLP, Unpublished.	Y	SUM
Sommer, E.W.	2007	Acute Oral Neurotoxicity Peak-Effect Study in Rats RCC Ltd, Report No. B33721 (Sumitomo QNT-0017) GLP, Unpublished.	Y	SUM
Sommer, E.W.	2008b	90-day Oral Neurotoxicity (Feeding) Study in Rats RCC Ltd, Report No. B36347 (Sumitomo QNT-0031) GLP, Unpublished.	Y	SUM
Sommer, E.W.	2008a	Acute Oral Neurotoxicity (Gavage) Study in Rats RCC Ltd, Report No. B36336 (Sumitomo QNT-0029) GLP, Unpublished.	Y	SUM
Sommer, E.W.	2009a	S-2188 Technical: Combined Chronic toxicity/oncogenicity (feeding) study in the Wistar rat Harlan Laboratories Ltd, report No. A08897 (Sumitomo QNT-0042) GLP, Unpublished.	Y	SUM

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Sommer, E.W.	2009b	S-2188 Technical: 78-week Oncogenicity (Feeding) study in the CD-1 Mouse Harlan Laboratories Ltd, report No. A08875 (Sumitomo QNT-0043) GLP, Unpublished.	Y	SUM
Sommer, E.W., Flade, D., Gretener, P. & Krinke, G.	2006	S-2188 Technical: 13-Week Repeated Dose Oral Toxicity (Feeding) Study in the Wister Rat RCC Ltd, Report No. A08886 (Sumitomo QNT-0009) GLP, Unpublished.	Y	SUM
Sommer, E.W., Flade, D., Gretener, P. & Weber, K.	2007	S-2188 Technical: 13-Week Repeated Dose Oral Toxicity (Feeding) Study in the CD-1 Mouse RCC Ltd, Report No. A08864 (Sumitomo QNT-0011) GLP, Unpublished.	Y	SUM
Sugimoto K.	2008	*In vivo* dermal absorption study of S-2188 50WG formulation in rats. Kumatomo Laboratory, Mitsubishi Chemical Safety Institute Ltd., Japan, Project No. P070639 Sumitomo Chemical Co., Ltd. Report No QNM-0031 GLP, Unpublished	Y	SUM
Yamada, T.	2010a	*In vitro* evaluation for role of nuclear receptor CAR in S-2188-induced mRNA expression of CYP2B1, UGT1A, and UGT2B1 Environmental Health Science Laboratory, Study No. S1524 (Sumitomo QNT-0049) Not GLP, Unpublished	Y	SUM
Yamada, T.	2010b	An evaluation of the human relevance of the liver and thyroid tumors observed in male rats treated with fenpyrazamine (S-2188) based on mode of action Sumitomo Chemical Co., Ltd. Report No. QNT-0050 Not GLP, Unpublished	Y	SUM
Wollny, H-E.	2007	Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT) with S-2188 Technical Grade RCC Cytotest Cell Research GmbH, Report No. 1043100 (Sumitomo QNT-0015) GLP, Unpublished.	Y	SUM

7.3 Environmental hazard assessment

7.3.1 Fate and Behaviour in the environment

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Burwood, C. & Scholey, A.	2006	S-2188: Assessment of ready biodegradability by measurement of carbon dioxide evolution Covance Laboratories Ltd, Report No. 0333/261-D2149 Sumitomo Chemical Co., Ltd. QNM-0011 GLP, Unpublished.	Y	SUM
Jarvis, T. & Callow, B	2009a	Determination of normalised rates of degradation for S-2188 from four soils incubated under laboratory conditions Sumitomo Chemical Co. Ltd: QNM-0037 Non-GLP, Unpublished	Y	SUM
Jarvis, T. & Callow, B	2009b	Determination of rates of degradation for S-2188 from a water sediment study incubated under laboratory conditions. Sumitomo Chemical Co. Ltd: QNM-0040 Non-GLP, Unpublished	Y	SUM
Lewis, C.J.	2007	[^{14}C]S-2188: Hydrolytic Stability Covance Laboratories Ltd, Report No. 0333/257-D2149 Sumitomo Chemical Co., Ltd. QNM-0017 GLP, Unpublished.	Y	SUM
Lewis, C.J. & Scholey, A.	2006a	[^{14}C]S-2188: Aerobic soil metabolism and degradation Covance Laboratories Ltd, Report No. 0333/256-D2149 Sumitomo Chemical Co., Ltd. QNM-0016 GLP, Unpublished.	Y	SUM
Lewis, C.J. & Scholey, A.	2006b	[^{14}C]S-2188: Adsorption/Desorption in soil Covance Laboratories Ltd, Report No. 0333/255-D2149 Sumitomo Chemical Co., Ltd. QNM-0012 GLP, Unpublished.	Y	SUM
Lewis, C.J. & Troth, K.	2007c	[^{14}C]S-2188: Photodegradation on a soil surface Covance Laboratories Ltd, Report No. 0333/259-D2149 Sumitomo Chemical Co., Ltd. QNM-0020 GLP, Unpublished.	Y	SUM
Lewis, C.J. & Troth, K.	2007e	[^{14}C]S-2188: Photodegradation and quantum yield in sterile, aqueous solution Covance Laboratories Ltd, Report No. 0333/258-D2149 Sumitomo Chemical Co., Ltd. QNM-0029 GLP, Unpublished.	Y	SUM

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Lewis, C.J. & Troth, K.	2007f	[^{14}C]S-2188: Degradation and retention in water-sediment systems Covance Laboratories Ltd, Report No. D2149-0333/260 Sumitomo Chemical Co., Ltd. QNM-0028 GLP, Unpublished.	Y	SUM
Peatman, M.H.	2008	S-2188: Storage stability of residues in EU soil stored deep frozen Covance Laboratories Ltd, Report No. 0333/268-D2149 Sumitomo Chemical Co. Ltd Report No.: QNM-0036 GLP, Unpublished.	Y	SUM
Peatman, M.H. & Brice, A	2009	S-2188: The Dissipation of Residues in Soil in Northern and Southern Europe Covance Laboratories Ltd, Report No. 0333/266-D2149 Sumitomo Chemical Co. Ltd Report No.: QNM-0038 GLP, Unpublished.	Y	SUM

7.3.2 Aquatic Toxicity

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Cafarella, M.A.	2006a	S-2188 Technical Grade – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions Springborn Smithers Laboratories (USA) Wareham, MA, USA Report No.: 13048.6504 (Sumitomo QNW-0002) GLP, Unpublished	Y	SUM
Cafarella, M.A.	2006b	S-2188 Technical Grade – Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Conditions Springborn Smithers Laboratories (USA) Wareham, MA, USA Report No.: 13048.6505 (Sumitomo QNW-0006) GLP, Unpublished	Y	SUM
Cafarella, M.A.	2006c	S-2188 Technical Grade – Early Life-Stage Toxicity Test with Rainbow Trout (*Oncorhynchus mykiss*) Springborn Smithers Laboratories (USA) Wareham, MA, USA Report No.: 13048.6506 (Sumitomo QNW-0011) Study No.: GLP, Unpublished	Y	SUM
Hoberg, J.R.	2006a	S-2188 Technical Grade – Acute Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata* Springborn Smithers Laboratories (USA) Wareham, MA, USA	Y	SUM

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
		Report No.: 13048.6509 (Sumitomo QNW-0004) GLP, Unpublished		
Panthani, A.M. & Herczog, K.J.S.	2007	Bioconcentration of [^{14}C]S-2188 by Bluegill Sunfish (*Lepomis macrochirus*) Ricerca Biosciences, LLC, Environmental Sciences Department Concord, OH, USA Report No.: 019492-1 (Sumitomo QNM-0018) GLP, Unpublished	Y	SUM
Putt, A.E.	2006a	S-2188 Technical Grade – Acute Toxicity to Water Fleas, (*Daphnia magna*) Under Flow-Through Conditions Springborn Smithers Laboratories (USA) Wareham, MA, USA Report No.: 13048.6507 (Sumitomo QNW-0007) Study No.: GLP, Unpublished	Y	SUM
Putt, A.E.	2006b	S-2188 Technical Grade – Full Life-Cycle Toxicity Test with Water Fleas, *Daphnia magna* Under Flow-Through Conditions Springborn Smithers Laboratories (USA) Wareham, MA, USA Report No.: 13048.6508 (Sumitomo QNW-0012) GLP, Unpublished	Y	SUM

8 ANNEXES