Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Spirotetramat (ISO)

EC number: not allocated
CAS number: 203313-25-1

CLH-O-0000001688-63-02/A2

Adopted

10 September 2013

ANNEX 1 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON SPIROTETRAMAT (ISO)

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

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

Substance name: Spirotetramat (ISO)
EC number:
CAS number: 203313-25-1
Dossier submitter: Austria

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
09/11/2012	France		Member State	1
Comment received				
We agree with the classification proposal for human health and the environment (Skin sensitisation, Eye irritation, Reproductive toxicity, Hazardous to the aquatic environment).				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted				

Date	Country	Organisation	Type of Organisation	Comment number
08/11/2012	Germany		Member State	2
Comment received				

The German CA supports to establish a harmonised classification and labelling for Spirotetramat, which is a new active ingredient in plant protection products.

1.3 Proposed harmonised classification and labelling based on Regulation (EC) No 1272/2008 and/ or DSD criteria

The first part of this section should probably be the presentation of the classification of "Spirotetramat" (cf. Table 2) that means no S-phrases, no Signal word, no Symbols and no Precautionary statements but Hazard class and category.

Concerning labelling based on Regulation (EC) No 1272/2008 (after Table 3) we like to remark the following:
- The applicable pictograms (GHS07, GHS08 and GHS 09) are missing and should therefore be added.
- The Hazard statement H400 is part of the classification but not of the labelling and should therefore be omitted here.
- The Precautionary statement "(P102)" should be added in case the substance may be available for the consumer/ general public.
- Furthermore Precautionary statements (first aid) like "P305 + P351 + P338" and/or "P337 + P313" are missing we think.
- Is "P373" a typing error?
- Finally is assignment of "EUH401" intended for active substances too?

Concerning labelling based on Directive 67/548/EEC (after Table 4) we like to remark the following:
- The indication of danger/symbol "Xi" can be omitted because we have "Xn".
- The S-phrase 2 should be as usual for substances in brackets "(S2-)".
- Having "S36/37/39" "S24/25" is in our opinion dispensable and can be deleted.

- The number of S-phrases is relatively large; some of them (S27/28, S56, S57) are quite unusual compared to similar labels (R-phrases).
- Furthermore some S-phrases (S13, S56) are not applicable if the substance is not likely to be used by the consumer/ general public.
- On the other hand taking into account the properties of the substance and the fact that it is a powder the assignment of "S22" should be considered.

Dossier Submitter's Response

Comment noted, the revised proposal is:

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

CLP Regulation	Skin Sens. 1; H317 Eye Irrit. 2; H319 Repr. 2, H361fd Aquatic Acute 1; H400; M-Factor 1 Aquatic Chronic 1; H410; M-Factor 1
Directive 67/548/EEC (Dangerous Substances Directive; DSD)	Xi, R36 R43 Repr. Cat. 3; R62 - 63 N; R50/53

after Table 3

Labelling:

Signal word:	Warning
Symbols:	
Hazard statements:	H317, H319, H361fd,
Precautionary statements:	P201, 202, 260, 263, 264, 270, 272, 280, P273, P391, P501

after Table 4

Labelling:

Indication of danger:	Xn, N
R-phrases:	R36, R43, R62, R63, R50/53
S-phrases:	S2, 13, 20/21, 24/25, 27/28, 36/37/39, S56, S57, S60, S61

RAC's response

Noted

Date	Country	Organisation	Type of Organisation	Comment number
30/10/2012	Germany	Bayer CropScience AG	BehalfOfAnOrganisation / Company-Manufacturer	3

Comment received

please refer to the attached statement plus related bibliography

ECHA comment: The document "Spirotetramat BCS comments to the CLH-Report (dated 28 AUG 2012 –Version no 4)" was submitted as a separate attachment [Attachment 1]. The "General Comments" section of the document is copied below:

General comments (Summary):

Several comments are made in the next chapters for the reproductive toxicity studies (i.e. definitive 2-gen study and 1-gen dose range finder [DRF] study), highlighting that the **sperm cell effect/testicular toxicity in rats is a high dose phenomenon** with an overall

reproductive NOAEL of 320 mg/kg and a threshold level at >320 – 400 mg/kg bw/day. The low toxic potency, the relatively high doses with the prolonged exposure period needed to induce spermatid injury, and the reversibility are based on special toxicokinetics of the anionic spirotetramat metabolites. The negatively charged main rat metabolites (i.e. enol and desmethyl-enol) require **active** elimination by organic anion transporter (OAT) proteins located in the renal tubule cells as confirmed by an in vitro assay (Kühne, 2011) submitted to the RMS in 2011 which has not yet considered in this CLH report.
Organic anion transporters mediate monovalent anion transport through the cell membranes and can become saturated after exaggerated repeated high experimental doses. This leads to a transition from "first order" to "zero-order" kinetics, implying that the elimination rate of the anionic spirotetramat metabolites is no longer proportional to the systemic drug concentration beyond threshold doses of > 320 - 400 mg/kg bw/day (as stated also on page 28, line 15).
Furthermore, it is plausible that the kidney toxicity observed (only at high doses) in 6000 ppm F1 males has resulted in a downregulation of renal transporter protein expression and function. This may also impair the OAT-mediated transport capacity in high dose animals, further lowering the renal elimination rate of anionic spirotetramat metabolites at these high doses.
The low toxic potency is confirmed by the high NOAEL of 148 mg/kg bw/day in the 90-day rat study and by the lack of testicular toxicity in 6000 ppm P(arental)-generation males at 320 and 419 mg/kg bw/day in the 1-gen DRF study and the definitive 2-gen reproductive toxicity study, respectively.
The onset of testicular toxicity at high doses only is demonstrated by the discrepancy in reproductive function outcomes at 6000 ppm between P- and F1-generation males in the 2-gen and the 1-gen DRF study (note that in the 1-gen DRF study F1 male offspring were exposed to the same dietary levels as the P-generation males, but only for a shortened time period until 8 to 9 weeks of age in so-called F1 eight-to-nine-weeks old "interim" males).
In the definitive 2-gen study, 6000 ppm P-gen males exhibited no sperm cell abnormalities following average daily doses of 419.3 mg/kg bw. Only 6000 ppm F1-gen males showed abnormal sperms, however at a higher average daily dose of 486.7 mg/kg bw demonstrating the existence of a steep dose-response relationship beginning at high doses.
The difference in active ingredient intakes between both generations of the 2-gen study is related to the fact that F1-gen males were younger (7 wks old; 189.4 g body weight) at start of the premating period compared to P-gen males (9 wks old; 277.2 g bw), which resulted in a higher mean food / test substance intake in F1-gen males (486.7 mg test substance/kg bw/day) compared to P-gen males (419.3 mg test substance/kg bw/day). Thus, the reproductive toxicity is a function of dose and not a progressive effect over successive generations, as stated in the CLH report on page 56, last paragraph; conclusion of point 4.11.1.1).
The mouse conjugated the enol with glucuronic acid to a high extent, while the rat did not conjugate the enol. The ability of the mouse to conjugate a fraction of this major spirotetramat metabolite with glucuronic acid shunts a portion of the free enol for elimination by other "membrane transporter proteins" (i.e. conjugate transporters), preventing saturation of the renal elimination process at high doses and thus protecting mice from spirotetramat-induced testicular toxicity at comparable high dose levels. The absence of kidney toxicity in mice at the limit dose may also represent a factor contributing to the species differences in the elimination capacity. Human liver cells proved to conjugate the enol also to some extent, making it likely that the elimination of spirotetramat metabolites is facilitated by the concomitant use of conjugate transporters as well. Even if humans would be as sensitive as rats, the expected complete recovery of testicular toxicity, the high-dose phenomenon and the repeated-dose prerequisite render it very unlikely that humans, even after high-dose accidental exposure or self-poisoning, will develop testicular toxicity. A very high (>6000-fold) margin of safety exists between the overall rat testicular NOAEL (320 mg/kg bw) and the maximum allowable dietary / occupational human exposure level (AOEL; ADI: 0.05 mg/kg bw/d).
Based on the above rationale, Bayer CropScience is of the opinion that it is not justified to

classify BYI 08330 as a reproductive toxicant as it is highly unlikely that such effects can occur in humans even under the worst case scenarios of high dose accidental poisoning or even self-poisoning attempts.
This view is supported by a comment from Directive 67/548: *"Even when clear effects have been demonstrated in animal studies the relevance for humans may be doubtful because of the doses administered, for example, where effects have been demonstrated only at high doses, or where marked toxicokinetic differences exist."*
In this sense, the kinetics of saturable processes in the metabolism and elimination of xenobiotics at high doses must be considered when evaluating human health hazards.
Similar toxicokinetic peculiarities are demonstrated for caffeine, which also triggers reproductive (testes atrophy) and developmental (skeletal malformations) toxicity in rats at high experimental bolus doses of 250 mg/kg bw/day, when the elimination capacity becomes saturated (Christian and Brent, 2001). Anticipated human exposure to caffeine is approximately 50-fold higher compared to spirotetramat residue levels, and a concern on human health is not anticipated for caffeine if taken at reasonable daily doses.
Thus, the need for classification and labelling with R62 and R63 should be discussed again.

Dossier Submitter's Response

In addition to testicular histopathology observed following subchronic and chronic exposure of male rats to spirotetramat, evidence of male reproductive toxicity was provided in the 2-generation reproductive toxicity study. Abnormal sperm cells were reported in F1-generation male rats treated with 6000 ppm (419 mg/kg bw/day) spirotetramat in the diet, and decreased reproductive performance was also observed in one of these males. Similar results were obtained in the 1-generation reproductive toxicity range-finding study, in which decreased sperm motility and progression and increased abnormal sperm cells in the epididymides were observed in F1 males at ≥6000 ppm (320 mg/kg bw/day). The highest dose level of 10000 ppm, equivalent to 538 mg/kg bw/d, was associated with no fertility in parental generation animals. There were no implantation sites noted in the females due to treatment-related effects on sperm cells of males at this dose level (increased numbers of abnormal sperms, reduced epididymal sperm counts, decline in both motility and progression of epididymal sperm cells). Absolute and relative weight of the cauda epididymis was decreased in parental males. Histopathology showed abnormal sperm cells of minimal to moderate severity in the epididymis and the cauda epididymis.
Renal toxicity was also observed in F1 adults in the 2-, but not 1-generation reproductive toxicity study.
Offspring toxicity was limited to decreased body weights in both studies, observed in F_1 and F_2 pups respectively of both sexes during lactation at 6000 ppm (320 and 419.3 mg/kg bw/day respectively). Decreased body weights were also observed in parental animals at the same dose. The difference in dose between the two studies was attributed to increased food consumption of parental animals in the 2-generation study.
Development of the sexual organs of offspring (balano-preputial separation, vaginal opening) was unaffected in both studies. Developmental toxicity in the absence of maternal toxicity was not observed in either the rat or rabbit.
In February 2008 the notifier submitted a position paper (*High dose reproductive effects in male rats and their relevance to humans; Temerowski M., 2008*). It was stated that the effects on testicular spermatogenesis were attributed to the BYI 08330-enol, which is the main metabolite in the rat. BYI 08330-enol is further metabolised by oxidation reactions to BYI 08330-desmethylenol, BYI 08330-enol-alcohol and BYI 08330-ketohydroxy. Oxidation products accounted for approximately 14%. Conjugation was not detected. In the mouse, conjugation of BYI 08330-enol with glucuronic acid accounted for approximately 30%. In human liver cells, conjugation to BYI 08330-enol-glucuronic acid was 6%. The in vitro conjugation rate is dependent on the concentration used and declined in mice from 30% to 9% and in humans from 6% to 2% at liver concentrations of 19 µg/g and 190 µg/g BYI 08330, respectively. Glucuronidation of the BYI 08330-enol in mice leads to much lower systemic levels of free BYI 08330-enol when compared to the rat. The conjugation enables the mouse to utilize separate active transport systems in the kidneys, thus avoiding a saturation of the elimination process. Thus, the utilization of different transport systems renders the mouse less sensitive to BYI 08330-mediated testicular toxicity when compared to the rat. Based on the

metabolic similarity between mice and humans, it is likely that humans are also less sensitive to BYI 08330-mediated testicular toxicity than rats.
This statement of the notifier can not be agreed to. In contrast to mice, for humans the ability to conjugate BYI 08330-enol with glucuronic acid is fivefold lower than for the mouse (dependent on the concentration, in humans 6% respectively 2%, in mice 30% respectively 9%). Therefore a similarity in the metabolic pathway can not be followed. As for humans conjugation is only 2% at high doses, it can not be assumed that humans are less sensitive to Spirotetramat than rats.
Mechanistic studies were provided by the notifier and evaluated already in the DAR. In an investigative study designed to explore the time of onset of testicular toxicity in rats, decreased epididymal sperm counts were recorded after 10 days of treatment with 1000 mg/kg bw/day spirotetramat by gavage. Repeated dosing, therefore, is necessary to produce male reproductive toxicity in rats (*Kennel, P. 2005. BYI 08330: Evaluation of the potential reproductive toxicity in the male rat following daily oral administration by gavage. Bayer CropScience, 355, rue Dostoievski, BP 153, 06903 Sophia Antipolis Cedex, France. Laboratory Report No. SA 041181, May 23, 2005.)*
In a second investigative study *(Tinwell, H. 2006. BYI 08330-ENOL: Investigation of the testicular/sperm toxicity in the rat following 21 days of exposure by gavage. Bayer CropScience, 355, rue Dostoievski, BP 153, 06903 Sophia Antipolis Cedex, France. Laboratory Report No. SA 06011, June 30, 2006)*, male rats were treated by gavage with the enol metabolite of spirotetramat for 21 days at a dose of 800 mg/kg bw/day. Spermatotoxicity, abnormal sperm, and Sertoli cell vacuolation were observed in the testes-epididymides of treated animals. Based on these and similar results with the parent compound, it was concluded that male reproductive toxicity in rats is caused either by the parent compound alone or by its enol metabolite following enzymatic cleavage.
2011the notifier submitted an new *in vitro assay:* Inhibitory Potential of BYI08330-enol and BYI08330-desmethylenol as inhibitors on hOAT1, hOAT3 and hOAT4 in transfected HEK-cells (Kühne, A. 2011).
Based on pharmakokinetik data and pharmakokinetik modelling it is assumed that the BYI08330-enol and the BYI08330-desmethylenol will be distributed and eliminated (mainly via urine) by membrane transporters. Both metabolites are weak acids and thus present as monovalent anions at physiological pH, and therefore cannot diffuse efficiently through cell membranes.
At spirotetramat doses above 300 mg/kg bw/day, active transport capacity seems to become saturated leading to an accumulation of these main metabolites in the body. This is also suggested by a mechanistic study in rats (Kennel, 2005, M-252001-01-2). More than 10 daily high doses of spirotetramat were necessary to elicit sperm cell effects in rats.
In order to investigate whether BYI08330-enol ("E") and BYI08330-desmethylenol ("DME") are substrates or inhibitors of human organic anion transporters (hOAT), transporter assays using hOAT-transfected human embryonic kidney (HEK)-cell lines were performed. The aim of the study is to characterize whether E and DME are interacting with hOAT1-mediated p-aminohippuric acid (PAH) uptake and with hOAT3- as well as hOAT4-mediated estrone sulfate (ES) uptake, respectively.
In the ADME study in rats (Klempner, 2006; M-268709-02-2), maximum plasma concentrations (C_{max}) of approximately 10 to 550 µM of E and DME have been reached in male rats after a single low and moderate dose of spirotetramat (i.e. 2 and 100 mg/kg bw). Therefore, 20 µM and 200 µM of the enol and the desmethyl-enol were used in these OAT-interaction studies
Two concentrations of PAH and ES at the Km and 1/10 Km value, determined by PortaCellTec biosciences, were used to characterize the inhibitory potential of E and DME. Probenecid, a well known competitive inhibitor of hOAT1 and hOAT3, and Sulfobromophthalein disodium salt hydrate (BSP), an inhibitor of hOAT4, were used in the experiments as positive controls.
The results of the studies can be summarized as follows:
hOAT1-mediated [^{3}H]PAH uptake was significantly inhibited by 200 µM E by a maximum value of 36±2% (100 µM PAH). The second test substance DME showed no interaction with the OAT1-mediated PAH uptake.
hOAT3-mediated [^{3}H]ES uptake was significant inhibited by both concentrations of E by a maximum value of 34±2% (20 µM) and 76±1 % (200 µM). The test substance DME had a much lower

inhibitory effect on the OAT3-mediated ES-uptake (1 µM) with values up to a maximum 10±8% (20 µM) and 24±4% (200µM).
hOAT4- mediated [^{3}H]ES uptake was significantly reduced by both test substances. The inhibitory effect was concentration dependent with a maximum value at the lower ES concentration (1 µM) of 73±1 (200 µM E) and 62±0.3 % (200 µM DME), respectively.
In conclusion, BYI08330-enol and BYI08330-desmethylenol clearly interact with the human OAT transporters. Membrane transporter proteins involved in the renal elimination of xenobiotics may become saturated after exaggerated high experimental doses. This may lead to a transition from first order kinetics to zero-order elimination kinetics. Thus, identification of transporter interactions may help in predicting the pharmacokinetics of drugs and explaining its toxic effects at high dose levels. Since substrates affinity and inhibitory potential of human, mouse and rat OATs are highly overlapping, it is inferred that the BYI08330-enol and the BYI08330-desmethylenol are also eliminated via rat organic anion transporter orthologs in the kidneys.
Because the enol metabolite of spirotetramat was found to be partly conjugated by glucuronic acid in mice and humans (Totis, 2006; M-274118-01-2), other important transporters like OATPs, mainly expressed in the liver, and also the efflux multidrug transporters (e.g. MDR1, MRP2, MRP4) could also be involved in the elimination of this conjugated metabolite.

RAC's response

It is agreed that effects of spirotetramat on the male reproductive system are observed in rats at the highest doses tested and such effects are not reported in mice and dogs.
The following points are however noted:

- It cannot be excluded that mechanistic elements other that toxicokinetics may explain the species specific sensitivity
- Existence of a more prominent glucuronidation pathway is identified in mice in an *in vitro* study but it is not known whether it would result in lower *in vivo* systemic levels of spirotetramat and its metabolites at high dose.
- *In vitro* data shows that glucuronidation pathway is five time lower in human than in mouse hepatocytes and the relevance of mouse model as a model similar to humans cannot be accepted.
- In human hepatocytes, due to limited metabolisation of the enol metabolite into further metabolites and limited conjugation, the level of enol metabolite formed *in vitro* is greater than in mouse or rat hepatocytes. This metabolite is likely to be involved in reproductive toxicity of spirotetramat so that toxicokinetic data does not dismiss the relevance of effects for humans.

It is also noted that consideration of aspects related to risk assessment are not relevant for classification that aims to identify the hazardous intrinsic properties of the substance.

Date	Country	Organisation	Type of Organisation	Comment number
25/10/2012	Spain		Member State	4

Comment received

p.6 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria. The Spanish CA agrees with the Austrian proposal to classify spirotetramat as: Eye Irrit. 2, H319; skin sens.1, H317; Repr.2, H361fd according to Regulation EC 1272/2008 and as Xn; R36, R43, Repr. Cat.3; R62-63 according to Directive 67/548/EC.

Dossier Submitter's Response

Noted

RAC's response

Noted

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
08/11/2012	Germany		Member State	5

Comment received

p. 50-53: We support not to classify Spirotetramat for carcinogenicity

Dossier Submitter's Response
Noted
RAC's response
Noted

MUTAGENICITY

Date	**Country**	**Organisation**	**Type of Organisation**	**Comment number**
08/11/2012	Germany		Member State	6
Comment received				
p. 48 & 49: We support not to classify Spirotetramat for mutagenicity				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted				

TOXICITY TO REPRODUCTION

Date	**Country**	**Organisation**	**Type of Organisation**	**Comment number**
09/11/2012	Sweden		Member State	7
Comment received				

General
SE does not support the proposed classification of spirotetramat regarding fertility as a reproductive toxicant, category 2. Based on the robust finding of testis toxicity in rats and the absence of information that casts doubt on the human relevance of this finding, spirotetramat fulfills the criteria for classification regarding fertility as a reproductive toxicant category 1B. SE supports the proposed classification of spirotetramat regarding developmental toxicity as a reproductive toxicant category 2.

Effects on fertility
Information provided from histopathology in the 90 day sub chronic toxicity study in the rat showed that spirotetramat cause testis toxicity at the 10 000 ppm (616 mg/kg/day). The toxicity was revealed as abnormal spermatozoa and hypospermia in the epididymidis, decreased testicular weight and testicular degeneration and vacuolization. These effects occurred at high/medium incidences and at a dose level that did not cause marked general toxicity (as compared to the controls, a reduced terminal body weight was recorded but no adverse clinical signs or mortalities were observed). Histopathological examination of recovery animals (after 4 weeks) indicated that these changes were partially reversible (signs of toxicity were still present in 1 animal out of 10) but no sperm analysis or mating trial was performed. In the 1yr chronic toxicity study in rats, signs of testis toxicity (exfoliated germ cells/debris in the epidymidis and abnormal spermatozoa) were also observed at the high dose level (7500 ppm, 414 mg/kg/day). Follow-up studies in rat with the enol metabolite of spirotetramat produced overall similar testis toxicity as the one observed after administration of spirotetramat, making it likely that it is the enol metabolite that is responsible for the observed effects.
Infertility was recorded at the 10 000 ppm dose level (538 mg/kg/day) in the parental generation in the multigeneration dose-range finding study. A significant decline in motility and progression of epididymal sperm as well as a slight decline in epididymal sperm counts and a significant increase in number of abnormal sperms were recorded in males at this dose level. Histopathology revealed abnormal sperm in the epididymidis (all animals, severity grade minimal to moderate) but no effects on the epithelial lining of the semniferous tubules. A slight decline in sperm motility and progression as well as an increased incidence of abnormal sperms was also recorded for the F1 males at the 6000 ppm (400 mg/kg) dose level. Furthermore, similar effects on sperm morphology was also observed in the 6000 ppm F1 males in the multigeneration study, were the single male that was infertile was the same male that had the highest incidence of abnormal sperms and also displayed abnormal sperm in the epididymis at the histopathological evaluation.
No testis toxicity was observed in studies in dogs, although this species was less tolerable towards spirotetramat and consequently lower dose levels (top dose 6400ppm, 104mg/kg) were used as compared the dose level used in the rat (10 000 ppm, 616 mg/kg). No toxicity of any kind was observed in the mouse at doses up to and including the highest dose level used, 7 000 ppm (1305/1515 mg/kg/day (M/F) in the 90 day sub-chronic toxicity study.

An in vitro comparative metabolism study using hepatocytes from male rats, mice and humans was performed (section 4.1.1 and section 4.11.4). Conjugation of the enol metabolite with glucuronic acid was observed for mice and humans whereas no conjugation was seen in the rat hepatocyte incubation (however results from in vivo disposition study in rats indicate that glucoronidation of the enol metabolite do occur although only as a minor metabolic pathway, see section 4.1.1). Based on the result from this in vitro comparative metabolism study, industry concludes that based on the metabolic similarities between mice and humans it is also likely that the humans are less sensitive as compared to the rats to spirotetramat induced testicular toxicity.
However, the information provided from the comparative metabolism study (sections 4.1.1 and 4.11.4) do not provide convincing and clear mechanistic information that show that the enol metabolite would be formed in such a minute magnitude or detoxified in such an extent that one should consider the testis finding in rats as having limited or no relevance for humans. As stated on page 72 the ability to conjugate spirotetramat-enol is fivefold lower for humans as compared to mouse, and at high doses the extent of conjugation (in vitro) of the enol metabolite is only 2% in humans. However, for transparency, details regarding the experimental design of the in vitro comparative metabolism study should be included in section 4.1.1 so that the robustness of these results can be properly assessed. It is also unclear to the reader if there are species differences in the activation of spirotetramat to its enol metabolite due to poor data presentation in section 4.1.1. We would also find it helpful if the discussion in para 4.11.4 considered the overall robustness of the in vitro result and what influence inter-individual differences in the human population regarding the glucoronidation pathway (including the transporters involved in the kidney excretion of the enol-glucoronidate) have on the interpretation of the result. Furthermore possible species differences regarding the activation rate of spirotetramat to the enol metabolite should also be discussed.
In summary, there is no information that cast doubts on the human relevance of the observed testis finding, i.e. testis toxicity leading to functional effects in the dose finding study to the multigeneration study as evidenced by infertility at the high dose level. Therefore, based on this finding spirotetramat fulfills the criteria for being classified regarding fertility as a reproductive toxicant in category 1B.

Developmental toxicity
We support the proposed classification of spirotetramat regarding developmental toxicity as a reproductive toxicant in category 2.
The basis for this classification is the presence of an increased incidence of malformations of dysplasia of the forelimb bone and an increased incidence of altered appearance of sacral vertebral arch (pelvic shift) in the high dose group in the oral developmental toxicity study in the rat (Klaus 2004). The recorded incidences were outside the concurrent and historical control data. In addition one case of supernumerary lumbar vertebra was also recorded. Some maternal toxicity was seen at this dose level (a 20% reduction in body weight gain during the period of gestation). A delayed ossification of several bones was also recorded which most likely can be related to the lower fetal weight (85% of controls) observed at this dose level and partly to the recorded maternal toxicity. A clear increased incidence of wavy ribs and of the 14 rib was also recorded in the high dose group. A tendency to delayed ossification (mainly affecting the distal phalanges of the toes and digits) as well as an increased incidence of wavy ribs were also observed at the low and intermediate dose levels. These findings were recorded at dose levels where no effects were seen on fetal weights and no maternal toxicity was observed.

Specific comments
• Section 4.11.5. Please provide a rationale for why spirotertramat is proposed to be classified regarding fertility as a category 2, and not as a 1B reproductive toxicant.
• Please clarify if the in vivo and in vitro data presented in section 4.1.1 originates from the position paper mentioned in section 4.11.4.
• Section 4.1.1. is very condensed and contains information that is derived from multiple studies. This information needs to be presented in a more structured way so it is clear what results are derived from which study. Also information should be added so the reader can understand how the comparative in vitro metabolism studies were performed. What was the source for the hepatocytes and what positive controls were used? What was the number of biological repeats (i.e. was the results reproducible if hepatocytes from another animal/donor was used) and at what time of incubation were the different metabolites analyzed? What was the rationale for the chosen incubation concentrations of spiroteramat (19 µg/g and 190 µg/g) and how did they relate to exposure in the in vivo tox studies etc. One suggestion would be to add a table that presents information on

amount/percentage of the different metabolites formed in the hepatocyte experiments from the different species (perhaps one could just copy a table that might be provided in the position paper by Temerowski that is mentioned in section 4.11.4.). Furthermore to get a grip on the inter-individual variations regarding metabolism in humans it is also important that information is included that clarifies how this aspect has been taken into consideration when designing the human hepatocyte experiment.

• To help the reader to absorb the information in section 4.1.1. We also suggest that a figure which shows the metabolic pathway of spirotetramat is included. In this figure one could add information that indicate which pathways are dominating in which species and also indicate the metabolite that dominates in rat plasma.
• In addition, there are also some more editorial comments
o Should it be "extent of absorption" instead of absorption rate (3rd sentence in section 4.1.1 and further down in that section
o Lower part of section 4.1.1. on page 27. Please clarify in which matrix that conjugation product was recorded.
o Last 6 rows in para 4.1.1. on page 28. Should it not be "extent of conjugation"? Since no in vivo data is available from the mouse, the statement that glucoronidation of the enol metabolite leads to a much lower systematic levels of the free enol metabolite when compared to the rat, is actually a conclusion/speculation drawn from in vitro data. Please clarify this sentence to avoid misunderstanding.
o The P in the abbreviation PBPK (page 28) stands for physiological and not pharmacologically as stated in the text.
• Section 4.11.4. The information that is presented after the reference to the position paper by Temerowski:
o Please clarify when presented information is from in vivo studies and when it is from the comparative in vitro metabolism studies, and when statements actually are conclusions/speculations derived from in vitro data. Se example provided above for section 4.1.1.
o The sentence "Conjugation was not detected." is correct if it refers to rat in vitro metabolism, but when reading section 4.1.1. it is clear that conjugation of the enol metabolite is a minor pathway in the in vivo situation. Please clarify.
o We lack a discussion in section 4.11.4. around how robust the data from the comparative in vitro metabolism studies really are. For example no information is provided that makes it possible for the reader to decide if 6% really differs from 2% and even if 30% differs from 9% and therefore one can ask how solid the statement "the in vitro conjugation rate is dependent on the concentration used" really is. Another aspect that needs attention in the discussion is that there is no data on possible inter-individual differences for the glucoronidation pathway (including the transporters involved in the kidney excretion of the enol glucornidate) and how that would impact the pattern of metabolites formed in humans.

Dossier Submitter's Response

Please refer to Dossier Submitter's Response to the Comments of Bayer CropScience AG from 30/10/2012 above.

RAC's response

Noted.
For developmental toxicity, it is noted that the interpretation of the data is generally shared with the exception for interpretation of findings on dysplasic forelimb bone. The incidence at high dose is 1.5% and exceed concurrent control incidence (0%) and historical control mean value (1.3%) but historical control range goes up to 4.3ù so that the incidence of this finding is considered to be within the historical control value and its relationship to treatment is unclear.

Date	Country	Organisation	Type of Organisation	Comment number
08/11/2012	United Kingdom		Member State	8

Comment received

Fertility
p56: In a dose range-finding study in the rat (Young 2006), at the 538 mg/kg bw dose level there was "no fertility was in the P-generation animals". It is not clear what is meant by this statement. It is also not clear whether the animals mated or not (i.e., were the females checked for the presence

of sperm/vaginal plugs)?

Developmental Toxicity
P69. It would be helpful if Table 24 gave an indication of the statistical significance of the foetal malformations observed, and an indication of their occurrence in the historical controls.

P72. Section 4.11.4: summary and discussion of reproductive toxicity. There is a lot of information in the reproductive toxicity section, and it is difficult for the reader to identify the key effects that have led the dossier submitter to their conclusion on classification.

In particular, it is not clear why the dossier submitter has proposed Category 3 (DSD)/Category 2 (CLP), rather than Category 2 (DSD)/Category 1B (CLP). For example, there is clear evidence of an adverse effect on fertility in male rats. The notifier has proposed a mechanism to suggest that these effects are not relevant to humans, however this mechanism is dismissed by the dossier submitter. An explanation should be included as to why the effects do not warrant classification in Cat 2 (DSD)/Cat 1B (CLP).

It would help the reader if the dossier submitter highlighted the effects that are considered to be relevant for classification, and compared them to the classification criteria under both pieces of legislation.

Dossier Submitter's Response

Fertility:
Males and females were exposed to the test material for ten weeks prior to mating. Mating was accomplished by co-housing one female with one male for up to seven consecutive days. During the mating phase vaginal smears were taken each morning and examined for the presence of sperm and/or internal vaginal plug. Females found to be inseminated were placed in a polycarbonate nesting cage. The day on which insemination was observed in the vaginal smear was designated day 0 of gestation for that female.
In order to evaluate those females which may have been inseminated without exhibiting sperm in the vaginal smear or an internal vaginal plug, all remaining females were placed in polycarbonate nesting cages following the 7-day mating period.
Developmental Toxicity Rabbit:
Retarded ossification of the 5th sternebra was seen only in the 10 mg/kg dose group and of 8th caudal vertebral arches in the 10 mg/kg and 40 mg/kg groups. All values regarding retarded ossification at the 10 mg/kg and 40 mg/kg levels lay well within the normal range of scattering for these findings in developmental toxicity studies in the strain of rabbits used. A dose dependency was neither evident for these findings and statistical significance was not seen when calculation was done on a litter basis. Therefore, a treatment related effect is excluded for the isolated findings of retarded ossification at the 10 mg/kg and 40 mg/kg levels.
The study data revealed the following mean percentages for 5th sternebra, unossified:
18.2 – 30.3* – 26.7 – 18.2.
The significant difference in the low dose group is covered by the HCD.

HCD for 5th sternebra, unossified:
23.6 – 33.8
24.1 – 43.0
18.9 – 40.3

The study data revealed the following mean percentages for the presence of the 8th caudal vertebral arch:
Right: 53.5 – 33.8** - 25.6** - 45%
Left: 53.5 – 33.8** - 25.6** - 45%

The significant differences in the low and mid dose group are covered by the HCD.

HCD for 8th caudal vertebral arch – present:
Right: 10.1 – 62.1%
Left: 10.1 – 62.1 %

Historical Data of Control Groups (2004)

External and Visceral Deviations of Fetuses

Species: **Rabbit** Strain: **CHBB:HM**

Study	Number of Examined Litters	Litters with Findings n	Litters with Findings %	Number of Examined Fetuses	Fetuses with Findings n	Fetuses with Findings %	Type of Finding
T0063272	18	2	11.1	134	3	2.2	liver whitish discoloration
		1	5.6		1	0.7	apex of the heart bluish discolored
T1073920	19	1	5.3	151	2	1.3	liver whitish discoloration
T3073931+	19	2	10.5	141	3	2.1	liver whitish discoloration
T6073925+	20	1	5.0	137	2	1.5	thymus enlarged
		1	5.0		1	0.7	liver whitish discoloration
		1	5.0		1	0.7	small accessory liver lobe
		1	5.0		3	2.2	stomach gaseous
T8073927	19	-	-	156	-	-	-

no historical control data in 2005
+ not checked by quality assurance

Historical Data of Control Groups (2006)

External and Visceral Deviations of Fetuses

Species: **Rabbit** Strain: **CHBB:HM**

Study	Number of Examined Litters	Litters with Findings n	Litters with Findings %	Number of Examined Fetuses	Fetuses with Findings n	Fetuses with Findings %	Type of Finding
T2073363	20	1	5.0	146	1	0.7	reddish brown mass in nasal cavity
		1	5.0		2	1.4	slight dilation of nasal cavity
		4	20.0		5	3.4	slight dilation of lateral brain ventricle(s)
		1	5.0		1	0.7	slight dilation of 3rd brain ventricle
		1	5.0		7	4.8	liver spongy
		1	5.0		3	2.1	liver pale
		2	10.0		2	1.4	whitish discoloration of liver
		1	5.0		2	1.4	distinct liver lobulation
T5073366	20	2	10.0	135	6	4.4	distinct liver lobulation
		1	5.0		1	0.7	whitish discoloration of liver
T5076390	20	3	15.0	160	6	3.8	slight dilation of lateral brain ventricle(s)
		1	5.0		1	0.6	distinct liver lobulation
		1	5.0		1	0.6	liver black vesicle
T9076394	17	1	5.9	136	1	0.7	whitish discoloration of liver

Historical Data of Control Groups (2007)

External and Visceral Deviations of Fetuses

Species: **Rabbit** Strain: **CHBB:HM**

Study	Number of Examined Litters	Litters with Findings n	Litters with Findings %	Number of Examined Fetuses	Fetuses with Findings n	Fetuses with Findings %	Type of Finding
T3077298	19	1	5.3	128	1	0.8	liver: whitish discoloration
T9077302	18	1	5.6	131	1	0.8	kidney: pale
T3077306	19	1	5.3	98	1	1.0	liver: whitish discoloration

Historical Data of Control Groups (2008)

External and Visceral Deviations of Fetuses

Species: **Rabbit** Strain: **CHBB:HM**

Study	Number of Examined Litters	Litters with Findings n	Litters with Findings %	Number of Examined Fetuses	Fetuses with Findings n	Fetuses with Findings %	Type of Finding
T1078259+	19	1	5.3	134	2	1.5	liver: whitish discoloration
T3078260+	18	2	11.1	112	3	2.7	liver: whitish discoloration
		1	5.6		1	0.9	liver: distinct liver lobulation
		1	5.6		2	1.8	limbs: musculature less than normal at both forelimbs and both hind limbs

+ not checked by quality assurance
no historical control data in 2009

Historical Data of Control Groups (2010)

External and Visceral Deviations of Fetuses

Species: **Rabbit** Strain: **CHBB:HM**

Study	Number of Examined Litters	Litters with Findings n	Litters with Findings %	Number of Examined Fetuses	Fetuses with Findings n	Fetuses with Findings %	Type of Finding
T8080622+	18	3	16.7	129	3	2.3	retina slightly folded
		2	11.1	129	3	2.3	whitish discoloration of liver
		1	5.6	129	1	0.8	several clear vesicle(s) in the tissue of the left lower liver lobe

+ not checked by quality assurance

Historical Data of Control Groups (2011)

External and Visceral Deviations of Fetuses

Species: **Rabbit** Strain: **CHBB:HM**

Study	Number of Examined Litters	Litters with Findings n	Litters with Findings %	Number of Examined Fetuses	Fetuses with Findings n	Fetuses with Findings %	Type of Finding
T2081977#	18	3	16.7	129	3	2.3	retina slightly folded
		2	11.1	129	3	2.3	whitish discoloration of liver
T5082410#	18	2	11.1	129	3	2.3	whitish discoloration of liver
T2082408#	18	2	11.1	129	3	2.3	whitish discoloration of liver

not checked by quality assurance

Summary and discussion of reproductive toxicity:
Please refer to Dossier Submitter's Response to the Comments of Bayer CropScience AG from 30/10/2012 above.

RAC's response

Noted

Date	Country	Organisation	Type of Organisation	Comment number
08/11/2012	Germany		Member State	9

Comment received

P 54-72: We support to classify Spirotetramat for reproductive toxicity category 2. We support the allocation of H361f - suspected of damaging fertility. However, concerning the fertility a more detailed discussion is necessary for justification of category 2 versus category 1b because of the results from the dose-finding study of Young, 2006 where at a dose of 10000 ppm (about 600 mg/kg bw/day) no pregnancies in the P0 generation were observed.

For developmental toxicity the identification with d "may cause damage to the unborn child" is proposed because of malformations and deviations observed in the studies of Klaus, 2004. We would like to have a further/more detailed discussion of the incidence of these malformations and deviations with respect to historical controls to conclude on the necessity of a classification of Spirotetramat as "Repr. 2; H361fd". Whether the observed skeletal malformations are sufficient for an additional allocation of H361d should be decided by ECHA/RAC.

p. 54, table 19, line 1: Please note that NOAEL for reproductive effects should be 31 mg/kg bw, since the described effects on sperm occur on males and not in females

Dossier Submitter's Response

Please refer to Dossier Submitter's Response to the Comments of Bayer CropScience AG from 30/10/2012 above also.
In the developmental toxicity study in rats, toxicity to the offspring was observed in the presence of maternal toxicity, including decreased food consumption and body weight/gain, at 1000 mg/kg bw/day. Reduced fetal weight and increased incidences of skeletal malformations and skeletal deviations were observed at 1000 mg/kg bw/day. Malformations at the high dose included one case of supernumerary lumbar vertebra, one case of cleft palate and one case of co-arctation of aortic arch. One case of atrial septal defect of the heart and microphthalmia were observed in the control, low and high dose each, but not at the mid dose.
Four cases of dysplastic forelimb bones (1.5 %) and three cases of malformed sacral vertebral arches with pelvic shift (1.1 %) were observed in the high dose. Historical control data of the performing laboratory (Bayer HealthCare AG) for dysplastic forelimb bones in studies conducted in the years 1999 – 2004 showed 26 affected animals out of 1975 animals, a percentage of 1.3 % [range 0.4 – 4.3% due to one study conducted in the year 2000, were 10 animals out of 232 were affected (4.3%)]. An incidence of 1.5 % in the study with spirotetramat is therefore outside the concurrent control (0.4%) and the historical control data (1.3%).
Statistically significantly increased incidences of sacral vertebral alterations (1.1 %) were observed at a dose level of 1000 mg/kg bw spirotetramat in comparison to the concurrent controls (0.0 %). The incidence in historical controls in studies conducted 1999 – 2006 showed 2 affected animals out of 6554 animals, a percentage of 0.03 % (range 0 – 0.4%).

Percentage of main skeletal observations in the rat developmental toxicity study with Spirotetramat (%)

	Dose (mg/kg/d)				% of Historical control data
Observations	**0**	**20**	**140**	**1000**	
Dysplasia of forelimb bones (%)	0.4	0.7	0.0	1.5**	1.3 (26 out of 1975 animals) [1999 – 2004]
Altered appearance of sacral vertebral arch; pelvic shift (%)	0.0	0.0	0.0	1.1**	0.03 (2 out of 6554 animals) [1999 – 2006]

Statistically significantly increased incidences of wavy ribs were observed at all dose levels compared to concurrent and historical control values. Statistically significantly increased numbers of fetuses with 14th ribs were observed at a dose level of 1000 mg/kg/d spirotetramat.

Percentage of observations at the ribs

	Dose (mg/kg/d)				Historical control data (range)
Observations	**0**	**20**	**140**	**1000**	
Wavy ribs	5.5	17**	17.8**	57.5**	(2.7 – 15.1) [1996 – 2001]
14th rib	15	10.7	12.6	45.9**	(0.0 – 13) [1996 – 2000]

Historical Control Data for preimplantation loss and mean number of fetuses
Excerpt from HCD data supplied in the rat developmental toxicity studies with BYI 08330

HCD ***Pages 609-637 of report no T9062786***
Pages 727-733 of report no T7063008

Study	Study	Mean no of implantations	Preimplantat loss (% per	Mean no of	No of females

Year	Number	per female	female)	viable fetuses	affected
1996	T…240	12,0	3,0%	11,2	20
	T...260	11,2	2,3%	10,7	18
	T…291	12,3	1,7%	11,3	18
	T…247	12,7	1,5%	12,0	14
	T…298	12,4	1,8%	11,6	17
1997	T…255	13,0	1,5%	12,1	17
	T…860	11,4	1,8%	11,1	14
	T…250*	12,0	1,8%	11,4	15
	T…255	13,1	0,8%	12,4	9
1998	T…366	12,0	1,8%	11,5	19
	T…370	12,8	1,7%	11,7	15
	T…380	12,1	1,6%	11,4	17
	T…375	11,8	1,2%	10,9	11
	T…390	12,3	1,6%	11,4	18
1999	T…880	12,2	1,7%	11,6	14
	T…311	11,9	2,0%	11,0	12
	T…318	13,0	1,5%	12,2	11
2000	T…551	12,1	2,7%	11,6	14
2001	T…765	13,8	2,1%	12,9	17
	T…563	12,5	2,3%	11,9	16
	T…750	12,1	1,9%	11,6	14
	T…800	12,6	1,7%	12,0	14
2002	T…568	12,4	2,2%	11,2	20
	T…784	12,3	1,5%	11,7	19
	T…558	12,8	0,8%	12,2	10
	T…786	13,0	1,5%	12,4	12
	T…590	11,6	0,9%	11,2	13
	T…600	12,6	1,7%	11,9	12
	mean	**12,4**	**1,7%**	**11,6**	**15,0**
	min	**11,2**	**0,8%**	**10,7**	**9,0**
	max	**13,8**	**3,0%**	**12,9**	**20,0**

* intravenous

Preimplantation loss and mean number of fetuses in the Teratogenicity test by the oral route in the rat with Spirotetramat

Observation	**Dose (mg/kg bw/day)**			
	0	**20**	**140**	**1000**
Animals assigned (mated)	25	25	25	25
Animals pregnant	20	24	23	22
Preimplantation loss (%) (Preimplantation loss/ female)	10.0 (1.5 ± 1.61)	15.6 (2.4 ± 2.06)	**20.2 (3.0 ± 2.12)***	11.2 (1.6 ± 1.53)

* Statistically different ($p < 0.05$) from the control.

Preimplantation loss was slightly increased in the 140 mg/kg bw/day group, and thus mean number of implantations was marginally lower. However, preimplantation loss and mean number of fetuses available for evaluation lay in the range of historical control data. An impact on the outcome of the study due to unequal distribution of females in the different groups with respect to preimplantation loss and number of implantation sites was excluded.

Data on co-arctation of aortic arch and cleft palate in historical controls and/or low dose groups from other studies were provided by the applicant.
In the Teratogenicity test by the oral route in the rat with Spirotetramat, at the highest dose level of

1000 mg/kg bw/day, 1 case of cleft palate and 1 case of co-arctation of aortic arch were observed.

Historical Control Data for aortic arch changes:

750

T9062786 BYI 08330

Number of Fetuses (Litters) with Defects of the Great Vessels

- Data of Different Study Groups -

(1999 – 2001)

Species: **Rat** Strain: **Hsd Cpb:WU**

Year	Study	Control	Dose 1	Dose 2	Dose 3
1999	T9067880	-	-	-	-
	T2061311	-	-	-	-
	T9061318	-	1 aortic spur	2 (2) aortic spur	1 aortic spur
	T0061319 [a]		-	1 aortic spur	#
2000	T4068631 [b]	1 persistent truncus arteriosus with VSD	-	-	-
	T5068551 [c]	-	-	-	-
2001	T5067750	-	-	-	1 multiple malformation of vessels
	T1067765	-	-	-	-
	T8068563	-	-	-	-
	T6062800	-	-	-	-

( ) number of litters affected
no more dosage
a same control as study T9061318
b dermal application; vehicle 1 % aqueous carboxylmethylcellulose, not reported
c b.i.d. administration

Historical Control Data for cleft palate findings:

T7063008 954 BYI 08330

Number of Fetuses (Litters) with Cleft Palate/Cheilochisis in Developmental Toxicity Studies

- Data of Different Dose Groups - (2001 – 2003)

Species: Rat Strain: Wistar Hsd Cpb:WU

Year	Study	Vehicle	Control	Dose 1	Dose 2	Dose 3	Dose 4
2001	T0062796[a]	0.5% CMC suspension	1	-	-	-	#
2002	T7062991[a]	Ethanol / Solutol / Aqua demin. (1:4:5)	0	2[b] (1)	0	0	#
	T3063590	Aqua demin. with PEG 6000	0	1[c,d]	0	0	#
	T9062786	0.5% CMC suspension	0	0	0	1	#
	T9063000	0.5% aqueous tylose suspension	0	1 (slight)	0	0	#
2003	T4063005[a]	Ethanol / Solutol / Aqua demin. (1:4:5)	0	0	1[d]	0	0
	T6062954	HPM cellulose (1%) / Solutol (2%) / Aqua demin.	0	0	1[d](slight)	0	#
	T7063008	0.5% CMC suspension	0	0	1 (slight)	0	#
	T0063010	0.5% aqueous tylose suspension	0	0	0	2 (2) (slight)	#
	T7062955	aerosol	1 (slight)	0	0	0	#

( ) no. of litters affected
\# no more dosage
a pilot study with n = 7 inseminated females/dose group
b in one fetus as part of a multiple malformation
c cheilochisis and microglossia
d as part of a multiple malformation

CMC = Carboxymethylcellulose

RAC's response

For developmental toxicity, it is noted that the interpretation of the data by DS is generally shared with the exception for interpretation of findings on dysplasic forelimb bone. The incidence at high dose is 1.5% and exceed concurrent control incidence (0%) and historical control mean value (1.3%) but historical control range goes up to 4.3ù so that the incidence of this finding is considered to be within the historical control value and its relationship to treatment is unclear.

Date	Country	Organisation	Type of Organisation	Comment number
30/10/2012	Germany	Bayer CropScience AG	BehalfOfAnOrganisation / Company-Manufacturer	10

Comment received

please refer to the attached statement plus related bibliography

ECHA comment: The document "Spirotetramat BCS comments to the CLH-Report (dated 28 AUG 2012 –Version no 4)" was submitted as a separate attachment [Attachment 1]. The section "Special comments to the CLH report" of the document" is copied below:

Special comments to the CLH report

Point 2.2 Short Summary, Page 12; Line 7: *Abnormal sperm cells were reported in F1-generation male rats treated with 419 mg/kg bw/day spirotetramat in the diet.*
The reviewer rightly reports that sperm cell abnormalities are noted in the 6000 ppm F1 males in the definitive 2-gen reproductive toxicity study. However, the average daily dose in these F1 males was 486.7 mg/kg (instead of 419.3 mg/kg as written in the CLH report). Note that the dose of 419 mg/kg bw belongs to the 6000 ppm P-generation males, which showed **no** testicular effects!

As mentioned in the summary above, the discrepancy between reproductive function outcomes at 6000 ppm in P- and F1gen males is related to a higher food and active ingredient intake in the (younger) F1-gen males compared to P-gen males.
Page 12; Line 9: *"Similar results were obtained in the 1-generation reproductive toxicity range-finding study, in which decreased sperm motility and progression and increased abnormal sperm cells in the epididymides were observed in F1 males at 320 mg/kg bw/day"*
The dose of 320 mg/kg does not belong to the 6000 ppm F1 males. It belongs to the 6000 ppm P-gen males.
Similar to the definitive 2-gen study (see above), the 6000 ppm F1 (-eight-to-nine-week old interim) males consumed more feed as compared to the P-generation males of the 6000 ppm level. This was due to their lower body weight during the 3 week premating period (terminal body weight was approx. 260g in F1-interim males, compared to 438g in P-gen males). Food consumption (FC) data have not been measured in F1-interim males in the 3 week premating period (at an age of 7-9 weeks). However, FC data from 7-9 week old F1-gen males can reliably be taken from the definitive 2-gen repro study to determine the likely a.i. intake of F1-interim males for the dietary treatment duration starting after weaning (4 weeks of age until sacrifice in age week 8 or 9, see also table 2, below).
The likely mean a.i. intake of 7-9 weeks old F1-interim males for the 1st, 2nd, and 3rd premating week are 697, 617 and 538 mg/kg bw, respectively (source: food intake data of F1-males of the definitive 2-gen repro study, page 75 of report). Thus, F1-interim males have been exposed to a mean daily dose of > 538 mg/kg during the reduced (3 weeks) period of premating which is much higher than the dose calculated for the P-gen males (i.e. 320 mg/kg) of the range-finder study.
Page 12 ; line 13: The summary does not recognize that the incidence of malformations at the limit dose of 1000 mg/kg bw/day are marginally increased only. Single findings such as cleft

palate, co-arctation of aortic arch, etc, noted in the rat study appeared only once in a fetus of the high dose group. Since all of the few findings were different in type and were covered by historical control data (except for sacral vertebral arch changes: see details later), a potential of BYI 08330 to induce a specific type of malformation was not deduced from these findings. It should also be noted, that the overall rate of malformations (40.9%) observed at 1000 mg/kg bw/day still ranged at the upper limit of historical controls (40%).
The need for classification and labelling of spirotetramat as a reproductive toxicant (H361fd, or R63/R62) should be discussed because of the very high experimental doses required to induce developmental (1000 mg/kg bw/d) and reproductive (>320 mg/kg bw/d) effects in rats. The high developmental NOAEL of 140 mg/kg/day and the high (overall) male reproductive NOAEL in reproductive toxicity studies (320 mg/kg bw/d) confirms that there is no concern for human safety.
(4.7.1.1 repeated dose toxicity: oral) Page 41, 2nd last paragraph: *Only one recovery animal each showed abnormal spermatozoa or hypospermia …*
The marked hypospermia in one single recovery high dose animal (no KF4201) was considered unrelated to treatment, because it was secondary to a marked bilateral testes atrophy.
Justification: Bilateral testes atrophy with aspermia was also noted in one control recovery animal (no KF0208). Bilateral testes atrophy with concomitant hypospermia/aspermia is a spontaneous finding in this rat strain and should be differentiated from the treatment-induced finding of abnormal spermatozoa.
4.7.1.1 repeated dose toxicity: oral; page 42, Testis/epididymis: The very low incidences of epididymal exfoliated germ cell debris (3 out of 25 animals) and of abnormal spermatozoa (2/25) in the 1-year rat study at 7500 ppm (414 mg/kg bw/day) supports the applicants conclusion of a high dose effect abruptely appearing at doses above 400 mg/kg bw. The middose of 189 mg/kg bw/day was a clear NOAEL for testicular/epididymal histopathology.
Point 4.7.1.1, last paragraph - Brain Dilation (1-year dog study), Page 45: The CLH report assumes the brain (ventricle) dilation as a treatment-related finding. A position paper (Christenson 2008), provides support for the conclusion that the dilation originates from a hereditary pre-existing condition in our dog strain.
EPA considered already our arguments and has included a respective discussion in the OECD monograph finally concluding that the *"brain dilation was an equivocal effect and that it should not be used as the basis for the LOAEL in this study"*.
Parts of the EPA-discussion in the reviewer´s comment section of the OECD monograph were as follows:
"… brain dilation was not observed in any other study in the dog or in any other species in the database"
"*In addition, brain dilation has been observed sporadically in several Bayer dog studies of varying durations (from four weeks up to one year). Historically brain dilation, considered a congenital anomaly by veterinary pathologists, has been observed at incidences up to 5% in some dog populations*.
Furthermore, it is stated in the **last paragraph of chapter 4.7.1.1:** *" mild axonal degeneration was also detected in 1 female at 1800 ppm."*
However, minimal axonal degeneration in one female (VP3103) at 1800 ppm is considered a spontaneous finding and not related to treatment. Other "brain" findings, not reported in the CLH report were noted in control dogs as well, supporting our conclusion of a common spontaneous finding. Other findings in control female dogs of the 1-y dog study included:
brain, gray matter, vacuolization, diffuse (slight) (animal VP 0101)
brain, vacuolization, multifocal (minim) brainstem (animal VP 0103)
brain, degeneration, vacuolar, multifocal (moderate), lat thalamus, corpus caudatus (VP 0104)
Point 4.7.1.7 Summary of repeated dose toxicity, Line 13, Page 46: Dilated brain (ventricle) is considered not a treatment-related effect as the dilation originates from a hereditary pre-existing condition in our dog strain.

Point 4.10.1.1, page 51, Kidney histopathology: loop of Henle (instead of Henley)
Point 4.11.1.1, first paragraph, Page 55: We
We appreciate the extrapolation of the a.i. intake to 400 mg spirotetramat/kg bw/day for F1-gen males instead of adopting the dose of 320 mg/kg bw/day, which belongs to the 6000 ppm P-gen males of the dose-range finder study. This value of 400 mg/kg considers the higher feed intake in the much younger F1-generation males (like our calculations, as given below).
However, we would like to point out that the value of 400 mg/kg has not been picked up in the conclusion part of this section (see below) and in the summary section (page 12). On page 12 it is stated that *"abnormal sperm cells in the epididymides were observed in F1 males at 320 mg/kg bw/day"*. However, -to be consistent- it should read *"… were observed in F1 males at 400 mg/kg bw/day"*. The dose of 320 mg/kg bw/d is a clear NOAEL for reproductive toxicity.
4.11.1.1 Conclusion- Page 56: The CLH-report states at the bottom of this page: *"The target organ is the testes with progressive effects over successive generations: the F_1 males were affected at a lower dose than the P-generation males."*
We disagree with this statement: Food intake data provide support for the conclusion that the sperm cell toxicity noted in each of the 1st generations of the two reproductive toxicity studies (i.e dose range finder and definitive study) is a function of dose and not a progressive effect over successive generations.
To support our argumentation, detailed information on feed intake in young male rats is given as follows: As stated already earlier, the F1 "interim" males of the dose-range finder reproduction study were much younger at start of the premating period than the P-generation males (i.e. 7 vs 14 weeks) and were sacrificed already after 3 weeks of premating (age=8-9 weeks old), whereas P-gen males underwent 10 weeks of premating with an age of 24 weeks at term; see table 1). These factors led to a much higher average daily intake of spirotetramat in F1-interim males, compared to P-generation males.

Table 1: Age of 6000 ppm males (P gen) at start and end of the premating period (10 w) and calculated mean active ingredient (a.i.) intake during 10 w of premating
Age of F1-interim males (F1-int) at start and end of the premating period (3 w) and estimated mean a.i. intake during 3 w of premating (based on definitive study data, see tbl 2)

Reproduction range-finding study 6000 ppm male rats					
Gene-ration	BW at Day 0 premating period	Age at	BW at end of pre-mating period	Age at	AI intake (mg/kg) premating period
P-gen	325,8	14 wks	438,3	24 wks	320,1
F1-int	(189,4)	7 wks	(259,7)	9 wks	≥538

Day 0 is the day before start of food consumption measurement
Figures in brackets are taken from F1-gen males of the two-generation study, because of lack of food consumption measurement in F1-interim males (F1-offspring stayed with littermates in same cage for 3 weeks after weaning (see also table 2)

The mean a.i. intake of **538** mg/kg bw/day has been taken from F1-males of the 2-gen repro study (see table 2, grey shaded area) and can be adopted to the F1-interim males of the 1-gen repro study for which food consumption data was not measurable (offspring stayed with littermates until 6 weeks of age, see right part of table 2, in yellow).

Table 2:

		left part:	study data of 2-generation reproduction study: Premating, F1-parental male generation									
			Mean body weight (BW), food consumption (FC), food intake (FI) and active intake (AI) *									
			Note: Start measurement of food consumption at age of 7 weeks (=day 0-7 of treatment)									
		right part:	study design of 1-gen repro study: Age of animals during 6 week-dietary treatment									
	F1-generation males (2-gen repro study)								F1-gen interim males (1-gen repro study)			
	Animal	Treatment	Food cons.	Diet. Conc.	BW	FC	FI	AI intake	Animal	Treatment	Food c..	
	Age		measurem.		(d 0, 7, 14...)	/week	/week	/week	Age		measurem.	
	(weeks)	week	(days)	(ppm)	(g)	(g/animal/d)	(g/kg bw/d)	mg/kg bw/d)	(weeks)	week	(days)	
	1	*1 - milk*							1	*1 - milk*		
	2	*2 - milk*							2	*2 - milk*		
	3	*3 - milk*							3	*3 - milk*		
postweaning	4	1 - diet	FC not done since	6000					4	1 - diet	FC not done since	
dietary	5	2 - diet	offsprings stayed	6000					5	2 - diet	offsprings stayed	
treatment	6	3 - diet	with litter mates	6000			*> 118,1*		6	3 - diet	with litter mates	
	7	4 - diet	day 0 - 7	6000	189,4	22,2	118,1	696,8	7	4 - diet	*FC also not done*	
period of	8	5 - diet	day 7 - 14	6000	226,5	23,6	104,5	616,5	8	5 - diet	*during the last*	
food	9	6 - diet	day 14 - 21	6000	259,7	23,6	91,2	537,7	9	6 - diet	*3 weeks*	
consumpt.	10	7 - diet	day 21 - 28	6000	284,8	23,7	83,3	491,5				
measurem.	11	8 - diet	day 28 - 35	6000	303,1	23,9	79,1	466,3				
day 0-70	12	9 - diet	day 35 - 42	6000	320,2	23,9	75,1	442,9				
	13	10 - diet	day 42 - 49	6000	333,6	23,7	71,3	420,5				
(premating	14	11 - diet	day 49 - 56	6000	345,2	24,2	70,5	415,6				
period)	15	12 - diet	day 56 - 63	6000	357,3	24,4	68,5	404,2				
	16	13 - diet	day 63 - 70	6000	367,1	23,4	63,6	375,3				
mean a.i. intake (mg/kg bw/d) of F1-gen males based on wk 1-10 (see report)								486,7				
calculated mean a.i. intake (mg/kg bw/d) of F1-interim males based on wk 6 FC								537,7				

grey shaded area is consistent with the dietary treatment period of F1 generation interim males in the one-gen DRF study (max. 6 weeks; age at start of treatment: 4 weeks)
see page 22 of one-gen repro report no 201300-1: "At 21-days of age, a sufficient number of F1 pups/sex/litter were maintained and observed for vaginal opening and preputial separation. The F1-males were maintained for an additional 5 to 6 weeks prior to performing sperm analysis (motility, counts, and morphology)."

Thus, the difference in outcome in sperm cell effects at 6000 ppm (P-gen: negative; F1-interim: positive) in the one-gen study is confounded by the higher average daily food consumption of the (much younger) animals of the F1-interim males compared to the P-gen males. Consequently, the active ingredient intake was also much higher in the younger (F1-interim) males and the F1-interim males were not affected at a lower dose than the P-gen males.
Thus, the reproductive NOAEL in the one-generation dose range finder study is 320 mg/kg bw/day based on the lack of abnormal sperm cell parameters in P-gen males.

Page 58 (Histopathology chapter and Reproductive function chapter of the definitive reproductive toxicity study):
We agree with the evaluator that the 6000 ppm P-gen males (i.e. 419 mg/kg bw) showed no effects on testicular tissues as well as on sperm parameters. Sperm effects were noted only at 486.7 mg/kg in the 6000 ppm F1-gen males, supporting the conclusion of a steep doseresponse curve. Kidney effects were also observed only in F1-gen male and female rats.
Based on the overall weight of evidence from the reprotox studies, the NOAEL for testicular toxicity can be determined at a level of 320 mg/kg bw/day. LOAEL-doses of > 400 mg/kg induced sperm cell toxicity.
Male rat reproductive toxicity is considered a high dose phenomenon, even if the reproductive NOAEL in male rats must be determined formally at 70 mg/kg bw/d.

Part 4.11.3 Gross pathological findings, Page 70, 3rd paragraph: It is stated: *"No other treatment-related microscopic lesions were observed in the testis or epididymis on day 3 or day 10 or in the prostate at any sacrifice time."* The word "other" needs to be deleted, because there are no treatment-related effects after 3 and 10 days.
The finding of a minimal increase in intraluminal aberrant cells in the epididymis in two treated animals on day 3 was considered to be incidental and correspond to changes

commonly encountered in laboratory rats of this age. The lack of this finding on treatment day 10 also supports this conclusion.

Part 4.11.3 Sperm analysis, Page 70: It is stated: *"Small statistically significant decreases (-13% to -15%) were observed in the absolute and relative (to cauda epididymis weight) sperm count on day 10 and the absolute sperm count on day 21"*

Results of epididymal sperm counts have an inherent high variability, which is partly due to methodological issues. The apparent decrease in epididymal sperm count (188.5) on day 10 in treated animals is considered not treatment-related because the day 10 control mean (218.4) was relatively high compared to controls on day 3 (184.4).

Since testicular effects were not observed until day 21, it is unlikely that a reduction in spermatogenesis will have occurred which could have translated into lowered epididymal sperm counts on day 10 of treatment. Since epididymal storage function is not considered to be impaired by spirotetramat (no evidence of morphological changes in the anatomical structure of epididymal tissue) the small difference in sperm count observed on day 10 is likely due to chance variation.

The absence of a treatment-related sperm count decrease within 10 days of treatment in this study is indirectly supported by the results of a similar study applying the enol (causative metabolite for sperm cell changes) for 21 days: sperm counts were not affected after 21 days of treatment (absolute and relative sperm cell counts were 105% and 92.1% of controls, respectively, indicating a high degree of variability for this parameter. Our mechanistic data indicate that abnormal spermatid development represents the primary lesion in the pathomechanism after spirotetramat exposure. Changes in epididymal sperm cell counts clearly result from changes in testicular production and not from impaired storage function. This is supported by the marked reduction (-50%) in sperm cell counts, 41 days after treatment, which is not comparable with the relatively small changes noted after 3-21 days of exposure.

Part 4.11.3 Conclusion, Page 71, line 8: The underlined part of the following sentence requires a correction:

"The effect on the testes progressed to loss of elongating spermatids in the testes and an increase in severity of intraluminal aberrant cells and the presence of oligospermia in the epididymides after treatment for 10 days".

It should read after treatment for 41 days" (see results on page 26-27 of report SA04181; Doc No. MO-05-008901). Ten days of continuous treatment did not lead to morphological sperm cell effects as proven by histopathology examinations in this specifically tailored mechanistic gavage study with serial sacrifice time points. Relevant changes in sperm cell morphology have only been detected after treatment for 21 and 41 days, but not at 10 days of treatment.

Developmental Toxicity: On page 61 (visceral examination) one case of anophthalmia is missing. It belongs to the 0 mg/kg bw group.

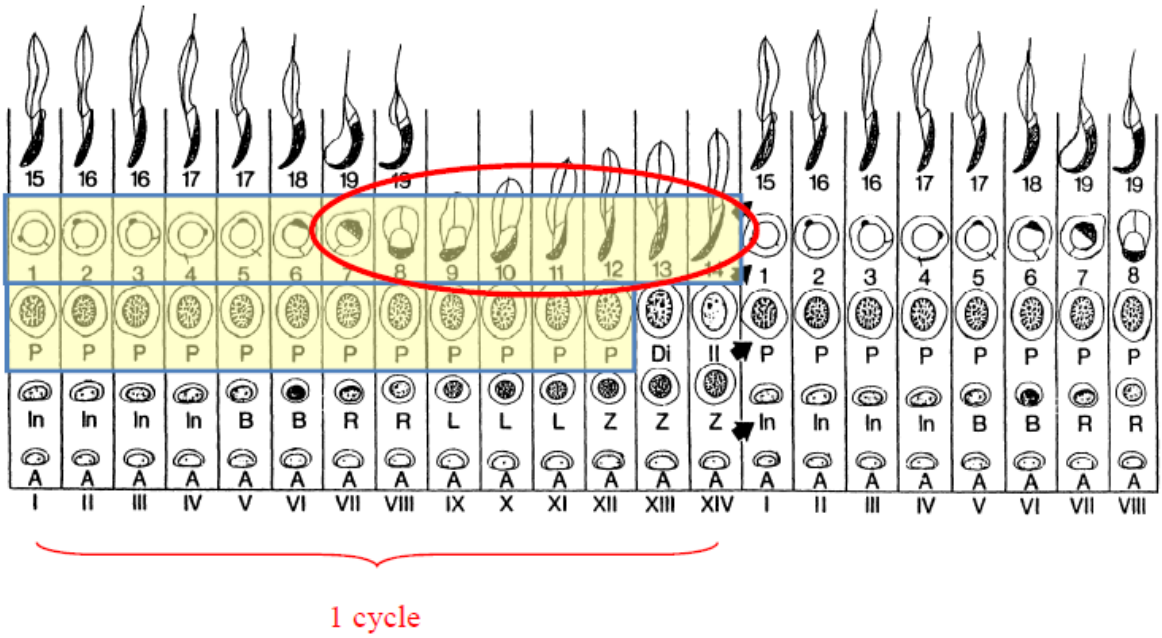
Page 73: Comment to historical control data on dysplastic bones: There were historical studies showing ten (year 2000: study T5068551) three and seven fetuses (year 1999: study T2062311 and T9061318) with forelimb dysplasia, demonstrating that the finding is a common and frequent abnormality found in this strain (Temerowski, 2009; page 7).

Page 73 (wavy ribs): The following sentence should be completed with the following addition (see underlined part) in order to be consistent with the conclusion of the RMS given on page 61: *"Statistically significantly increased incidences of wavy ribs were observed at all dose levels compared to concurrent and historical control values"* but this was not reproduced in the supplementary study at doses of 10, 35, and 140 mg/kg bw/day (Klaus, 2004). Thus, toxicological relevance is assumed at the 1000 mg/kg bw/day dose level only.

Overall, Bayer CropScience is of the opinion that BYI 08330 must not necessarily be classified as a developmental toxicant. The increased incidences of dysplastic forelimb bones and sacral vertebral alterations in rat fetuses (3 fetuses out of 3 litters) observed at the maternally toxic limit dose of 1,000 mg/kg bw/day do occur also spontaneously, and the study

incidence is either covered by historical control data (dysplastic forelimb bones) or (for sacral vertebral arch alterations) ranged only slightly above the maximum historical incidence in a study from 2004.
In this study (from 2004), the maximum incidences of 2 fetuses out of 2 litters were found in the mid dose accompanied by 1 fetus in the corresponding control group. Furthermore, sacral vertebral arch alterations were not noted at skeletal examinations in the spirotetramat dose range finder study, using a total litter number of 18 for the high dose levels of 800 and 1,000 mg/kg bw/day.
Note should also be taken of the specific toxicokinetic behavior of the anionic metabolites (saturation of elimination pathways) after repeated exposure to doses of 1000 mg/kg bw/day.
Page 73, 3rd paragraph (starting with " In a mechanistic study …): It is stated *"Therefore, the author of the study concluded that repeated dosing is necessary to produce male reproductive toxicity in rats, not considering the relatively prolonged period of spermatogenesis (about 60 days in man, 55 in rat). A single high dose at one critical point in development may be required, but the effects would not be seen until some time later."*
It is highly unlikely that step 7-8 spermatid injury may occur several weeks after a single high dose of spirotetramat, for the following reasons:
In the gavage study (Kennel, 2005) sperm cell toxicity was noted beginning at day 21 of continuous treatment with spirotetramat. In the 90-day feeding rat study, the recovery period of 28 days can be considered a follow-up period for observing "a potential delayed sperm cell injury". Supposing that the last high dose (i.e. on day 90) in the 90-day feeding study would be considered as an (isolated) single dose application followed by a treatment-free period of 28 days, the following assumptions are made:
- If a single high dose of spirotetramat would elicit morphological effects in step 7-8 spermatids 21 days after stop of treatment as well, one would expect similar incidences of sperm cell damage at the end of the 28-day recovery period of the 90 day study compared to those incidences noted at the end of the 90 day treatment period.
- However, the definite reversibility of the testicular findings at the end of the 28-day recovery period of the 90-day study clearly suggests that one single high dose is insufficient to induce delayed sperm cell toxicity in rats.
- Spermatid injury should have been detected at a much higher incidence at the end of the recovery period, if pachytene spermatocytes (see figure 1) would have been damaged by one single dose at the end of the 90-day treatment period and if their degeneration would be delayed to a later time when they undergo a critical event which depends on their previous (single dose) exposure to spirotetramat.
- Since spirotetramat induces an incomplete formation of the sperm head, it is likely that the Sertoli Cell (SC) is the primary target of spirotetramat. Vacuolative changes in SCs were noted in the mechanistic gavage study (Kennel, 2005). During the normal course of the spermatid differentiation into a spermatozoon, the nucleus and the cytoplasm in the sperm head undergo a number of extremely complex morphological modifications (i.e development of an acrosome, re-arrangement of cell organelles, redistribution of cytoplasm) requiring structural and metabolic support of the Sertoli cells.
- A steady accumulation of the enol following high-dose treatment of more than 320 mg/kg bw/day most likely leads to a deterioration of "stage-specific" Sertoli cell functions that are critical to the development of the step 7-14 spermatids.
- Although Sertoli cells are very sensitive to functional perturbation, they are remarkably resistant to cell death. Once, the exposure to spirotetramat is decreased below threshold levels, restoration of Sertoli cell functions is regained.

Figure 1: The scheme illustrates the cellular composition of the 14 stages (depicted by roman numerals) of the cycle of the seminiferous epithelium where each column represents a stage. The stages are defined by the step of development of the spermatid. As the cells advance through one cycle of spermatogenesis, they move to the top of the tubular epithelium and are replaced by another generation of cells from the basal site.
Pachytene (P) spermatocytes and spermatids (step 1-14) are depicted in the blue box. Approximately 21 days are required to develop from pachytene spermatocytes to step 7 spermatids which are known to be the first cells affected by spirotetramat. Step 7 to 14 spermatids (in red circle) have been shown to be affected following at least > 10 days of high dose (>320 mg/kg bw/day) treatment with spirotetramat.
(taken from Handbook of Toxicologic Pathology, Vol 2, Academic Press, 2002; page 794)



In conclusion, based on the reversibility of the testicular effects during a 28-day recovery period of the 90-day rat feeding study, it is highly unlikely that the damage of progenitor cells like pachytene spermatocytes could lead to a delayed appearance of degenerated spermatids (step 7-8) after >10 to 21 days following a single high dose of spirotetramat.
Spirotetramat induces effects on germ cells only after repeated doses and when elimination pathways for spirotetramat metabolites are overwhelmed by continuous high dose exposure.

References

1. Christenson (2008), Technical Grade Spirotetramat (BYI 08330): Further Examination of the Finding of Brain Dilatation in the One Year Dog Study, Bayer Document No. M-298505-01-1
2. Kuehne A. (2011). Inhibitory potential of BYI 08330-enol and BYI 08330-desmethylenol as inhibitors on hOAT1, hOAT3 and hOAT4 in transfected HEK-cell. PortaCellTech Study Report PCT-009-11, Document No. M-413065-01-1
3. Temerowski (2008), Spirotetramat (syn. BYI 08330): Request for historical control data, Document M-296860-01-1

ECHA note: These three references were submitted as separate attachments [Attachments 2-4]

Dossier Submitter's Response

Noted

RAC's response

Fertility:
These comments are noted and have been considered in details. They are not considered to impact the conclusion on classification of spirotetramat for fertility.
Developmental toxicity:
Noted.

RESPIRATORY SENSITISATION

Date	Country	Organisation	Type of Organisation	Comment number
08/11/2012	Germany		Member State	11
Comment received				
p. 38: We support not to classify Spirotetramat for respiratory sensitization.				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted				

HAZARDOUS TO THE AQUATIC ENVIRONMENT

Date	Country	Organisation	Type of Organisation	Comment number
09/11/2012	United Kingdom	Environment Agency	Behalf of An Organisation / National Authority	12

Comment received

Section 5.4.2.1
We do not think the oyster test assessing shell deposition should be viewed as an acute test, as it is not assessing a mortality endpoint. In classification terms the result is being compared with the acute Daphnia study, which is an immobilisation endpoint based test.

We also highlight that 96hr oyster shell deposition data were available in the previous ESR assessment of Tetrabromobisphenol A, but assessed as chronic effects.

There are other oyster tests where mortality is the endpoint (e.g. ASTM E725), although we appreciate such data are not available here.

Section 5.4.3
For the algal and aquatic plants acute data, there is one acute result below 1 mg/l, with the remainder all exceeding 1mg/l. The result below is the Skeletenema costatum test where the ErC50 = 0.98mg/l with the 95% confidence intervals straddling the 1mg/l threshold (0.92 – 1.05mg/l)

If the oyster data above are considered chronic data, this would mean there is only one data point across all the taxa that results in the substance being classified as acute aquatic 1. Due to this, we think it is scientifically appropriate to consider section 4.1.3.2.4.3 of the CLP guidance (Guidance on weight of evidence for substances for which more than one valid piece of data is available for a given data element). In particular we think an HC5 could be derived for the algal & aquatic plant endpoint, which would allow all these data to be used for the acute classification. It would also provide more confidence for the significance of the Skeletenema costatum result.

Dossier Submitter's Response

Comment noted, the revised proposal is:

We still prefer the Eastern Oyster endpoint (Crassostrea virginica) EC50 at 0.85 mg/L (see citations below).
This test was conducted under EPA Guideline OPPTS 850.1025 and is used to develop data on the **acute toxicity** (EC50 Shell deposition) to Eastern oysters.

According to Regulation (EC) No 1272/2008
4.1.1.2.2 > freshwater and marine species toxicity data are considered:
"Preferably data shall be derived using the standardised test methods referred to in Article 8(3). In practice data from other standardised test methods such as national methods shall also be used where they are considered as equivalent. Where valid data are available from non-standard testing and from non-testing methods, these shall be considered in classification provided they fulfil the requirements specified in section 1 of Annex XI to Regulation (EC) No 1907/2006. ***In general, both freshwater and marine species toxicity data are considered."***

According to Annex 9 Guidance on hazards to the aquatic environment UNO 2007
"Acute toxicity is generally expressed in terms of a concentration which is lethal to 50% of the test organisms (LC50, causes a measurable adverse effect to 50% of the test organisms (e.g. immobilization of daphnids), ***or leads to a 50% reduction in test (treated) organism responses from control (untreated) organism responses (e.g. growth rate in algae)."***

RAC's response

Concerning the use of oyster growth data, the guidance indicates a preference for toxicity data on crustaceans for the invertebrate trophic level, although data for other species can be used as pointed out in the Dossier Submitter's response. The consistency of species used for environmental classification purposes is an important and relevant consideration, to avoid both under- and over-classification. However, the RAC agrees to the use of the oyster growth data in this case, particularly because it is supported by the marine diatom result and is within a factor of 2-3 of the lowest acute result for both fish and aquatic insects.

The Dossier Submitter did not respond to the second point, which suggested the derivation of an HC_5 to take full account of the range of algal/aquatic plant data available. The RAC notes that concentrations were not well maintained in the algal studies, so results are conservatively based on geometric mean concentrations. Given the uncertainties in the exposure concentrations, the closeness of the most sensitive result to the classification threshold, and range of values for different species, the RAC considers that a species sensitivity distribution would be a reasonable way of analysing the available information (e.g. by deriving an HC_5). However, since invertebrates appear to be of similar sensitivity to the most sensitive algal species (at least for acute end points), there is no need to perform this calculation for the purposes of opinion development.

Date	Country	Organisation	Type of Organisation	Comment number
09/11/2012	Sweden		Member State	13

Comment received

For the environment SE supports classification of Austria (Cas No 203313-25-1) as specified in the proposal. SE agrees with the rationale for classification into the proposed hazard classes and differentiations.

The current proposal for consideration by RAC and harmonized classification is: Aquatic Acute 1; H400; M-factor 1 and Aquatic Chronic 1; H410,M-factor 1.
Sweden agrees with the classification proposal Aquatic Acute 1; H400; M-factor 1 and the Acute Chronic 1, H410, M-factor 1 even if we did have some discussions about the Acute Chronic 1, H410, M factor 1.
The classification Acute Chronic 1 is based on a Chironomus riparius toxicity test (NOEC EMERGENCY=0.1 mg/L) which is a sediment living organism and not an aquatic organism directly exposed by the substance in the water column.
It is however justified to use this organism for chronic classification (ref "Guidance on the Application of the CLP criteria/ECHA , Apr 2012, version 2) since the substance is an insecticide and will be expected to be toxic against insects like Chironomus riparius, and also is not biodegradable.

Dossier Submitter's Response

Noted

RAC's response

The RAC notes support for the proposal, but also some uncertainty over the use of a toxicity test including sediment. The RAC notes that test substance concentrations rapidly declined during the initial stages of the *Chironomus* test, and the substance was not detectable in overlying water by the end of the test. It is therefore unclear what caused the observed toxicity, and the test system might not have achieved equilibrium. In addition, it cannot be ruled out that the test organisms were exposed to the substance or metabolites adsorbed to the sediment surface. Therefore although the Dossier Submitter and commenting Member State consider the study to be valid, the RAC does not think it is relevant for classification purposes. This does not affect the proposal, because the classification conclusion is the same when the surrogate approach is followed for the invertebrate trophic group.

SKIN SENSITIZATION

Date	Country	Organisation	Type of Organisation	Comment number
09/11/2012	Sweden		Member State	14

Comment received

SE agrees that spirotetramat should be classified as a skin sensitizer based on a GPMT and an LLNA study. However, regarding the sub-categorisation it is not possible from the presented data to conclude on a sub-category. Regarding the GPMT the intradermal induction concentration should be ≤ 1% for consideration of sub-category 1A. According to the GPMT protocol (OECD TG 406) the induction concentration should be adjusted to the irritating properties of the test substance. Following the GPMT protocol the intradermal induction dose for spirotetramat was chosen to be 5%; thus in this case it is not possible to conclude on the sub-category; which is a weakness in the criteria. Regarding the LLNA the EC3-value needs to be calculated in order to subcategorise. EC3 ≤ 2% will refer a sensitizer to 1A.
In conclusion, SE agrees that spirotetramat should be classified as a skin sensitizer; however data presented in the CLH proposal does not allow sub-categorisation.

Dossier Submitter's Response

Noted

RAC's response

Considering potency, the EC-3 value was not derived in the available LLNA but a SI above 3 was reported at all concentrations tested including a SI of 3.4 at the lowest tested concentration of 1%. It can be concluded that the EC3 in this test is below 1%, which fulfils the criteria for subcategory 1A. In the GPMT, a single intradermal concentration of 5% was tested for induction and an incidence of 95% of animal sensitised was observed. It fulfils the criteria for subcategory 1B (more than 30% of animals sensitised at an intradermal induction concentration above 1%). Given the high incidence of sensitisation observed, it is however not excluded that spirotetramat may induce a significant level of sensitisation at concentrations below 1% that may indicate a potency in line with subcategory 1A.

Date	Country	Organisation	Type of Organisation	Comment number
08/11/2012	United Kingdom		Member State	15

Comment received

Sensitisation

Skin: The data set consists of a negative Guinea pig (Buehler) test, a positive Guinea pig (Magnusson and Kligman) test, a positive local lymph node assay (LLNA) and some positive human data. We agree that the data support classification as a sensitiser (Xi; R43 under DSD; skin sensitisation category 1, H317: May cause an allergic skin reaction under CLP).

The dossier submitter has proposed classification in subcategory 1A under the CLP Regulation. However, it is not clear from the data (as it is currently presented) that the criteria for this sub-classification are met.

According to the 2nd ATP to CLP, a skin sensitiser should be classified in subcategory 1A if it meets the following criteria:

Assay: Guinea pig maximisation test:
Result: ≥ 30% responding at ≤ 0.1% intradermal induction dose, or
≥ 60% responding at > 0.1% ≤ 1% intradermal induction dose

Assay: LLNA
Result: EC3 Value: ≤ 2%

The results of the guinea pig maximisation test (95% responding at intradermal induction dose of 5%) do not support classification in subcategory 1A.

The EC3 value for the LLNA is not reported in the dossier, therefore the reader cannot easily assess whether the assay supports classification in subcategory 1A (i.e., EC3 ≤ 2%). The data suggest that the EC 3 value will be ≤ 2% (indeed, they indicate it will be less than 1%), however this should be clearly stated in the results. We also suggest including a section where the results/data are compared with the classification criteria so that it is clear to the reader that classification in subcategory 1A is appropriate.

Dossier Submitter's Response

The stimulation index values of the test material were 5.9, 5.4, 4.3 and 3.4 at treatment concentrations of 10, 5, 2.5 and 1%, respectively. The stimulation indices of the positive control were 3.4, 1.8, 1.3 and 0.8 at treatment concentrations of 5, 2.5, 1 and 0.5%, respectively.

Stimulation Index:

Sample Description Test or Control Group	Stimulation Index (SI)
Control DMF	1.0
BYI 08330 10%	5.9
BYI 08330 5%	5.4
BYI 08330 2.5%	4.3
BYI 08330 1%	3.4
Isoeugenol 5%	3.4
Isoeugenol 2.5%	1.8
Isoeugenol 1%	1.3
Isoeugenol 0.5%	0.8

RAC's response

See response to previous comment (comment number 14).

Date	Country	Organisation	Type of Organisation	Comment number
08/11/2012	Germany		Member State	16

Comment received

Skin Sensitisation:
p. 38: We support to classify Spirotetramat for skin sensitisation, cat. 1 (H317- may cause an allergic skin reaction.
It should be noted that e.g. in point 1.2, Table 2 under "Current proposal for consideration" and "Resulting harmonised classification" the specification "Skin Sens. 1A" for Spirotetramat is missing. This should be added.

Dossier Submitter's Response

Noted

RAC's response

Noted

SERIOUS EYE DAMAGE / EYE IRRITATION

Date	Country	Organisation	Type of Organisation	Comment number
08/11/2012	Germany		Member State	17

Comment received

p. 35: We support to classify Spirotetramat for eye irritation, cat. 2 (H319 - causes serious eye irritation)

Dossier Submitter's Response

Noted

RAC's response

Noted

REFERENCES:

1. Christenson (2008), Technical Grade Spirotetramat (BYI 08330): Further Examination of the Finding of Brain Dilatation in the One Year Dog Study, Bayer Document No. M-298505-01-1
2. Kuehne A. (2011). Inhibitory potential of BYI 08330-enol and BYI 08330-desmethylenol as inhibitors on hOAT1, hOAT3 and hOAT4 in transfected HEK-cell. PortaCellTech Study Report PCT-009-11, Document No. M-413065-01-1
3. Temerowski (2008), Spirotetramat (syn. BYI 08330): Request for historical control data, Document M-296860-01-1

ATTACHMENTS RECEIVED: 4

1. "Spirotetramat BCS comments to the CLH-Report (dated 28 AUG 2012 –Version no 4)", 29 Oct 2012.
(Filename: **01_Spirotetramat_comments to CLH report_2012-10-29.pdf**), submitted on 30/10/2012 by Bayer CropScience

2. Christenson (2008), Technical Grade Spirotetramat (BYI 08330): Further Examination of the Finding of Brain Dilatation in the One Year Dog Study, Bayer Document No. M-298505-01-1
(Filename: **02_M-298505-01-1.pdf**), submitted on 30/10/2012 by Bayer CropScience

3. Kuehne A. (2011). Inhibitory potential of BYI 08330-enol and BYI 08330-desmethylenol as inhibitors on hOAT1, hOAT3 and hOAT4 in transfected HEK-cell. PortaCellTech Study Report PCT-009-11, Document No. M-413065-01-1
(Filename:**03_M-413065-01-1.pdf**), submitted on 30/10/2012 by Bayer CropScience

4. Temerowski (2008), Spirotetramat (syn. BYI 08330): Request for historical control data, Document M-296860-01-1
(Filename: **04_M-296860-01-1.pdf**), submitted on 30/10/2012 by Bayer CropScience

ECHA note: Attachment 1 is copied in the comments table. Attachments 2-4 are being provided as separate attachments