Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

spirodiclofen (ISO); 3-(2,4-dichlorophenyl)-2-oxo-1-xaspiro[4.5]dec-3-en-4-yl 2,2-dimethylbutyrate

EC Number: -

CAS Number: 148477-71-8

CLH-O-0000001412-86-135/F

Adopted

9 December 2016

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON SPIRODICLOFEN (ISO); 3-(2,4-DICHLOROPHENYL)-2-OXO-1-OXASPIRO[4.5]DEC-3-EN-4-YL 2,2-DIMETHYLBUTYRATE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: **Spirodiclofen (ISO); 3-(2,4-dichlorophenyl)-2-oxo-1-oxaspiro[4.5]dec-3-en-4-yl 2,2-dimethylbutyrate**
EC number: **-**
CAS number: **148477-71-8**
Dossier submitter: **The Netherlands**

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2015	Germany		MemberState	1
Comment received				
The German CA supports the proposed classification of spirodiclofen (ISO). Editorial Comments: • The reference substance dataset for spirodiclofen in IUCLID section 1.1 respectively IUCLID section 1.2 does not include information on the molecular weight of the substance. Furthermore, no structural formula, SMILES notation or InChI code is given in the reference substance dataset. Please add the missing information. • In IUCLID section 1.2 two impurities are given: N,N-dimethylacetamide and 3-(2,4-dichlorophenyl)-4- hydroxy-1-oxaspiro[4.5] dec-3-en-2-one. Both reference substance datasets do not include a structural formula, SMILES notation or InChI code. Furthermore for the impurity 3-(2,4-dichlorophenyl)-4- hydroxy-1-oxaspiro[4.5] dec-3-en-2-one the corresponding CAS No. and the molecular formula are missing as well. Please add the missing information. • As already mentioned two impurities are given in IUCLID section 1.2. According to the information given in the confidential Annex to the CLH report more impurities are present in the substance composition. The corresponding impurities should be given in section 1.2 of the IUCLID file and should be flagged confidential. Please add the missing information. • In the confidential Annex to the CLH report for spirodiclofen information on the substance composition are given. In the first passage of this document concentration values for the substance spirodiclofen and the impurities of the substance are given. The corresponding concentrations are given in mg/kg and are deviating from the values given in section 1 of the same document and the concentration values in the IUCLID file. Please amend the values in the first section of the confidential annex by replacing mg/kg using g/kg instead.				
Dossier Submitter's Response				
Thank you for the support.				

The comments are noted. However, the CLH report or IUCLID-file cannot be updated anymore at this stage of the CLH-process. In future, we will pay better attention to these issues and include this type of information when needed.
RAC's response
RAC appreciates the editorial comments from Germany although these do not lie within RAC's area of responsibility.

Date	Country	Organisation	Type of Organisation	Comment number
30.11.2015	Norway		MemberState	2
Comment received				
Norway supports the proposed classification and labelling for spirodiclofen.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
RAC appreciates the general support of the Norwegian CA on the proposed CLH of spirodiclofen.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
04.12.2015	Spain		MemberState	3
Comment received				
The Spanish CA agrees with the dossier submitter that classification for carcinogenicity is necessary for spirodiclofen under CLP classification criteria as Carc. 1B (H350: May cause cancer). In rat (Wistar), spirodiclofen induced neoplastic effects in testes and uterus. The tumors in the testes (Leydig cell tumors) are benign. The mechanistic studies showed that spirodiclofen clearly interferes with steroid hormone synthesis in the adrenals and gonads. The tumors in the uterus (uterus adenocarcinoma) are malignant. For both tumor types it cannot be excluded that these are relevant for humans, and these should be taken into account for classification of spirodiclofen for carcinogenicity in humans. In mice (CD-1), spirodiclofen induced a significantly increased of hepatocellular adenomas in males. Further, a dose-related increase (not statistically significant) of malignant hepatocellular tumor (carcinomas) was observed in male animals as well. The combined frequency (adenomas and carcinomas) was also significantly increased. As a potential irrelevance for humans is not clearly demonstrated, these should be taken into account for classification of spirodiclofen for carcinogenicity in humans. There was a combination of benign and malignant neoplasms of relevance for humans in two species and therefore, in our opinion there is sufficient evidence for classification spirodiclofen as a category 1B				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
RAC appreciates the comments from the Spanish CA and has taken into consideration the presented reasoning.				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON SPIRODICLOFEN (ISO); 3-(2,4-DICHLOROPHENYL)-2-OXO-1-OXASPIRO[4.5]DEC-3-EN-4-YL 2,2-DIMETHYLBUTYRATE

Date	Country	Organisation	Type of Organisation	Comment number
04.12.2015	Sweden		MemberState	4
Comment received				
We agree with the dossier submitter's proposal to classify Spirodiclofen as Carc. 1B (H350), since the available long-term oral carcinogenicity studies showed that spirodiclofen induced adenocarcinomas in the uterus and benign Leydig cell tumours in rats, and hepatocellular carcinomas and hepatocellular adenomas in mice. In view of these results it can be concluded that evidence matching the criteria for classification of spirodiclofen as a Category 1B carcinogen is available, i.e. a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in two or more species of animals.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
RAC appreciates the comments from the Swedish CA and has taken into consideration the presented reasoning.				

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2015	Germany		MemberState	5
Comment received				
Based on the data provided, the proposed classification in category 1B for carcinogenicity is in general supported. However, historical control data (HCD) should be taken into account as well as a recent publication on the Mode of Action of spirodiclofen (Yoshida et al. 2015). The reasons for suggesting a consideration of HCD are given below. For the assessment of carcinogenicity an oncogenicity testing study in CD-1 mice and a combined study on chronic toxicity and carcinogenicity in Wistar rats are available. In the oncogenicity study in CD-1 mice (Wahle 2000) significantly increased incidences of hepatocellular adenoma and a significantly increased combined frequency of hepatocellular adenomas and carcinomas were found in males of the mid- and high-dose group. A dose-related but not significant increase of hepatocellular carcinomas was found in male mice as well. In the combined chronic toxicity and carcinogenicity study in Wistar rats (Wimitzer et al. 2000) increased incidences in benign Leydig cell tumours and malignant uterus adenocarcinoma (both not statistically significant or dose-related) were found in the high-dose group. An occurrence of thyroid C-cell adenoma and carcinoma in female Wistar rats was considered to be irrelevant based on historical control data. In that context it remained unclear why the incidences of thyroid C-cell tumours were compared to HCD, while HCD were ignored in case of the other tumour types. Especially Leydig cell tumours and hepatocellular adenomas are known to occur spontaneously and with a high variability in certain rat and mice strains (Section 3.6.2.3.2 in Guidance on the application of the CLP criteria). Please include and discuss appropriate HCD. An inquiry on publicly available historical control data revealed (although certain limitations regarding differences in laboratories, animal specification, time window				

broadness and time window distancy existed) that the combined multiplicity of data indicates a relatively high spontaneous occurrence and variability in incidences of hepatocellular tumours in CD-1 mice (Maita et al. 1988, Chandra and Frith 1992, Giknis and Clifford 2000, Giknis and Clifford 2001, Giknis and Clifford 2005, Forster et al. 2014) and of Leydig cell tumours in Wistar rats (Bomhard and Rinke 1994, Eiben and Bomhard 1999, Walsh and Poteracki 1994, Poteracki and Walsh 1998, Giknis and Clifford 2003). Based on the above listed HCD, every observed tumour type, except the malignant uterus adenocarcinoma, can be considered to lie within the HCD. Please discuss the multitude of available HCD - if they sufficiently support a high variability and spontaneous occurrence of hepatocellular tumours and Leydig cell tumours - and a potential impact on classification.

Additionally it remained unclear why the incidences of Leydig cell tumours and uterus adenocarcinomas in table 42 were divided in "except deaths", "deaths only" and "combined incidences" without further discussion of e.g. early onset of tumour development/reduced latency. Please clarify.

Literature

Yoshida et al. (2015) "Predictive modes of action of pesticides in uterine adenocarcinoma development in rats" J Toxicol Pathol, 28, pp. 207-216.
Maita et al. (1988) "Mortality, major cause of moribundity, and spontaneous tumors in CD-1 mice" Toxicologic Pathology, 16 (3), pp. 340-349.
Chandra and Frith (1992) "Spontaneous neoplasms in aged CD-1 mice" Toxicology Letters, 61, pp. 67-74.
Giknis und Clifford (2000) "Spontaneous neoplastic lesions in the Crl:CD-1® (ICR)BR mouse" Charles River Laboratories.
Giknis und Clifford (2001) "Compilation of spontaneous neoplastic lesions and survival in Crl:CD® (SD) BR rats from control groups" Charles River Laboratories.
Giknis und Clifford (2005) "Spontaneous neoplastic lesions in the Crl:CD-1 (ICR) mouse in control groups from 18 months to 2 year studies" Charles River Laboratories.
Forster et al. (2014) "Lifetime carcinogenicity studies in the CD-1 mouse:Historical data for survival and neoplasms" Toxicology Letters, 229, p. S148.
Bomhard und Rinke (1994) "Frequency of spontaneous tumours in Wistar rats in 2-year studies" Exp Toxic Pathol, 46, pp. 17-29.
Eiben and Bomhard (1999) "Trends in mortality, body weights and tumor incidences of Wistar rats over 20 years" Exp Toxic Pathol, 51, pp. 523-536.
Walsh and Poteracki (1994) "Spontaneous neoplasms in control Wistar rats" Fundamental and applied toxicology, 22, pp. 65-72.
Poteracki and Walsh (1998) "Spontaneous neoplasms in control Wistar rats: A comparison of reviews" Toxicological sciences, 45, pp. 1-8.
Giknis and Clifford (2003) "Spontaneous neoplasms and survival in Wistar Han rats: compilation of control group data" Charles River Laboratories.

Dossier Submitter's Response

Thank you for the support.

The comments are noted.

- Thank you for drawing our attention to the recent publication of Yoshida et al. (2015). This publication is evaluated by us, and a short summary is presented below. This publication provides some information on the mode of action of spirodiclofen for its uterine carcinogenic activity, a pathway which is considered relevant for humans.

Yoshida et al. (2015) "Predictive modes of action of pesticides in uterine adenocarcinoma development in rats" J Toxicol Pathol, 28, pp. 207-216.
Yoshida et al (2015) evaluated chemicals (pesticides) for potential uterine carcinogenicity and attempted to predict their mechanism using parameters from mechanistic and toxicity studies. Five pathways for uterine carcinogenesis in rodents were presented (of which the first three appear to be accepted as major pathways): 1) estrogenic activity, 2) increased serum 17beta-estradiiol (E2) to progesterone (P4) ratio and 3) modulation of estrogen metabolism to produce 4-hydroxyestradiol via P450 induction, 4) inhibition of estrogen excretion, 5) increased aromatase in situ in the tumor.
Their evaluation of a total of 300 pesticides revealed that seven chemicals increased uterine tumor formation in rats, and the pathways of 4 chemicals (including spirodiclofen) could be predicted based on various mechanistic studies. The mode of action of spirodiclofen was predicted to be increased serum 17beta-estradiiol (E2) to progesterone (P4) ratio given that mechanistic studies showed that E2-levels were not changed while P4-levels were decreased.

- It is acknowledged that a comparison with historical control data for all relevant tumour types (tumour types with an increased incidence compared with study-controls) would be valuable. However, these data were not available to us for all relevant tumour types.
  References of (publically available) reports with historical control data were presented and it was suggested to use these for comparison with the tumour incidences of the rat and mouse carcinogenicity study with spirodiclofen. However, in our opinion historical control data should be derived from the same species/strain, the same laboratory and same time period. This is conform the CLP Guidance as section 3.6.2.3.2.a states "*The historical data must be from the same animal strain/species, and ideally, be from the same laboratory to minimise any potential confounding due to variations in laboratory conditions, study conditions, animal suppliers, husbandry etc. It is also known that tumour incidences in control animals can change over time, due to factors such as genetic drift, changes in diagnostic criteria for pathological changes/tumour types, and husbandry factors (including the standard diet used), so the historical data should be contemporary to the study being evaluated (e.g. within a period of up to around 5 years of the study). Historical data older than this should be used with caution and acknowledgement of its lower relevance and reliability. (RIVM, 2005; Fung et al, 1996; Greim et al, 2003).*"

  In conclusion, a comparison with relevant historical control data could have been included to further strengthen the evidence. Given that no relevant historical control data are available to us, this comparison was not performed.

- With respect to the subdivision of the incidences of Leydig cell tumours and uterus adenocarcinoma in table 42 in "except deaths" and "death only": it is acknowledged that there are no additional discussion points concerning this division.

RAC's response

RAC appreciates the comments from the German CA generally supporting classification of spirodiclofen as Carcinogen 1B. Regarding the point raised on historical control data for the tumours used for classification purposes, RAC notes that the German CA did not provide actual numerical data but relevant references. The two studies RAC uses (both in 2000) for

the evaluation of the carcinogenicity properties of spirodiclofen, are performed by Bayer AG and are not publicly available except for the data present in the registration dossier that the DS uses and presents. In both studies the data on the control group (n=50) are presented and discussed. In addition, RAC found some of the relevant references on the HCD provided by the German CA and prepared Table 1 for CD1 mice and Table 2 for Wistar rats:

Table 1 CD1 Mice

Study HCD	% liver adenomas	% liver carcinomas	% combined liver tumours	ODD study	Dose (ppm)	% liver adenomas	% liver carcinomas	% combined liver tumours
Males								
Maita *et al.*, 1988	26	9.1	35.4	Wahle, 2000	3500	10	6	16
Chandra and Frith, 1992	11	5.7	16.7		7000	12	10	22
Giknis and Clifford, 2000	10.46	5.29	15.8					
Females								
Maita *et al.*, 1988	5.17	0.9	6.07	Wahle, 2000	3500	6	4	10
Chandra and Frith, 1992	1.8	0.7	2.48		7000	2	4	6
Giknis and Clifford, 2000	0.99	0.66	1.64					

Two points are evident:

1. Data on HCD present high variation and are not consistent.
2. Incidents of liver adenomas, carcinomas and combined tumours in the Wahle 2000 study are higher than the HCD in some cases especially in females. A combination of benign and malignant hepatocellular tumours were observed in the CD-1 mice in both sexes at 2 doses in a statistically significant manner and a dose-dependent way in the males. A combination of benign and malignant hepatocellular tumours were observed in the CD-1 mice in both sexes at 2 doses in a statistically significant manner and a dose-dependent way in the males.

Table 2 Wistar Rats

Study HCD	% Benign Leydig cell tumours	Uterus adenocarcinomas	ODD study	Dose (ppm)	% Benign Leydig cell tumours	Uterus adenocarcinomas
Bomhard and Rinke, 1994	2.1-16.3 (7.0)	0.0-16.3 (7.8)	Wirnitzer *et al.* 2000	350	8	4
Eiben and Bomhard, 1999 (Bayer AG rats)	7.0	6.5				
Walsh and Poteracki, 1994	3.9	1.6		2500	20	28

	Giknis and Clifford, 2003	No reference	2.3				

() cumulative average

RAC agrees with the DS that these HCD discussed during PC are not relevant and should not be used to disregard the hepatocellular adenomas/carcinomas in mouse and the benign Leydig cell tumours in rat.

Regarding the historical control data, Industry has performed its own evaluation in the Wahle 2000 study. More specifically, historical control data from the literature suggest a rate of 0%-9.6% in male controls (n=499) and 0%-2.7% in females (n=497) in nominal 18-month studies. Data from five in-house studies conducted 1989-1998 show a rate for the combined hepatocellular neoplasms in controls of 4%-14% in male controls (n=250) and 0%-2% in female controls (n=250). While the male control numbers in the Wahle study are historically low, at 2%, the female values are consistent with historical data. Male frequencies at 3500 and 7000 ppm (16% and 20% respectively), and corresponding female values of 10% and 6% are above the range seen in either in-house or literature historical data.

With regard to the differentiation between "except deaths", "deaths only" and "combined incidences" RAC has taken into consideration the DE CA comment in the ODD. The same stands for the mechanistic study of Yoshida *et al.* 2015.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2015	Germany		MemberState	6
Comment received				
It is supported not to classify spirodiclofen for mutagenicity.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
RAC appreciates the general support of the German CA not to classify spirodiclofen for mutagenicity.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
04.12.2015	Spain		MemberState	7
Comment received				
As proposed by the dossier submitter, the Spanish CA supports to classify spirodiclofen for effects on sexual function and fertility as Repro 2 (H361f: Suspected of damaging fertility). In the 2-generation study with rats, weights of adrenals, ovaries and uterus in the F1 animals had changed. In the high dose group, decreases were observed in the number of spermatids in the testes and in the number of sperms in the epididymis. In addition, effects on the reproductive organs were observed in the repeated dose toxicity and carcinogenicity studies. Effects on testes were observed in all studied species (i.e. mouse, rat, dog), though most pronounced in dogs. These effects included increased testis weight (absolute + relative), hyperplasia, hypertrophy and vacuolisation of testis, but also oligo- and aspermia (in 4- and 14-week dog studies, 18-month mouse study). Further, changes				

of weight of uterus/oviduct and ovaries were observed in female animals. The mechanistic studies showed that spirodiclofen has a direct effect on steroid hormone synthesis, which is probably mediated by effects on general pathways (interference with formation of NADPH, which is an important co-substrate in several steps of the biosynthesis of steroid hormones) and that no androgenic, antiandrogenic, estrogenic or anti-estrogenic effects were noted in mechanistic studies. Further, it was shown that spirodiclofen might have a direct effect on the enzymes involved in the steroidogenesis in testis. These data indicate that the observed effects are not secondary to general toxic effects, but rather a direct effect of spirodiclofen. A direct effect of spirodiclofen on enzymes involved in the synthesis of steroidhormones in the testes (microsomal hydrogenases) could not be excluded. No information is available which indicate that the effects observed in dogs (including the underlying mechanisms) are not relevant for humans. Therefore, the effects on sexual function observed should be taken into account for classification for effects on sexual function and fertility.
Dossier Submitter's Response
Thank you for the support.
RAC's response
RAC appreciates the comments from the Spanish CA and has taken into consideration the presented reasoning.

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2015	Germany		MemberState	8
Comment received				
The proposed classification for reproductive toxicity (R2, H361f) is supported. Spirodiclofen fulfills the criteria for being classified as toxic to reproduction cat. 2.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
RAC appreciates the general support of the German CA to classify spirodiclofen as Repro 2, H361f.				

RESPIRATORY SENSITISATION

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2015	Germany		MemberState	9
Comment received				
It is supported not to classify spirodiclofen for respiratory sensitization.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
RAC appreciates the general support of the German CA not to classify spirodiclofen for respiratory sensitisation.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2015	Germany		MemberState	10
Comment received				
It is supported not to classify spirodiclofen for acute toxicity.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
RAC appreciates the general support of the German CA not to classify spirodiclofen for acute toxicity.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2015	Germany		MemberState	11
Comment received				
It is supported not to classify spirodiclofen for skin irritation/corrosion.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
RAC appreciates the general support of the German CA not to classify spirodiclofen for skin corrosion/irritation.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2015	Germany		MemberState	12
Comment received				
It is supported not to classify spirodiclofen for eye irritation.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
RAC appreciates the general support of the German CA not to classify spirodiclofen for eye irritation.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
04.12.2015	Spain		MemberState	13
Comment received				
The Spanish CA supports the proposed classification of spirodiclofen as Skin Sens. 1B, H317: May cause an allergic skin reaction, given that the response in the guinea pig Maximisation test (Stropp, 1996) was 40% at an intradermal induction dose of 5%.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
RAC appreciates the comments from the Spanish CA and has taken into consideration the presented reasoning.				

Date	Country	Organisation	Type of Organisation	Comment number
04.12.2015	Sweden		MemberState	14
Comment received				
We agree with the proposed classification of Spirodiclofen as Skin Sens. 1B (H317). The criteria for Cat. 1B classification based on results from the Guinea pig maximization test is ≥ 30% incidence of sensitized Guinea pigs with > 1% intradermal induction dose. In the Stropp (1996) study, a high intradermal induction dose was used (5%) where 40% of the Guinea pigs had a positive skin reaction after the first and second challenge (Table 25, p. 50 in CLH Report). Our conclusion is that these results meet the criteria for Cat. 1B. Moreover, classification in Category 1A can be excluded based on the limited number of animals with positive skin reactions at such a high intradermal induction dose.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
RAC appreciates the comments from the Swedish CA and has taken into consideration the presented reasoning.				

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2015	Germany		MemberState	15
Comment received				
It is supported to classify spirodiclofen as skin sensitizer cat.1B (H317). Spirodiclofen fulfills the criteria for being classified as skin sensitizer.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
RAC appreciates the general support of the German CA to classify spirodiclofen as Skin Sens. 1B; H317.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2015	Germany		MemberState	16
Comment received				
It is supported not to classify spirodiclofen for specific target organ toxicity after single exposure.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
RAC appreciates the general support of the German CA not to classify spirodiclofen for STOT SE.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
04.12.2015	France		MemberState	17

Comment received

The proposed classification for spirodiclofen includes a classification as STOT-RE 2 based on effects observed in dogs.
Haematological effects were observed in the 14-week dog study and consisted in a dose-related reduction of haemoglobin and haematocrit of about 20%. Such effects were not reproduced in the other dog studies: 4-week (decrease of Hb and Ht but apparently not dose-related according to table 31, no data on males and females separately, % of decrease not mentioned), 8-week and 1-year studies. Given the inconsistencies of these haematological effects between the dog studies and the absence of haematological effects in the other tested species, the relevance of the classification as STOT RE for these effects is questionable.
The other effects observed in dogs involved effects on reproductive organs, for which a classification as Repr 2 H361f is already proposed (and supported). Therefore, classification with STOT RE for these effects is not considered needed.
Finally, effects observed in adrenals and liver do not seem to be severe enough to warrant a classification.

Dossier Submitter's Response

The comments are noted. However, in our opinion the available data justify a proposal for STOT RE 2 based on the observed adverse effects in dog.
In dogs many parameters of various organ systems were affected and these included a.o. the haematological system, the liver and the adrenals.
Although effects on the haematological system were not observed in the 8-week and 1-year dog study, effects were observed in the 4-week and 14-week studies. Dose-related effects on Hb and Ht-levels and % erythrocytes were observed in the 14-week oral dog study, in which a 20% decline of these levels was observed at the highest dose level of 82.8 mg/kg bw/d (i.e. below the upper limit of 100 mg/kg bw/d for STOT RE 2). Also in the 4-week oral dog study, haematological parameters were affected and reduced erythrocytes, Hb and Ht were observed at ≥65.5 mg/kg bw/d. However, no information is available on the extent of decrease of these levels in this 4-wk dog study.
Further, the liver was found to be a target organ in the 4-week, 8-week, 14-week and 1-year dog studies. Effects included increased organ weight, increased biochemical parameters. Also hepatocellular necrosis was observed at 284.5 mg/kg bw/d in the 4-week study (i.e. below the upper limit of 300 mg/kg bw/d for STOT RE 2), 55.9 mg/kg bw/d in the 8-week study (i.e. below the upper limit of 150 mg/kg bw/d for STOT RE 2), 82.8 mg/kg bw/d in the 14-week study (i.e. below the upper limit of 100 mg/kg bw/d for STOT RE 2).

It is acknowledged that some effects individually would not fulfil the classification criteria for STOT RE. However, according to section 3.9.1.4 of the CLP Guidance "Assessment shall take into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs". Further, according to section 3.9.2.5.2 of the CLP Guidance, a reduction in Hb of ≥20% would fulfil the classification criteria. In addition, necrosis is also one of the effects which fulfil the classification criteria as mentioned in and illustrated in an example in section 3.9.6.2.1.

In our opinion, it cannot be excluded that the observed effects in dogs are relevant for evaluating potential effects of spirodiclofen in humans. Therefore, the observed effects

cannot be ignored and should be taken into account for potential classsifcation of spirodiclofen for STOT RE. Given that the effective dose levels are below the upper limit of STOT RE 2, classification as STOT RE 2 is warranted.

RAC's response

RAC believes that in the available repeated dose toxicity studies in **dogs** (4-week, 8-week, 14-week and 1-year) many parameters of various organ systems were affected including the haematological system, the liver and the adrenals.

The observed adrenal effects in the dog studies (cytoplasmic vacuolisation and mononuclear cell infiltration adrenal cortex effects) are not considered severe, in combination with the CLP guidance values (tables 3.9.2 and 3.9.3) and do not support STOT RE classification.

Effects on the haematological system were not observed in the 8-week and 1-year dog studies. However, they were seen in the 4-week and 14-week dog studies. In both studies the effect-levels were below the CLP guidance values for STOT RE 2. In the 4-week study reduced erythrocytes, Hb and Ht were observed but not quantified. In the 14-week study though, a dose related effect on Hb and Ht levels and % erythrocytes was seen and at the highest dose level a 20 % decline of these parameters was observed which is considered a consistent and adverse effect in haematology (Guidance on the application of CLP criteria, Annex 3.9.2.7.3.(c)).

The liver was a target organ in the dog studies. Effects included increased organ weight and increased biochemical parameters. Hepatocellular necrosis was also observed at effect-levels below the CLP guidance values for STOT RE 2 classification.

In the following Table an overview of effects on sexual function/fertility parameters and reproductive organs in available repeated dose toxicity, carcinogenicity and reproductive toxicity studies is presented.

Study		males	females
Leser, Romeike (1998)	**13-wk oral mouse repeated dose toxicity study** 0, 100, 1000, 10000 ppm	**≥ 1000 ppm** slight ↓bw, 8 % ↑ r (dose-related) testes weights Hypertrophy/activation of Leydig cells (testes) 1/10, 1/10, **9/10**, 10/10 Average Severity (1)	**≥ 1000 ppm** no effects
		≥ 10000 ppm 12% ↑ r testes weights Hypertrophy/activation of Leydig cells (testes) 1/10, 1/10, 9/10, **10/10** Average Severity (2.3) Vacuolation of Leydig cells 7/10 Average Severity (1.1)	**≥ 10000 ppm** slight ↓bw, 10% ↑ weight ovaries
Wahle (2000)	**18-month mouse carcinogenicity study** 0, 25, 3500, 7000 ppm	**≥ 3500 ppm** no mortality, ↓ bw (statistically not consistent) ↑ food consumption ↑ar testis weight Hypertrophy/hyperplasia interstitial cells testis	**≥ 3500 ppm** no mortality, ↓ bw (statistically not consistent) terminal body weight ↓ significantly
		≥ 7000 ppm **Epididymides** Aspermia: 15/50, 15/50, 15/50, 26/50, ↑ s	**≥ 7000 ppm** no mortality, ↓ (statistically not consistent) body weight, terminal body weight was significantly ↓

		average severity: 4.3, 4.2, 4.8, 4.7 **Testes** 23% ↑r testes weight Hypertrophy/hyperplasia of interstitial cells 6/50, 6/50, 26/50, 31/50 Average Severity: 1.2, 1.3, 1.8, 2.5	**Ovaries** 38% ↓ r ovaries wt	
Krotlinger, GeiB (2000)	**4-wk oral rat** f, 0,100, 500, 5000 ppm	–	**≥ 5000 ppm** no mortality, no bw change, ↓17% r weight ovaries	
Wirnitzer, Romeike - 1998	**14-week oral rat** 0, 100, 500, 2500, 12500 ppm	**≥ 12500 ppm:** 10% ↑r testes weight, no mortality, no clinical signs, ↓ s bw m, ↓ water consumption ↓ s food consumption	**≥ 12500 ppm:** ↓ s bw f	
Wirnitzer 2000	**108-week rat carcinogenicity study** 0, 50, 100, 350, 2500 ppm	**≥ 350 ppm:** no effects **≥ 2500 ppm:** no mortalities, no clinical signs ↓s bw, ↑ food consumption ↑[r] testis weight Focal Leydig cell hyperplasia 4/31, 4/30, 4/36, 6/31, 19/41 ↑[s]	**≥ 350 ppm:** 33% ↑ar ovaries weight **≥ 2500 ppm:** no mortalities, no clinical signs, ↓s bw, ↑ food consumption	
Wetzig, Romeike, Sander (2001)	**4-week oral dog** 0, 400, 2000, 10000 ppm	**≥ 2000 ppm**: no general toxicity effects Leydig cell vacuolation 2/2 (1,1) **≥ 10000 ppm:** no general toxicity effects Leydig cell vacuolation 2/2 (3,1) Leydig cell hypertrophy/activation 1/2 (3) Immature testes/prostate, 1/2 (2) Massive oligospermia, slight spermic debris 1/2 (5)	**≥ 2000 ppm:** no general toxicity effects 33% ↑ar weight uterus **≥ 10000 ppm:** no general toxicity effects 43 % ↑ar weight ovaries 18 % ↑ar weight uterus	
Wetzig, Hartmann (2001b)	**8-week oral dog** 0, 100, 2000 ppm	**≥ 100 ppm:** no general toxicity effects ↓ar wt prostate (dr), 13 % ↓r wt prostate Degeneration germinal epithelium 1/5 (2) **≥ 2000 ppm:** no general toxicity effects Hypertrophy and vacuolization of Leydig cells (testes) 5/5 (3,2,3,2,2) Degeneration germinal epithelium 4/5 (2,1,1,1)	-	
Wetzig, Hartmann (2001a)	**14-week oral dog** 0, 200, 630, 2000 ppm	**≥ 200 ppm**: 52% ↓r weight prostate **≥ 630 ppm:** ↓ bw Testes Vacuolization Leydig cells, 2/4 (2,3) Hypertrophy Leydig cells, 2/4 (2,2) Epididymides Aspermia, 1/4	**≥ 200 ppm**: ↓ r weight uterus **≥ 630 ppm:** ↓ bw ↓ r weight uterus	

		Oligospermia, 2/4 (2,2) Immature prostate, 1/4 (4) **≥ 2000 ppm:** ↓ bw Testes Degeneration germinal epithelium, 2/4 Vacuolization Leydig cells, 4/4 (3,2,2,3) Hypertrophy Leydig cells, 3/4 (3,3,4) Epididymides Aspermia, 2/4 Immature prostate, 4/4 (4,3,3,4)	**≥ 2000 ppm:** ↓ bw 48% ↓ r weight uterus 15% ↓ r weight ovaries	
Wetzig, Ruh-Fehlert (2001)	**52-week oral dog** 0, 20, 50, 150, 600 ppm	**≥ 20 ppm:** no general toxicity effects ↑ ar testes weight **≥ 50 ppm:** no general toxicity effects ↑ ar testes weight, ↑ ar epididymis weight **≥ 150 ppm:** no general toxicity effects ↑ ar testes weight, ↑ ar epididymis weight Focal tubular degeneration testes, 1/4 (1) **≥ 600 ppm:** no general toxicity effects 30% ↑ r testes wt, 17% ↑ r epididymis wt 19% ↑ ar prostate weight Vacuolization Leydig cells, 4/4 (1,2,1,1) Hypertrophy Leydig cells, 1/4 (2) Focal tubular degeneration testes, 1/4 (2)	**≥ 20 ppm:** no general toxicity effects ↓ ar uterus/oviduct weight 29% ↓ r uterus/oviduct weight	
Krottlinger, Sander (1999)	4-wk dermal rat	–	–	
Eiben (2000)	**2-generation study rat** 0, 70, 350 & 1750 ppm	**F0:** ↓ bw dose related **≥ 70 ppm:** ↓ bw ↑sr prostate weight ↓srepididymides weight, ↓sr seminal vesicles **≥ 350 ppm:** ↓ s bw ↑sr prostate weight ↓srepididymides weight, ↓sr seminal vesicles **≥ 1750 ppm:**↓ s bw ↑sr testes weight **Testes** (diminished in size) 0/25, 1/25, 1/25, **4/25**	**F0:** ↓ bw dose related **≥ 70 ppm:** – **≥ 350 ppm:** bw **≥ 1750 ppm:**↓ s bw –	

		Epididymides (diminished in size) 0/25, 1/25, 1/25, **4/25** **F1:**↓ bw dose related **≥ 350 ppm:** ↓ bw **≥ 1750 ppm:** ↓ s bw, ↑s food consumption **Mating/fertility/gestation*** spermatids per mg testis: -23% sperms per mg epididymides: -18% **Testes*** atrophy, diffuse: 0/25, 1/25, 1/25, 4/25** **Epididymides*** Oligospermia: 0/25, 1/25, 1/25, 4/25 Atrophy: 0/25, 1/25, 1/25, 4/25 **Prostate*** Atrophy: 0/25, 0/25, 0/25, 3/25	**F1** **≥ 350 ppm:** – **≥ 1750 ppm:** ↓ s bw ↑ ar uterus & ovaries weight

* Effects were observed in four specific animals where there was a sever decrease in body weight.
** The testes atrophy was within the HCD range.
a: absolute, r: relative, s: statistically significant, bw: body weight

In conclusion, RAC agrees with the DS's proposal to classify spirodiclofen as STOT RE 2 (H373) based on the dog studies and the assessment that takes into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs. The classification should apply to all routes of exposure with no specific organ specified.

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2015	Germany		MemberState	18

Comment received

It is supported to classify spirodiclofen for specific target organ toxicity. Several target organs (liver, prostate) fulfil the criteria for being classified STOT-RE cat. 2. However, for the adrenal effects, due to the rather low dose levels the effects occur at, category 1 seems more appropriate.

Dossier Submitter's Response

Thank for the support for the classification for STOT RE.
The comments concerning the category are noted. In his comments, the Member State Germany considers the adrenal effects sufficient for classification for specific target organ toxicity in category 1.

Adrenal effects were observed in mouse, rat and dog:

- ➔ Mouse
  - Oral
    - 13-week study: increased adrenal organ weight and cytoplasmic vacuolisation (≥ 233.6 mg/kg bw/d; i.e. above the upper limit of 100 mg/kg bw/d for STOT RE 2), degeneration of cortical cells and mononuclear infiltrate (2685.2 mg/kg bw/d; i.e. above the upper limit of 100 mg/kg bw/d for STOT RE 2)

- 18-month study: increased adrenal organ weight and vacuolisation (≥ 610 mg/kg bw/d; i.e. above the upper limit for STOT RE 2)

➔ Rat

- Oral
  - 4-week study: no adrenal effects observed
  - 14-week study: increased adrenal organ weight (≥851.4 mg/kg bw/d; i.e. above the upper limit for STOT RE 2), cortical vacuolisation (6.6+32.1 mg/kg bw/d: within range of historical controls, ≥166.9 mg/kg bw/d: above the upper limit for STOT RE 2)
  - 108-week study: increased adrenal organ weight (≥2.04 mg/kg bw/d), cytoplasmic vacuolisation and adrenocorticocellular hyperthrophy (110.14 mg/kg bw/d; i.e. above the upper limit for STOT RE 2)
  - 2-generation study: increased adrenal weight P0-animals (134.8-139.2 mg/kg bw/d), vacuolisation of adrenal gland P0-females (27.6 mg/kg bw/d)
  - 13-week neurotoxicity study: no adrenal effects described
  - 4-week immunotoxicity study: no adrenal effects described
- Dermal
  - 4-week study: reduced adrenal weight (1000 mg/kg bw/d; i.e. above the upper limit for STOT RE 2)

➔ Dog

- Oral
  - 4-week study: increased adrenal weights, cytoplasmic vacuolisation (≥ 65.5 mg/kg bw/d; i.e. below the upper limit of 300 mg/kg bw/d for STOT RE 2 classification, above the upper limit of 30 mg/kg bw/d for STOT RE 1 classification)
  - 8-week study: increased adrenal weight, cytoplasmic vacuolisation, mononuclear cell infiltration adrenal cortex (≥2.9 mg/kg bw/d; i.e. below the upper limit of 150 mg/kg bw/d for STOT RE 2 classification, below the upper limit of 15 mg/kg bw/d for STOT RE 1 classification)
  - 14-week study: increased adrenal weight (≥27.3 mg/kg bw/d; i.e. below the upper limit of 100 mg/kg bw/d for STOT RE 2 classification, above the upper limit of 10 mg/g bw/d for STOT RE 1 classification), cytoplasmic vacuolisation, mononuclear cell infiltration adrenal cortex (≥8 mg/kg bw/d; i.e. below the upper limit of 100 mg/kg bw/d for STOT RE 2 classification, below the upper limit of 10 mg/g bw/d for STOT RE 1 classification)
  - 52-week study: increased adrenal weight (≥0.57 mg/kg bw/d; i.e. below the upper limit of 25 mg/kg bw/d for STOT RE 2 classification, below the upper limit of 2.5 mg/kg bw/d for STOT RE 1), vacuolisation of adrenals (≥4.54 mg/kg bw/d; i.e. below the upper limit of 25 mg/kg bw/d for STOT RE 2 classification, above the upper limit of 2.5 mg/kg bw/d for STOT RE 1)

Although most of the adrenal effects were observed at effective dose levels below the upper limit for STOT RE 2 and therefore do not warrant classification, some of the adrenal effects in dogs were observed below the upper limit for STOT RE 2 classification and even below the upper limit for STOT RE 1 classification. Effects included increased adrenal weight, cytoplasmic vacuolisation and mononuclear cell infiltration in the adrenal cortex.

Although these effect clearly point towards the adrenals as target organ, these effects are considered not severe enough to fulfil the classification criteria (i.e. no evidence of marked organ damage cf. CLP Guidance). In summary, the adrenal effects were, at least in mouse and rat, observed at effective dose levels above the upper limit for STOT RE 2, and in general do not fulfil the classification criteria based on observed severity, no evidence of marked organ damage or dysfunction cf. CLP-guidance. Therefore, in the opinion of the Dossier Submitter these adrenal effects do not warrant classification for STOT RE.
RAC's response
RAC appreciates the German CA comment on STOT RE classification. RAC uses only the dog studies for classification not the mice or rat studies. From the dog studies, the adrenal effects are not considered severe enough to be used for classification purposes (see response to comment 17). From the other effects in dogs (hematological parameters, liver) classification in Cat 2 is proposed.

OTHER HAZARDS AND ENDPOINTS – Aspiration Hazard

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2015	Germany		MemberState	19
Comment received				
It is supported not to classify spirodiclofen for aspiration hazard.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
RAC appreciates the general support of the German CA not to classify spirodiclofen for aspiration hazard.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
04.12.2015	France		MemberState	20
Comment received				
We agree with the classification and M factor proposed for Environmental hazards.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
RAC appreciates the general support of the French CA to classify spirodiclofen as Aquatic Chronic 1; H410, M=10.				

Date	Country	Organisation	Type of Organisation	Comment number
03.12.2015	Finland		MemberState	21
Comment received				
We support the proposed classification for environmental hazards Aquatic Chronic 1 – with M-factor of 10 for Spirodiclofen.				
Dossier Submitter's Response				
Thank you for the support.				

RAC's response
RAC appreciates the general support of the Finnish CA to classify spirodiclofen as Aquatic Chronic 1; H410, M=10.

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2015	Germany		MemberState	22
Comment received				
page 9: Proposed harmonised classification and labelling based on CLP Regulation: We support the proposed environmental classification and labeling as Aquatic chronic 1 (H410) as well as the M-Factor of 10.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
RAC appreciates the general support of the German CA to classify spirodiclofen as Aquatic Chronic 1; H410, M=10.				