Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Desmedipham (ISO); ethyl 3-phenylcarbamoyloxyphenylcarbamate

EC Number: 237-198-5
CAS Number: 13684-56-5; (125579-95-5); (153703-69-6)

CLH-O-0000001412-86-294/F

Adopted

20 September 2019

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON DESMEDIPHAM (ISO); ETHYL 3-PHENYLCARBAMOYLOXYPHENYLCARBAMATE

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: desmedipham (ISO); ethyl 3-phenylcarbamoyloxyphenylcarbamate
EC number: 237-198-5
CAS number: 13684-56-5
Dossier submitter: Finland

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
11.02.2019	Germany		MemberState	1
Comment received				
For us it is unclear which substance is discussed for inclusion in Annex VI. ethyl 3-phenylcarbamoyloxyphenylcarbamate including relevant impurities (table 1 and 3) or the pure substance.				
Dossier Submitter's Response				
Thank you for your comment. The CLH report discusses the active substance ethyl 3-phenylcarbamoyloxyphenylcarbamate (desmedipham) including relevant impurities. Please see also response to comment number 4.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
12.02.2019	France	<confidential>	Company-Manufacturer	2
Comment received				

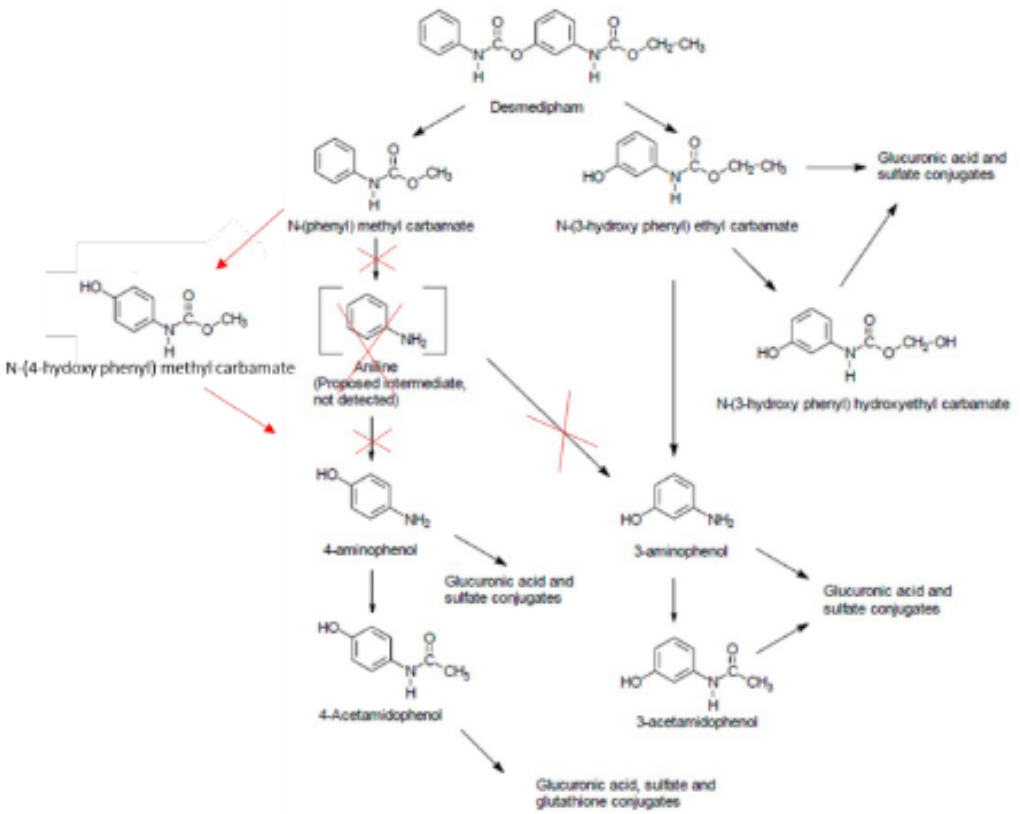
10. Health hazards -Read-across

We agree that the classification proposal should only be based on the data on desmedipham itself, but not with the proposal that this should be supported by read-across from phenmedipham and their assumed common metabolites. We disagree with the statement that the chemical structure, chemical properties, breakdown products and toxicological profiles of desmedipham and phenmedipham are similar since it is known that even within the same compound classes between compounds with similar structures differences in their toxicological profiles can occur.From the metabolic pathways (shown in the table under chapter 10. in the CLH report) it is obvious that the metabolic pathway show differences so that this does not support the stated similarity of both molecules. Some of the endpoints are similar, like the hematological effects, however, depending from the study type and species, the potency and reference doses are different.

Aniline formation

The statement that in the metabolism of desmedipham formation of aniline may happen was not confirmed in the ADME studies since aniline was not detected in them. It is also unlikely due to the fact as stated in the CLH report "that in the metabolic pathway of desmedipham, the first metabolite of the PC ring radiolabelled form was PMC (phenyl methyl carbamate), which was supposed to convert to aniline and then further rapidly to 4-aminophenol and at last acetylated to 4-acetaminophenol. However, aniline was not detected in metabolism studies in rat." Based on the quick conversion to 4-aminophenol apparently no aniline is occurring at measurable amounts if at all which is demonstrated by the fact that no aniline was detected in the ADME studies.
Aniline was proposed as an intermediate metabolite. However, several considerations are opposed to this proposal:

1. Aniline is known to be metabolized to ortho- or para-hydroxylated metabolites (4-aminophenol (80%) and 2 aminophenol (12%)) thus conversion to meta-aminophenol (3-aminophenol) is a very minor pathway (<0.1%) in mammalian metabolism.
2. There was no indication of 2-aminophenol.
3. Further known metabolites of aniline include N-conjugated metabolites (N-glucuronide, phenylsulfonic acid, acetanilide: none of these was observed upon analysis of desmedipham metabolism.
4. For N-substituted aniline derivatives such as N-(phenyl) methyl carbamate again hydroxylation at the para position is the major route of metabolism. For the structurally related Acetanilide only 1% formation of aniline was observed.
5. Thus, the observed formation of 4-aminophenol is most likely occurring by first 4-hydoxylation followed by de-carbamoylation at the N-position



Taken together, the absence of aniline itself and the expected typical metabolites of aniline are strongly opposed even to an intermediate formation of aniline. Also, the knowledge about metabolism of N-substituted aniline derivatives which results in 4-hydoxylation rather than unmasking the amino group give further support to the proposal that aniline is not formed, not even as an intermediate.

It is stated in the CLH report that "There are slight differences in the substances formed during metabolism between the two substances. The first steps of metabolism pathways seem to be slightly different. The –NHCOO- group in between the aromatic rings is metabolised in the first step to –NH2 and HO- in phenmedipham and to –NHCOOCH3 and HO- for desmedipham. However, both substances are suggested to produce compounds which have aromatic amine structure. Some of the identified metabolites are common for both substances such as 3-aminophenol and various acetamidophenols. Phenmedipham is also suggested to metabolise to acetamidocarboxylic and salicylic acids, which are not identified in the toxicokinetic studies of desmedipham. Not detected in the studies but phenmedipham is also suggested to produce aniline."
Like we disagree with the statement that aniline occurs as metabolite of desmedipham as explained before, we also disagree with the speculation that phenmedipham could produce aniline (for further arguments regarding phenmedipham see CLH argument paper about phenmedipham).

Genotoxicity in the read-across tables

We disagree with the presentation of the OECD 474 studies on phenmedipham as +/- and the conclusion that in vivo exposure has not been shown for phenmedipham (table page 13 of the DMP CLH report). For further explanation, we refer to our comments on the CLH report for phenmedipham.

Under 10.10.10 Conclusion on classification and labelling for reproductive toxicity the CLH report concludes that "Based on developmental toxicity effects seen in reproductive toxicity studies in rats and rabbits, classification of desmedipham for Repr. 2, H361d (Suspected of damaging the unborn child) is proposed."

We disagree with this proposal.
It is stated that in a developmental toxicity study in rats (Becker, 1985) incidences of palatoschisis/micrognathia, agnathia/ open eyes might have been affected. The following table gives an overview about these findings:

End point	Dose levels (mg/kg bw/day)			
	0	10	100	1000
Number of dams	25	22	23	23
Dams with abortion		1[a)]		1[b)]
Implantations	292	255	280	257
Embryonic resorptions (% of implantations)	10(3.4)	22(8.6)	15(5.4)	15(5.8)
Live fetuses (% of implantations)	282(96)	233(91)	265(94)	242(94)
Fetus weight	4.8	4.8	4.8	4.2
Major malformations				
Number of fetuses investigated	282	233	265	242
Runt (number of litters)				1(1)
Omphalocoele (number of litters)			1(1)	
Agnathia (inferior)and open eyes (number of litters)			1(1)	
Palatoschisis and slight micrognathia (inferior)				6(1)*
Palatoschisis, distinct micrognatia and dysplastic tail (number of litters)				1(1)*
Visceral investigations				
Number of fetuses investigated	138	118	133	123
Renal pelvis dilated bilaterally (number of litters)		1(1)	2(1)	
Skeletal investigations				
Number of fetuses investigated	144	115	132	119
Number of skeletal abnormalies[1)] (number of litters)	10(8)	12(7)	2(2)	20(11)[c)]
Shortened rib (right side no. 13) (number of litters)			1(1)	
Supernumerary rib (left side)[2)]	15	8	18	29
Supernumerary rib (right side)[2)]	14	8	22	25
Calculated total number of implants affected (%)[3)]	20(6.8)	35(13.7)	22(7.9)	42(16.3)

* The same litter, **1)** Abnormalities included; absent sternebrae, abnormally shaped sternebrae, dumbbell shaped thoracic vertebral body, longitudinally split sternebrae, and wavy ribs, **2)** Abnormalities were not listed together with the individual number of fetuses/litter and so the total number of fetuses with supernumerary rib (unilateral or bilateral) remained unclear, **3)** Implants affected = resorptions, major malformations, visceral and skeletal abnormalities, **a)** One female excluded from the evaluation (no fetuses on day 21 post coitum, only 2 implantation, colporrhagia observed on day 15 of gestation), **b)** One female excluded from the evaluation based on administration error seen in thorax (15 corpora lutea, 9 embryonic resorptions, 5 live fetuses), **c)** Three of these fetuses (3/20) also had palatoschisis
In this study

The report remarks: "Although the incidence of externally visible malformations appears to be a direct effect of Desmedipham Technical, all these anomalies were noted in the historical background data of this Wistar rat strain. The absence of any of these findings in the vehicle control groups of these studies may be considered to be incidental. The toxic hemolytic anemia caused by Desmedipham Technical at high doses might have enhanced the overall frequency of those malformations which were noted spontaneously in fetuses of this Wistar rat strain. Therefore, Desmedipham Technical should not be considered to reveal either embryotoxic or teratogenic potency in the rat."

Agnathia (inferior) and open eyes occurred only in one fetus in the mid-dose (100 mg/kg bw), but not in the highest dose group so that there is no dose-relationship of these findings so that these findings cannot be regarded as treatment effect. Palatoschisis and slight micrognathia (inferior) occurred only at the highest dose of 1000 mg/kg bw, but only in fetuses of one litter so that based on the litter incidence

there is no indication of treatment effect. Also one female fetus was mentioned as 'runt' at the highest dose. The finding 'runt' is a term which is not used nowadays anymore since it is too unspecific and subjective because it is used when a pup seems small, and no other change is seen in such a pup. This is however, no malformation but only a development retardation secondary to maternal toxicity. Sometimes as rough term a fetus is called 'runt' if the weight of such animal is half of that of the average weight of the litter such fetus belongs to. The one 'runt' had a weight of 1.7 g and was in a litter with an average litter weight of 3.7±1.0 g so that it was almost half or higher if the lower variation range is considered so that the definition as 'runt' does not appear correct. Thus this 'finding' should not be regarded as of concern or for classification. In this study increased methemoglobin concentrations were observed in all treated groups which most likely were responsible for such delays due to hypoxia. Thus, the discussed effects would be secondary to maternal hypoxia and not to a direct compound-related developmental effect.
Therefore, no teratogenic potential can be derived from the results of this study since only normal background findings were seen; especially based on the affected litter numbers which is the more relevant parameter no dose-related or treatment effect is obvious.

Barton, 1991 study

It is stated that in the [redacted] 1991 study in Sprague-Dawley rats, the incidences of cleft palate were changed. It is further stated in the CLH report that classification for developmental toxicity is proposed based on the incidence of cleft palate in the [redacted] 1991 study. This finding occurred only at a high dose of 1,000 mg/kg bw/day in the presence of maternal toxicity (indicating hemolytic anaemia) and as such can be considered as a secondary effect to maternal toxicity. Concern is further reduced by its occurrence in only one litter, further supporting a maternal factor rather than a specific developmental effect of the test substance."
We agree to the conclusion of UK in the commenting phase that a classification for developmental toxicity is not warranted. In this rat teratogenicity study ([redacted] 1991 M-146990-01-1) cleft palate was observed in 3 fetuses from the same litter at 1000 mg/kg bw/day. In this study, at the highest desmedipham dose of 1000 mg/kg bw severe maternal toxicity was seen as indicated by reduced food consumption and body weight gain, by discoloration of the urine and by increased spleen weight. At 250 mg/kg/day, occasionally animals with discolored urine and a slightly increased spleen weight were observed. Furthermore, from another developmental toxicity study ([redacted]., 1985a, M-146758-01-1) distinctly increased methemoglobin and Heinz body levels are known from 10 and 100 mg/kg bw on, respectively. From studies with repeated administration in rats an increase of methemoglobin levels from 30 mg/kg bw on is known. Therefore, the cleft palate incidences were seen in a highly maternal toxic dose which most likely was beyond MTD. Malformations can occur secondary to hypoxia (methemoglobinamia), also fetal cleft lip and other congenital abnormalities have also been linked to maternal hypoxia, ([redacted]. (2007): The effect of hypoxia in development. Birth Defects Research Part C: Embryo Today: Reviews, 81(3), 215-228). Thus, the discussed effects would be secondary to maternal hypoxia and not to a direct compound-related developmental effect.

At the maternal-toxic dose of 1000 mg/kg bw some skeletal and visceral effects were addressed in the report which says "At the maternally toxic dose level of 1000 mg desmedipham/kg bw/day, there were unequivocal adverse effects on foetal development, indicated by reduced foetal weight and reduced state of skeletal ossification. In the 2 litters with the lowest mean foetal weight, there were 5 foetuses with abnormalities of the palate or interventricular septum, and it was considered that these abnormalities were further indications of effects on patterns of growth during foetal development."
The latter two abnormalities are regarded as consequence of the maternal toxicity, furthermore it can be seen from the following table that the incidences of the interventricular septal defect were within the HCD and thus should not be of concern.

The following table shows selected findings and historical data ranges:

Finding	Dose (mg/kg bw)				HCD[a]
	0	60	250	1000	
Interventricular septal defect					
Fetal incidence (%)	0	0	0	0.62	0.67
Litter incidence (%)	0	0	0	4.8	8.33
Cleft palate					
Fetal incidence (%)	0	0	0	0.93	
Litter incidence (%)	0	0	0	4.8	

[a] from "Historical Control Data for Development and Reproductive Toxicity Studies using the Crl:CD® BR Rat, Compiled by MARTA (Middle Atlantic Reproduction and Teratology Association), Edited by Patricia L/Lang, Ph.D. Consultants Toxicology, September, 1993, M-259312-01-1

With regard to post-implantation loss, only in one of the developmental toxicity studies in rabbits ([redacted], 1984) an increased incidence of this parameter was noted. The study report concludes that this was mainly caused by the high maternal toxicity at the highest dose which caused an increased incidence of abortions. The following table gives an overview of the data of this study.

Parameter/Finding	Dose (mg/kg bw)			
	0	50	150	450
Body weight gain (g) days 0-28 p.c. (% of control)	580	483 (-16.7)	460 (-20.1)	279 (-51.4)
Incidences of abortions[a]				
partial	0/14	0/15	0/13	1/14
total	0/14	0/15	1/13	1/14
Postimplantation loss (%)	11.7	17.8	12.9	26.8

p.c. = post coitum
[a] of pregnant animals

The table shows that the postimplantation loss was increased at the highest dose of 450 mg/kg bw. It can be seen that at the highest dose a clear reduction of the body weight gains occurred with -51.4 % which started already at 150 mg/kg bw and that at the highest dose the number of abortions was increased. Thus a clear correlation between the body weight effects, the incidences of abortions and the postimplantation losses exists. Thus, we conclude that this is not a direct effect of DMP, but rather a secondary effect of the high maternal toxicity. This is supported by the lack of such findings in all other developmental toxicity studies in rabbits at doses which did not cause body weight effects.

The developmental toxicity study in rabbits by [redacted] 1991 which used doses of 0, 30, 90 and 270 mg/kg bw with some body effects at 270 mg/kg bw which however were less severe than in the aforementioned study, did not indicate an increase in postimplantation loss as shown in the relevant table from the report:

Parameter/Finding	Dose (mg/kg bw)			
	0	30	90	270
Body weight gain (g) days 0-29 p.c. (% of control)	480	470 (-2.1)	460 (-4.2)	350 (-27.1)
Number pregnant animals	13	14	14	14
Total implants	136	97	143	137
Total live implants (% of total implants)	123 (90)	91 (94)	123 (86)	116 (85)
Total dead implants	13 (10)	6 (6)	20 (14)	21 (15)
Total early deaths	4 (3)	3 (3)	10 (7)	13 (9)
Total late deaths	4 (3)	1 (1)	5 (3)	1 (1)
Total fetal deaths	5 (4)	2 (2)	5 (3)	7 (5)

(% of total implants)				

It can be seen that there was no severe maternal toxicity based on the body weight gains and lack of abortions at the highest dose and also the incidences of deaths were not affected as in the [redacted] 1984 study which supports the discussed relevance of the maternal toxicity for the postimplantation loss in the [redacted] 1984 study.

It is overall concluded that the developmental effects in the studies with desmedipham were caused by strong maternal toxicity and not by a direct developmental toxic potential of this compound.

Under 10.12 Specific target organ toxicity-repeated exposure, the CLH report proposes "Although the severity of the haemotoxic effects represents a borderline case, multiple less severe and dose-related effects with regenerative capacity involving several organs were observed rather consistently in oral repeated dose toxicity studies in three species at the dose levels approximately equal to the STOT-RE 2 guidance values. These effects are considered sufficient for classification. Therefore, classification of desmedipham for STOT-RE 2 ("H373: May cause damage to organs (blood) through prolonged or repeated oral exposure") is proposed."

The following tables give an overview of the results for methemoglobin levels and Heinz bodies in the different studies:

Suter et al., 1985, Methemoglobin levels in the 90-day rat study

Dose (ppm)	Males					Females				
	0	6	30	60	300	0	6	30	60	300
Met-Hb (%) (changes in % of control)										
Week 4/5	1.1	0.9 (-18.2)	1.2 (+9.1)	1.1 (0)	2.8* (+154.5)	0.7	0.8 (+14.3)	0.8 (+14.3)	0.9 (+28.6)	1.6* (+128.6)
Week 9	0.8	0.9 (+12.5)	1.1 (+37.5)	1.2* (+50)	3.5* (+337.5)	0.8	0.8 (0)	1.0 (+25)	1.1* (+37.5)	2.7* (+237.5)
Week 12/13	1.1	1.1 (0)	1.0 (-9.1)	1.5 (+36.4)	2.4* (+118.2)	0.8	0.9 (+12.5)	0.8 (0)	0.9 (+12.5)	1.4* (+7.5)
Week 16/17	0.8	0.9 (+12.5)	0.9 (+12.5)	1.0* (+25)	1.1* (+37.5)	0.8	1.0 (+25)	0.8 (0)	0.8 (0)	0.8 (0)
Heinz bodies (‰)										
Week 4/5	1	0	0	0	0	0	0	0	0	0
Week 9	0	0	0	0	0	0	0	0	0	0
Week 12/13	0	0	0	0	0	0	0	0	0	0
Week 16/17	0	0	0	0	1	1	0	0	0	0

* = Dunnett-test based on pooled variance or Steel-test significant at 5% level

It can be seen that after week 4/5 only at 300 ppm the methemoglobin level was increased approximately twice of the control, whereas at week 9 also at 60 ppm much lower, but statistically significant increases were seen. However the values of 1.2 and 1.1 % for males and females, respectively, were minimal and close to the variation range of the controls in this study. Therefore,

these slight deviations are regarded as due to variability. Also the minimal increases at 60 and 300 ppm after the recovery period are due to variability and also close to the variation range of the controls. Statistical significance was most likely only due to the fact that by chance the control values were at the lower range of the normal control values. Also the fact that only males and not the females showed this spurious finding supports that this is not a treatment-related effect. Furthermore, these methemoglobin levels are much lower than the level of 4% which is regarded as maximum non-adverse level ([redacted] 2005, Food and Chemical Toxicology 43 (2005) 1569-1593).
If the discussed hematological effects were assumed as real treatment-related effects, spleen effects which could be the consequence of these effects would be expected at this dose. However, no histopathological changes were observed at the highest dose so that histopathological examinations of the lower doses were not necessary and not performed in this study.
Any changes in the spleen at the lower doses are not expected since in the other subchronic rat studies hemosiderosis as the possible subsequent finding in the spleen was not seen at doses which are in the range of the doses below 300 ppm.
An overview of the spleen findings in this and another subchronic study is given in the following table:

Spleen effects in subchronic rat studies

Study (Doses)	Spleen effects	LOAEL	NOAEL
[redacted]., 1985 (0, 300, 1200, 4800 ppm)	Spleen enlarged and dark red to black in color (m)	4800 ppm (415 mg/kg bw)	1200 ppm (97 mg/kg bw)
	Increased hematopoiesis (m, f)	300 ppm (24 (m), 27 (f) mg/kg bw)	No lower dose than 300 ppm in this study
[redacted] 1987 (0, 160, 800 and 4000 ppm)	Spleen weight increase (m, f)	4000 ppm (275 (m), 339 (f) mg/kg bw)	800 ppm (54 (m), 60 (f) mg/kg bw)
	Hemosiderosis, minimal to moderate (f)	800 ppm (60 mg/kg bw (f))	160 ppm (12.3 mg/kg bw (f)) 800 ppm (54 mg/kg bw (m))

m = males; f = females

Thus, the minimal changes at the highest dose of 300 ppm which are regarded as due to variability do not warrant a STOT-Re classification.
Since in the other toxicity studies mostly higher doses were used, the aforementioned study from [redacted] 1985 is the relevant study for discussion of the hematological effects and their relevance for warranting classification.

The CLH report states that the "effects on thyroid system in available repeated dose toxicity studies (increased thyroid weight, follicular hypertrophy, decreased T3 and T4) did not always occur at doses which also caused signs of liver toxicity (1987, RAR B.6.3.2/04; 1991, RAR B.6.3.2/09)."

We disagree with this conclusion.
An evaluation of findings in the toxicology studies with desmedipham in which thyroid effects were observed, showed that at doses with thyroid effects also hepatic effects, like liver enzyme induction and subsequent liver effects occurred. However, no thyroid tumors occurred in the two conducted rat carcinogenicity studies. Also in dogs thyroid effects were observed at doses with liver effects. No liver and also no thyroid effects were seen in mice, neither in the 90-day nor in the oncogenicity study which further supports the causal relationship between the liver and thyroid effects.
A special study in rats was conducted with desmedipham to investigate the MOA of the thyroid effects. From the results of the MOA study, it can be concluded that the thyroid effects are not caused by a direct

mechanism, but by the known rodent-specific mechanism via liver enzyme induction, especially of UDPGT which is responsible for an increased catabolism of thyroid hormones. The reduced thyroid hormone levels subsequently trigger a TSH feedback release which over longer time periods stimulates the thyroid tissues leading to the observed thyroid findings however desmedipham did not cause thyroid tumors. This MOA is similar to that of phenobarbital which caused similar thyroid changes up to thyroid tumors in rodents but not in humans. This MOA is a well-known example in literature of a mainly rodent-specific effect which has no relevance to humans. All these effects are secondary to induction of hepatic enzymes as a primary key event. This document summarizes all relevant existing toxicological data and concludes that the thyroid effects are caused by a liver enzyme-mediated MOA that is not relevant to human. This is also valid for the thyroid findings in dogs in which a similar MOA like that in rats is known from literature ([redacted] 2000).

An overview of the thyroid and liver effects in the different species and study types is given in the following tables.

Rat studies

90-day rat dietary toxicity study (M-146746-01-1, [redacted] 1984)

In this study, Wistar rats received desmedipham at concentrations of 0, 300, 1200 and 4800 ppm via the diet for 13 weeks.
The pathological evaluation showed mainly toxic hemolytic anemia and a compensatory erythrocytic response. Reactive and compensatory processes in response to hemolysis were seen in spleen, liver, and kidneys. At 4800 ppm, swollen or enlarged spleens were noted, increased hemopoiesis in the spleen at 1200 and 4800 ppm and extramedullary hemopoiesis and/or iron deposition in Kupffer cells in the liver and brown pigmentation, partly iron-positive in the renal tubular epithelium at 1200 and 4800 ppm. Relative liver weights were increased at 1200 and 4800 ppm, at the same doses follicular hyperplasia in the thyroid gland was noted, at 1200 ppm in 8 males and 10 females and at 4800 ppm in 8 males. and 10 females. The thyroid weights were not determined in this study. 300 ppm is regarded as NOAEL for the thyroid findings. The thyroid findings and relative liver weights are summarized in the following table.

Overview of thyroid findings and relative liver weights in the 90-day rat study

Parameter	Unit	Dose Level (ppm)							
		Males				Females			
		0	300	1200	4800	0	300	1200	4800
Group size	N	10	10	10	10	10	10	10	10
TMI	(mg/kg bw/day)	-	24	97	415	-	27	109	378
Thyroid hormones		Not measured				Not measured			
Thyroid weight		Not measured				Not measured			
Follicular hyperplasia	Week 13	0/10	1/10	8/10	8/10	0/10	0/10	10/10	10/10
Liver weight rel. (%)	Week 13	2.447	2.485	2.729	3.233**	2.681	2.921	3.162**	3.351**

** p<0.01

It can be seen that the relative liver weight increases occurred thus at the same doses at which follicular hyperplasia in the thyroid gland was noted, at 1200 ppm and 4800 ppm.

90-day rat dietary toxicity study (M-146760-01-1, [redacted] 1984)

In this 90-day study, 25 Wistar rats per group and sex received desmedipham at concentrations of 0, 6, 30, 60 and 300 ppm via the diet for 13 weeks. 10 animals per group and sex were observed for an additional recovery period of 4 weeks without treatment. Clinical biochemistry data indicated slightly lower T4 values for both sexes of the 300 ppm group at weeks 12/13 of treatment, and for 300 ppm males at termination of the recovery phase. No treatment-related organ weight changes of the thyroids were observed after the 13-week treatment or the recovery phase. No treatment-related macroscopic and microscopic findings were observed after 13 and 17 weeks, respectively. The relevance of the slight T4 changes may be questioned and they were rather due to variation than to a real thyroid effect since no thyroid weight changes and no morphologic changes in the thyroids occurred. This is in agreement with the liver weights which were only slightly affected in males. An overview of the thyroid effects is given in the following table.

Overview of thyroid findings in the 90-day rat study

Parameter	Unit	Dose Level (ppm)									
		Males					Females				
		0	6	30	60	300	0	6	30	60	300
Group size	N	25	25	25	25	25	25	25	25	25	25
Dose/bw	(mg/kg bw/day)	-	0.5	2.6	5.2	26	-	0.5	2.7	5.6	27
Relative liver weight	%	3.28	3.30	3.26	3.30	3.39	3.47	3.38	3.24*	3.26*	3.32
T3 (nmol/l)	4/5	0.67	0.55	0.56	0.58	0.69	0.72	0.77	0.82	0.80	0.74
	12/13	1.20	1.14	1.11	1.25	1.33	1.27	1.25	1.34	1.36	1.08
	16/17	1.05	0.95	0.88	0.74*	0.83	0.87	0.80	0.75	0.75	0.68
T4 (nmol/l)	4/5	66.2	62.6	60.6	56.6*	60.4	52.0	50.5	55.2	54.8	45.8
	12/13	35.4	32.0	31.4	30.7	24.8*	21.3	17.1	23.9	18.5	6.6*
	16/17	33.4	30.2	27.3	27.9	25.6*	18.0	15.2	14.4	14.6	11.7
Thyroid Weight	g	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
	% bw	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	% brain	1.12	1.14	1.15	1.10	1.08	1.02	1.03	1.02	0.96	1.01
Thyroid Pathology		No effect					No effect				

* $p<0.05$

90-day rat dietary toxicity study (M-146976-01-1, [redacted], 1987)

In this 90-day study, 10 Sprague-Dawley rats per group and sex received desmedipham at concentrations of 0, 160, 800 and 4000 ppm via the diet for 13 weeks. Higher adjusted liver weights were recorded for 4000 ppm males (11.3 % versus control, statistically significant) and females in which also a rather distinct increase of 9.3 % compared with controls occurred. Higher thyroid (male weights adjusted, female weights unadjusted) weights were recorded in 4000 ppm males and females. Minimal to moderate follicular cell hypertrophy of the thyroids was noted mainly in 4000 ppm males and females, and thus at doses at which also liver effects occurred. An overview of thyroid and liver findings is given in the following table.

Overview of thyroid, pituitary and liver findings in the 90-day rat study

Parameter	Unit	Dose Level (ppm)							
		Males				Females			
		0	160	800	4000	0	160	800	4000
Group size	N	10	10	10	10	10	10	10	10
Dose/bw	(mg/kg bw/day)	-	10.6	54	275	-	12.3	60	339
Liver weight (adjusted for final bw)	g	21.2	19.9	19.2	23.6*	10.7	10.9	10.9	11.7
Thyroid Weight	absolute g	0.019	0.020	0.020	0.024	0.015	0.014	0.014	0.019**
	adjusted+ g	0.018	0.019	0.020	0.026**	na	na	na	na
Thyroid follicular cell hypertrophy	Minimal	0	1	4	6	0	0	3	6
	Moderate	0	0	0	2	0	0	0	3
	Total	0	1	4	8	0	0	3	9

+ Under use of final body weight as covariate
Na = not calculated in report
* P<0.05; ** P<0.01

52-week rat dietary toxicity study (M-146979-01-1, [redacted] 1990)

In this study, 20 Sprague-Dawley rats per group and sex received desmedipham at concentrations of 0, 100, 400 and 1200 ppm via the diet for 52 weeks. Liver weights were slightly increased in females. Histopathologically, in 1200 ppm males Kupfer cell pigmentation in the liver was increased. No thyroid findings were observed. Absolute liver weights were not affected, however, relative liver weights were not provided in the report. Since the final body weights were lower at 1200 ppm in both sexes, there seems to be an increased relative liver weight at the highest dose. An overview of the liver and thyroid effects is given in the following table.

Overview of thyroid and liver findings in the 90-day rat study

Parameter	Unit	Dose Level (ppm)							
		Males				Females			
		0	100	400	1200	0	100	400	1200
Group size	N	20	20	20	20	20	20	20	20
Dose/bw	(mg/kg bw/day)	-	6.5	25.2	75	-	8.0	31.7	97.1
Liver weight absol.	g	21.36	21.69	21.04	20.02	12.74	14.82*	13.80	13.10
Thyroid weight	g	Not determined				Not determined			
Thyroid – no abnormality detected		20/20	-	1/1	18/20	19/20	1/1	-	19/20
Thyroid pathology		No treatment-related effect				No treatment-related effect			

* P<0.05

With regard to thyroid pathology only a few incidences of benign C-cell adenomas and one focus of C-cell hyperplasia occurred which were not dose- and thus not treatment-related. Thus, as remarked in the table above the thyroids did not show pathological effects.

2-year rat dietary toxicity study (M-146980-01-1, [redacted], 1991)

In this study, 50 male and female Sprague-Dawley rats received desmedipham at concentrations of 0, 100, 400 and 1200 ppm via the diet for 104 weeks.

Thyroid weights were not determined in this study. A higher incidence of deposition of hemosiderin pigment was noted in the liver of 1200 ppm males and in the spleen of 800 ppm and 1200 ppm females. No histopathological changes in the thyroids were observed, as can be seen in the following table.

Overview of thyroid findings in the 2-year rat study

Parameter	Unit	Dose Level (ppm)							
		Males				Females			
		0	100	400	1200	0	100	400	1200
Group size	N	50	50	50	50	50	50	50	50
Dose/bw	(mg/kg bw/day)	-	5.4	21.6	64.4	-	6.9	28.4	86.5
Liver weight (co-variance analysis)	g	19.13	20.35	20.35	21.00	16.48	16.32	17.66	17.76
Thyroid Weight	g	Not determined				Not determined			
Thyroid – no abnormality detected		34/50	16/18	16/27	38/49	40/50	21/25	22/30	41/50
Thyroid histopathology		No treatment-related effect				No treatment-related effect			

In this study histopathologically some incidences of benign C-cell adenomas and of C-cell hyperplasia were seen which however were not dose- and thus not treatment-related. Thus, as remarked in the table above the thyroids pathologically did not show treatment effects.

2-year rat dietary toxicity study (M-146766-01-1, [redacted] 1986)

In this study, 70 male and female Wistar rats received desmedipham at concentrations of 0, 60, 300 and 1500 ppm via the diet for 2 years. Clinical chemistry data indicated as sign of liver effects an increased total bilirubin level for male and female rats at 1500 ppm after 12 months of treatment and for 1500 ppm females after 24 months of treatment. Furthermore, a decreased triiodothyronine (T3) level was noted for female rats at 300 and 1500 ppm after 12 months and a decreased thyroxine (T4) level for female rats at 300 ppm after 12 and 24 months of treatment, and for both sexes at 1500 ppm after 12 and 24 months of treatment. These findings occurred in parallel to liver effects since slightly higher relative liver weights were seen in all female dose groups at the terminal sacrifice.
In the thyroid gland, diffuse follicular hyperplasia was observed at interim and terminal sacrifice at a higher incidence in 300 and 1500 ppm males and in 1500 ppm females. Diffuse or multifocal c-cell hyperplasia occurred at a higher incidence and severity in 300 and 1500 males at the terminal but not at the interim sacrifice. An overview of the liver and thyroid effects are given in the following table.

Overview of thyroid and liver findings in the 2-year rat study

Parameter	Unit	Dose Level (ppm)							
		Males				Females			
		0	60	300	1500	0	60	300	1500
Group size	N	50	50	50	50	50	50	50	50
Dose/bw	(mg/kg bw/ day)	-	3.2	16	80	-	3.9	20	100
Liver weight	rel (% bw)	2.82	2.86	2.81	2.92	2.94	4.24*	3.32*	3.37*
T3 (nmol/L)	12 months	1.39	1.36	1.43	1.42	1.40	1.33	1.22*	1.10*
	24 months	1.36	1.32	1.22	1.10	1.38	1.16*	1.29	1.21
T4 (nmol/L)	12 months	60.3	54.1	56.3	44.2*	36.3	34.3	29.2*	24.7*
	24 months	27.1	26.0	23.5	21.5*	33.9	29.1*	20.3*	12.9*
Thyroid Weight (12 months)	g	0.04	0.03	0.04	0.04	0.03	0.03	0.03	0.03
	% bw	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	% brain	1.94	1.57	1.72	2.01	1.50	1.51	1.44	1.71
Thyroid Weight (24 months)	g	0.05	0.04	0.04	0.04	0.03	0.03	0.06	0.03
	% bw	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01
	% brain	2.20	1.71	1.93	1.83	1.48	1.64	2.93	1.49
Follicular cell hyperplasia	12 months	1/10	4/10	5/10	6/10	1/10	1/10	2/10	4/10
	24 months	6/60	9/60	14/59	25/59	3/59	5/59	5/59	12/60
C cell hyperplasia	12 months	2/10	4/10	0/10	4/10	1/10	4/10	2/10	1/10
	24 months	20/60	21/60	29/59	34/59	28/59	29/59	28/59	26/60

* P<0.05

Rat 2-generation study (M-146764-01-1, [redacted] 1986)

In this study, desmedipham was administered orally to the F0 generation of Wistar rats during an 80-day pre-pairing, pairing, gestation and lactation periods for breeding of the F1A and FIB litters. Following the weaning of the FIB litters on day 21 post-partum, the pups were reared for a further seven days on the test diet.
Slight parental toxic effects in the F0 and F1 parent animals and progeny of the 1250 ppm group were noted. In the 250 ppm group, only infrequently reduced body weights of the F1A pups and increased spleen weights in the F0 females, were observed. In the F1 generation, minimal to slight increases in hemopoiesis and hemosiderosis in the liver of rats of the 1250 ppm group, slightly enhanced incidence and severity of erythropoiesis and hemosiderosis in the spleen of rats at 250 ppm and 1250 ppm, and slightly increased erythropoietic activity in the bone marrow of a few rats at 50, 250, and 1250 ppm were seen as response to hemolytic anemia. Although not statistically significant, the relative liver weights appeared to be slightly increased at the highest dose in males and females. In the thyroid, follicular hyperplasia was observed which was not regarded as a direct organ lesion but rather as a change indicating altered thyroid function.
The liver and thyroid effects are shown in the following table.

Thyroid effects in the 2-generation rat study

Parameter	Unit	Dose Level (ppm)							
		Males				Females			
		0	50	250	1250	0	50	250	1250
Dose/bw	(mg/kg/day)	-	4	20	90	-	6	30	140
Relative liver weights (F0 P*)	%	3.063	3.148	2.935	3.044	4.363	4.613	4.537	4.694
Thyroid Weight	g	Not determined				Not determined			
Follicular cell hyperplasia		0/26	1/26	5/25	11/26	0/26	2/25	3/25	12/25

* P = parents

Conclusion of the rat studies with regard to liver and thyroid effects

It is concluded that in the rat studies the effects on thyroids occurred only at doses which also caused liver effects and signs of general toxicity, like hemolytic anemia and liver effects so that the thyroid findings were no primary toxicological effects. It is concluded that the thyroid effects are secondary to the observed liver effects. Especially liver enzyme induction is known to cause an increased metabolism and excretion of thyroid hormones which leads to a feedback mechanism of a TSH release with higher TSH levels which over longer time periods leads to a stimulation of the thyroids.

This relationship between the thyroid and the liver effects was demonstrated in a MOA study in rats.

Thyroid MOA study in rats

To investigate the MOA of the thyroid findings a special MOA study in rats was performed. In this study, desmedipham was administered via the diet to groups of male Wistar rats (15/group) for at least 28 days at concentrations of 0, 1500 and 4000 ppm, corresponding to 94 and 252 mg/kg bw/day. All animals were observed for mortality and clinical signs daily, body weight and food consumption were measured weekly. A detailed physical examination was performed weekly throughout the study. Before necropsy a blood sample was collected from the retro-orbital venous plexus of each animal for possible further analysis. All animals were necropsied, brain and liver were weighed. Liver, thyroid gland and pituitary gland were sampled. Two pieces of both the median and the left lobe of the liver plus the pituitary gland from each animal were collected, and flash frozen in liquid nitrogen and stored at approximately -74°C ± 10°C for gene transcript analyses by quantitative Polymerase Chain Reaction (q-PCR). In addition, the thyroid gland was collected and flash frozen in liquid nitrogen and stored at approximately -74°C + 10°C for possible gene transcript analyses. The remaining portions of the liver from all animals were homogenized for microsomal preparations for possible further analysis.

In the 4000 ppm group, at necropsy, mean relative liver weight was higher when compared to the controls. Total cytochrome P-450 content was increased by 1.2-fold, T4-GT activity by 1.3 fold and PROD activity by 1.7-fold (Table 13). In the liver, the up-regulated phase I enzyme gene transcripts were Cyp2b1 and Cyp4a1 when compared to the controls. Regarding the phase II enzyme gene transcripts, Ugt1a6, Ugt2b1 and Ephx1 were up-regulated when compared to the controls. In the pituitary gland, Tshb gene transcripts were slightly up-regulated (not statistically significant) compared to the controls.
At 1500 ppm PROD activity was increased by 1.3-fold versus controls. In the liver, the up-regulated phase I enzyme gene transcripts were Cyp2b1 and Cyp4a1. Regarding the phase II enzyme gene transcripts, Ugt1a6 and Ephx1 were up-regulated. Ugt2b1 gene transcripts were very slightly up-regulated ((not statistically significant) when compared to the controls.
In the pituitary gland, Tshb gene transcripts were very slightly up-regulated (not statistically significant) when compared to the controls.
An overview of the key events of this MOA as demonstrated in this study is given in the following tables.

Liver enzyme induction results

Parameter	Control 0 ppm	Desmedipham 1500 ppm	Desmedipham 4000 ppm
Total P450 nmol/mg protein	0.70 ± 0.16a (100.0 ± 22.3)	0.75 ± 0.21 (108.1 ± 30.3)	0.86 ± 0.13** (123.7 ± 18.6)
T4-GT pmol T4 glucuronide formed/min/mg protein	2.20 ± 0.33 (100.0 ± 15.0)	2.50 ± 0.75 (113.4 ± 34.0)	2.78 ± 0.70** (126.1 ± 32.0)
PROD pmol resorufin formed/min/mg protein	2.11 ± 0.71 (100.0 ± 33.6)	2.78 ± 0.65* (132.2 ± 30.8)	3.61 ± 1.20*** (171.3 ± 57.0)
BROD pmol resorufin formed /min/mg protein	23.42 ± 8.46 (100.0 ± 36.1)	23.37 ± 5.76 (99.8 ± 24.6)	24.90 ± 6.49 (106.3 ± 27.7)

Values are Mean ± SD. Values in parenthesis are mean % control ± SD. n = 15 per group. A Student's t-test (2-sided) was performed on the results; *statistically different from control p<0.05; **p<0.01; *** p<0.001.

Gene transcript results

Organs		Mean relative quantity ± standard deviation of gene transcripts (% change compared to control mean values)		
Liver	Gene transcripts	Control	Desmedipham 1500 ppm	Desmedipham 4000 ppm
	Cyp1a1	0.21 ± 0.236	0.80 ± 1.435	0.51 ± 0.647
	Cyp2b1	0.73 ± 0.547	2.51** ± 2.250 (+244%)	7.71** ± 6.893 (+956%)
	Cyp3a3	0.87 ± 0.249	1.04 ± 0.332	1.01 ± 0.270
	Cyp4a1	0.88 ± 0.325	1.48** ± 0.511 (+68%)	3.60** ± 1.262 (+309%)
	Ephx1	0.85 ± 0.169	1.11** ± 0.297 (+31%)	1.32** ± 0.377 (+55%)
	Gstm4	0.77 ± 0.172	0.83 ± 0.154	0.82 ± 0.118
	Sult2a2	0.74 ± 0.388	0.81 ± 0.420	0.91 ± 0.507
	Ugt1a6	0.75 ± 0.243	1.16** ± 0.355 (+55%)	1.28** ± 0.318 (+71%)
	Ugt2b1	0.39 ± 0.320	0.53 ± 0.248 (+36%)	0.73* ± 0.429 (+87%)
	Por	1.27 ± 0.494	1.51 ± 1.051	1.42 ± 0.482
Pituitary gland	Tshb	1.12 ± 0.616	1.37 ± 0.751 (+22%)	1.55 ± 0.869 (+38%)

Values are Mean ± SD. Values in parenthesis are % of control;
*statistically different from control p<0.05; **p<0.01; *** p<0.001.

It is concluded from this study that the thyroid effects can be explained as a consequence of liver enzyme induction because some evidence of CAR activation was observed qualitatively through increased PROD activity and induction of Phase II enzymes (Ugt1a6, Ugt2b1 transcripts; T4-GT increased) associated with an increase of TSH (Tshb transcript increased). Although the magnitude of changes of

key parameters investigated in this study was not very high compared with that of phenobarbital, the typical phenobarbital-like MOA for inducing thyroid effects in rodents was clearly demonstrated. This weak potency also explains that the liver enzyme and secondary thyroid effects did not lead to thyroid tumors as seen with other similar non-genotoxic liver enzyme inducers, like phenobarbital.

Thus, it is concluded that the typical phenobarbital-like MOA for inducing thyroid effects in rodents was clearly demonstrated. This rodent-specific MOA is not considered relevant for humans.

An additional important fact is that no thyroid tumors occurred, thus the potency of the thyroid effects is small.

Additional consideration of ToxCast and Tox21 Activity Data for Desmedipham

The predictions of ToxCast and Tox21 data are summarized in this chapter. A systems biology model that integrates eighteen ToxCast/Tox21 assay endpoints for ER-based pathway activity is negative for desmedipham, indicating a prediction that desmedipham does not act as an estrogen or anti-estrogen. A systems biology model that integrates nine ToxCast/Tox21 assay endpoints for AR-based pathway activity is negative for desmedipham, indicating a prediction that desmedipham does not act as an androgen or anti-androgen. Desmedipham was negative in the two aromatase inhibition assays in the ToxCast/Tox21 assay suite and not tested in the 20 H295R assays for steroidogenesis (10 hormones analyzed in positive and negative directions). Desmedipham was negative in four thyroid receptor alpha-related assays in ToxCast/Tox21; there is no suggestion that desmedipham interacts directly with thyroid hormone receptors.

Thus it can be concluded from the ToxCast and Tox21 Activity Data:

- they do not indicate a clear biological effect or suggest a mode of action for toxicity.

- one ToxCast positive result is consistent with the observed thyroid findings (ATG_PXRE_CIS_up), but this single assay is not sufficient to predict thyroid effects.

- no specific evidence of endocrine activity was observed for desmedipham in ToxCast.

Overall, it can be concluded that no evidence of an endocrine potential of desmedipham was detected in ToxCast/Tox21.

Overall conclusion on liver and thyroid findings

The effects on thyroids occurred at doses which also caused signs of general toxicity, like hemolytic anemia and especially liver effects and it is postulated that the thyroid effects are secondary to the observed liver effects. Especially liver enzyme induction is known to cause an increased metabolism and excretion of thyroid hormones which leads to a feedback mechanism of a TSH release with higher TSH levels which over longer time periods leads to a stimulation of the thyroids. This relationship between the thyroid and the liver effects was demonstrated in a MOA study in rats. This study demonstrated that the thyroid effects can be explained as a consequence of liver enzyme induction because some evidence of CAR activation was observed qualitatively through increased PROD activity and especially through induction of Phase II enzymes (Ugt1a6, Ugt2b1 transcripts; T4-GT increased) together with an increase of TSH (Tshb transcript increased). Although the magnitude of changes of key parameters investigated in this study was not very high such MOA for inducing thyroid effects in rodents was clearly demonstrated. This weak potency also explains that the liver enzyme and secondary thyroid effects did not lead to thyroid tumors as seen with other similar non-genotoxic liver enzyme indices,

like phenobarbital. This rodent-specific MOA is not considered relevant for humans. Also from the ToxCast and Tox21 Activity Data no evidence of thyroid effects can be derived. Furthermore, males appeared to be more sensitive than females, which is a characteristic of liver-mediated thyroid changes. In case of thyroid toxicants acting directly on the thyroid (i.e. by inhibiting thyroid hormone biosynthesis) females would be equally sensitive to males which is not the case with desmedipham.

There is no relevance of such findings to humans due to differences in thyroid physiology between rodents and humans (Capen, 1997; Dellarco et al 2006). The main reasons for the difference in response between rodents and humans are as follows:

- Rodents are more sensitive to thyroid hormone changes
- Rodents have enhanced thyroid hormone elimination
- Thyroxin binding globulin is major plasma protein in humans (which acts as a buffer), but not in rodents, as a consequence, the concentration of unbound T4 is greater in rodents than humans, resulting in greater susceptibility to metabolism and excretion and compensatory increase in thyroid follicular cell turnover

ECHA note – An attachment was submitted with the comment above. Refer to public attachment DMP_ECHA commenting_task force_sanitized.pdf

Dossier Submitter's Response

Thank you for your comment. We agree that aniline was not detected in metabolism studies in rat. Aniline was proposed as a intermediate metabolite from PC ring part (dRAR B6.1.1/03, B6.1.1/06). Aniline is very transient in nature and may rapidly and completely be converted to more polar products 4-aminophenol and further to 4-acetamidophenol. Based on the results from the toxicokinetic study desmedipham is metabolised to compounds which have aromatic amine structure. It is well known that aromatic amines have potential to induce haematological effects. The classification proposal is based on the data on desmedipham itself and supported by read-across.

Reproductive toxicity/developmental effects

Cleft palate/micrognathia/agnathia

We agree that regarding the findings of cleft palate/micrognathia/agnathia) there are uncertainties (effect of maternal toxicity, genetic background). However, there were incidences of cleft palate observed in two separate studies and in two different rat strains, and malformations of the jaw in two studies in one rat strain. Furthermore, in none of these studies were there similar observations made in the control animals arguing against the sole role for the background in these incidences. We agree that congenital malformations may occur secondary to hypoxia. Nevertheless, taking into account the uncertainties and since it cannot be excluded that the observed malformations are treatment-related, category 2 was proposed.

Runts

The expression "runt" is used in the study reports (1985a, b). There is a definition for "runt" in the ECETOC guidance (2002): small foetus < half the size of litter mates; they are considered malformations and of high concern. There were observations made of runts in two separate studies with desmedipham although with low incidence but at maternally non-toxic dose. In one teratogenicity study with phenmedipham (RAR B.6.6.2/02), which is structurally very close to desmedipham, runts there were observed both in the range finding study and in the main study. For phenmedipham it was concluded that occurrence of runts could not be considered as a secondary non-specific consequence of other toxic effects.

Interventricular septal defects
We agree that the visceral malformations affecting the cardiovascular tissue could be attributed to a genetic background. However for the same arguments as for cleft palate/jaw malformations the fact that the incidences were not evenly distributed across treated groups and the control might speak for a treatment-related effect. Taking into account the remaining uncertainty category 2 was proposed.

Rabbit studies
We agree that the post-implantation losses in the 1984 study with Chincilla hybrid rabbit may have been due to maternal toxicity and no classification for these effects were proposed. However in the 1991 study with the New England White rabbit the dose 90 mg/kg was not clearly maternally toxic. Since there were observations of early embryonic effects at this dose and since there was a dose dependent increase in the caudal pelvic shift, it was concluded that the observations raise concern for developmentally adverse effects.

Effects on methemoglobin levels and spleens in rats

In the 90-day rat study (dRAR B.6.3.2/03, 1985), after 4/5 and 9 weeks, statistically significant increase in MetHb was seen at the highest dose level of 300 ppm (26-27 mg/kg bw/day) in males and females (MetHb increase 338 % in males and 238 % in females compared to controls, respectively). Although the MetHb values (% of Hb) were below 4 %, we do not agree that these increases can be regarded as due to normal variability. The dose levels relevant for STOT RE Cat 2 classification are in a range of $0 < C \leq 100$ mg/kg bw/day.

We agree that MetHb value (1.1 %) in males at 300 ppm after recovery period (week 16/17) are close to the variation range of the control values this study (0.8-1.1 %). The increase was reported as statistically significant when compared to the relevant control group. Concerning spleen effects, we agree that the spleen was not microscopically examined from all rats at 6, 30 and 60 ppm. However, increased erythropoiesis in the spleen was noted in males at 300 ppm (14/15 vs 3/15 in controls) that could be considered as sign of compensatory changes in the blood system. Similar effects on spleen (i.e haematopoiesis, hemosiderosis and congestion of the spleen) and blood were seen in other 90-day repeated dose toxicity studies in rats (i.e. dRAR B.6.3.2/02, 1984a and dRAR B.6.3.2/04, 1987).

We note that haemotoxic effects were seen also in mice and dogs at the dose levels relevant for STOT RE 2 classification, not only in rats. Following oral administration for rats, mice and dogs, the effects observed are consistent with effects pointing towards methemoglobinemia, leading to changes in red blood cell parameters and slight hemolytic anemia, increased activities of the bone marrow, kidney, liver and spleen – the organs mainly involved in the turnover of red blood cells – and compensatory hematopoiesis.

Overall, we consider that the severity of the haemotoxic effects is not high and represents a borderline case for classification. Multiple less severe and dose related haemotoxic effects with regenerative capacity involving several organs were observed rather consistently in oral repeated dose toxicity studies in three species (rats, mice and dogs) at dose levels approximately equal to the STOT RE 2 guidance values. Therefore, we consider these effects sufficient for classification.

Effects on thyroids at doses with liver effects

In the 90-day study in rats (dRAR B.6.3.2/04, 1987) higher relative liver weights (statistically significant) with higher thyroid weights and thyroid follicular cell hypertrophy were recorded at the dose level of 4 000 ppm (275 mg/kg bw/day). However, at the dose levels relevant for STOT RE 2 classification (≤ 800 ppm) follicular cell hypertrophy with low incidence was seen in both sexes (incidence 4/10 in males and 3/10 in females at 800 ppm vs 0/10 in controls, respectively) but liver weights were not statistically significantly changed. No significant changes in thyroid gland weight were seen.

In the 90-day study in dogs (RAR B.6.3.2/09, 1991), at the doses relevant for STOT RE classification, the incidence of follicular cell hypertrophy was increased in females and males (incidences 0/4, 4/4 and 4/4; 0/4, 2/4 and 4/4 at 0, 500 and 1 500 ppm, respectively). Thyroid glands were increased in weight at 1 500 ppm in females and absolute pituitary weights were increased in males at 500 ppm (29 %). The liver weight was not statistically significantly changed.

We do not agree that in the available studies the effects on thyroids occurred only at doses which also caused liver effects. Overall, we consider that the thyroid effects observed in oral repeated dose toxicity studies at the dose levels relevant for STOT RE 2 classification are not considered sufficiently severe to justify classification. Therefore, we do not propose classification for effects on thyroid gland.

RAC's response

Read-across
RAC does not support a read-across from phenmedipham. There is a conclusive database on desmedipham itself and the toxicological similarity between the two substances is not sufficient for phenmedipham to make a meaningful contribution to the assessment of desmedipham.

Developmental toxicity
RAC considers classification in Category 2 for developmental toxicity justified mainly based on the following findings:

- Micrognathia (B.6.6.2/02, /03)
- Cleft palate (B.6.6.2/02, /05)
- Interventricular septal defect (B.6.6.2/05)

RAC agrees that the incidences of malformations were low and that the findings occurred together with some maternal toxicity. This reduces the concern and is reflected by RAC's conclusion to classify in classification in Cat. 2 instead of 1B.

Haematotoxicity
RAC notes that other studies in addition to B.6.3.2/03 have to be included in the assessment (e.g., B.6.3.2/10, B.6.5/03) and these show some haematotoxicity at doses relevant for classification. However, none of the classification criteria for haemolytic anemia provided in the Guidance on the application of the CLP (CLP guidance) criteria is met. Therefore, RAC agrees that classification is not warranted.

Thyroid-related effects
Thank you for the presentation of the data. Although the MoA study in rats indicates that induction of liver enzymes may be responsible for the thyroid effects, alternative MoAs (e.g., interference with thyroid hormone synthesis) have to be excluded in order for the liver-mediated MoA to be fully demonstrated.

Date	Country	Organisation	Type of Organisation	Comment number
12.02.2019	Denmark		MemberState	3
Comment received				
We propose to make it clear that the CLH was only open for carcinogenicity, reproductive toxicity and specific target organ toxicity on human health. Hence, other endpoint such as skin sensitisation and genotoxicity classification has not been taken into consideration. This is important as often the harmonized classification after renewals of pesticides are considered to cover all toxicological endpoints.				
Dossier Submitter's Response				
Thank you for your comment. We have followed ECHA´s instructions in order to make clear in the CLH report which hazards classes are opened for public consultation.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
15.02.2019	France		MemberState	4
Comment received				
FR, Page 2: the relevant impurity aniline have a maximal content at 0.5 mg/kg in the active substance desmedipham, according to EFSA Journal 2018; 16 (1):5150. The maximum content of 0.5 g/kg (0.05%) should be added in table 3 for aniline.				
Dossier Submitter's Response				
Thank you for your comment.				
RAC's response				
Noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
11.02.2019	Germany		MemberState	5
Comment received				
The EFSA conclusion (EFSA Journal 2018;16(1):5150) recommends Carc. 2 classification for desmedipham based on the incidences of ovarian tubular adenomas and pulmonary adenomas. Whilst the evidence is weak, the increase in pulmonary tumours in females in the 80-week mouse study is of particular concern and needs careful consideration by the RAC. A trend test indicated a significant (p = 0.03) increase in pulmonary adenomas, however the increases in pulmonary adenocarcinomas and total tumours were border-line significant, p = 0.06 and 0.07 respectively. In addition, the incidences also lie outside the HCD ranges given, although it must also be noted that the HCD are questionable as they come from published studies and some are more than 5 years older than the study itself.				
Dossier Submitter's Response				
Thank you for your comment. A slight dose-related increase in the incidences of pulmonary adenomas in desmedipham treated Crl:CD-1 BR female mice was observed in one study. We agree that RAC should carefully consider the relevance of this finding for carcinogenicity classification. As elaborated in the clh report this mice strain has variable spontaneous incidence of lung adenoma, there were no differences in tumour incidences in males or in adenocarcinoma incidences in females, and the maximum tolerated dose was exceeded in high dose females in this study. Findings in the lungs that could be related to tumor formation (increased accumulation of macrophages) were more evident in males than in females in this study and no preneoplastic leasons in lungs were reported in either sex.				

Moreover there were no indications of oncogenicity in lungs in other studies with desmedipham. Therefore we conclude that it is obscure whether this finding is treatment-related.

According to RAR the incidences of benign tumors in this study were not tested by Peto/fatal incidence analyses. To our knowledge the applicant has not submitted a Peto analysis/trend test for lung adenomas. In case you have conducted a trend test on this data we thank you for your contribution.

RAC's response

The increase in pulmonary adenomas (B.6.5/04) was no statistically significant on pairwise comparison and was not accompanied by non-neoplastic findings. There was no increase in pulmonary adenomas in males. Therefore, this finding is not considered to warrant classification.

Overall, RAC agrees with the DS's proposal of no classification.

Date	Country	Organisation	Type of Organisation	Comment number
12.02.2019	France	<confidential>	Company-Manufacturer	6

Comment received

Under 10.9.2 the CLH report states that the "incidences of pituitary adenomas of pars distalis in all Wistar KFM Han male groups", "Ovarian tubular adenomas" and "variable spontaneous incidence of lung adenoma" are considered as "borderline evidence between category 2 and no classification, but finally too weak and inconsistent and as such, not sufficient for category 2 classification. However, based on pituitary and lung adenomas classification of desmedipham for category 2 for carcinogenicity could be argued."

Although the CLH report states that the discussed factors weaken the available evidence and decrease the level of concern regarding the carcinogenicity concern for humans, we want to provide further arguments to support non-classification.

Pituitary adenomas of pars distalis ([redacted] (1986): 2-year rat (Wistar KFM-Han))

The following table gives an overview of the pituitary adenoma incidences at the interim kill after 52 weeks and at study termination after 104 weeks:

Pituitary adenomas	Doses (ppm)							
	Males				Females			
	0	60	300	1500	0	60	300	1500
Interim	1/10	-	-	4/10	1/10	-	0/1	0/10
Terminal	21/58	15/27	20/24	27/59	42/59	33/37	36/39	41/60

It can be seen that the pituitary tumor incidences do not follow a clear dose-response relationship, no dose-related incidence increase was seen in females and in males only a slight increase in the incidence at 1500 ppm. In the other long-term rat studies no effect on pituitary tumor incidences were seen. The total incidences for this benign tumor are 36.2 % in controls and 45 % in the highest dose group. HCD from the same laboratory and the same rat strain (Wistar Hannover) which conducted this study are available, the data for males from studies conducted from 1982 – 1989 are summarized in the following table:

RCC Study identification	1	2	3	4	5	6	7	8	9	10	min-max
Sex	m	m	m	m	m	m	m	m	m	m	
Experimental years	1982-1984	1981-1983	1982-1985	1982-1984	1982-1985	1983-1985	1983-1986	1984-1986	1983-1986	1984-1986	

Adenoma, pars anterior incidence (%)	26	29	22	26	19	20	22	51	20	19	
Adenoma, pars anterior incidence (%)	54,2	29,9	22,4	54,2	19,6	40	32,4	51	33,9	37,3	19.6 - 54.2

Also published HCD can be used for comparison. Published data for Wistar rats in 2-year studies from 1975 to 1980 (Bomhard et al, 1994; Exp. Toxic. Pathol. 1994; 46: 17-29) show HCD ranges for pituitary adenomas of 3.4 to 34.8 % in males and of 8.2 to 44.9 % in females. It is obvious that already the control incidence in males with 32 % is at the higher end of this HCD range so that the slightly increased incidence at the highest dose should be covered by the background range of this rat batch which was used in the Suter, 1986 study.
In another publication about carcinogenicity studies in HanWistar rats from the years 1981 to 1998 (Tennekes et al, 2004; Regulatory Toxicology and Pharmacology 40 (2004) 18-27) ranges of 14-71 % are reported. This HCD range clearly covers the observed incidences for pituitary adenomas in males in the study from [redacted] 1986. An overview is given in the following table:

Parameters	**Doses (ppm)**				**HCD**
Males	0	60	300	1500	
Pituitary adenoma incidences					
Interim	1/10	-	-	4/10	
Terminal	21/58	15/27	20/24	27/59	
Total (%)	32			45	14 – 71*

HCD: historical control data
* Tennekes et al, 2004; Regulatory Toxicology and Pharmacology 40 (2004) 18-27

This table shows that a published HCD range in HanWistar rats, i.e. the same strain as used in the [redacted] study and from the same time period, clearly covers the observed incidences for pituitary adenomas in the study from Suter et al, 1986.
Furthermore, there are two HCD studies with this strain and from this time range in the RITA database. In this database for males a HCD range of 34 – 46 % and for females of 60.4 – 68.0 % is given (RITA, lesion-related incidence data, report created: 19-Mar-2017). Also these HCD data from RITA cover the pituitary adenoma incidences of the Suter, 1996 study.
Further HCD information as provided for this finding for a study with phenmedipham for the same rat strain, Han Wistar rats, also support a HCD range of up to 45 % (see HCD data from Huntingdon Research Centre, Feb. 26, 2016 for phenmedipham study from [redacted] 2004 (M-240148-01-1)). Also the total number of tumors was not increased by treatment in this study.

Therefore it is concluded that the pituitary adenoma incidences in the study from Suter et al, 1986 do not show a clear dose-related pattern and are covered by the HCD prevalent in HanWistar rats at the time period of the study conduct and do not show a treatment-related effect on the incidences of pituitary adenomas. This is further supported by the fact that also in other long-term studies no effect on pituitary tumors was seen.
Therefore it is concluded that the pituitary adenoma incidences in the study from Suter et al, 1986 are covered by the HCD prevalent in HanWistar rats at the time period of the study conduct and do not show a treatment-related effect on the incidences of pituitary adenomas. Also in other long-term studies no effect on pituitary tumors was seen.

Thus we agree with the CLH conclusion that this finding does not warrant classification.

Ovarian tubular adenomas ([redacted] 1986b)

The following table gives an overview of ovarian tumor incidences in female mice at final sacrifice and of historical control data for uterine and ovarian tumor incidences in a 104-week mouse oncogenicity study in NMRI mice ([redacted], 1986b) (experimental phase: July 1983 – July 1985) were requested.:

Organ/tumor type	Dose (ppm) - females				HCD (%)[a]
Incidences in %	0	30	150	750	
Ovaries					
Cystadenoma		2.1			
Theca/granulosa cell tumor	8.2	8.5	22.9	14.3	0 – 27.8[b]
Sertoli cell tumor	14.3		2.1	6.1	0 – 4.2
Tubular adenoma	2.0	12.8	14.6	8.2	0 – 4.4
Luteoma	2.0		4.2	4.1	0 – 6.8
Hemangioma	2.0				

HCD: historical control data

[a] Bomhard (1993): Frequency of spontaneous tumours in NMRI mice in 21-month studies. Exp. Toxic. Pathol. 1993; 45: 269-289

[b] malignant and benign tumors together

It is obvious that none of the tumor incidences in the ovaries were dose-related. They were compared with published HCD for NMRI mice from 18 oncogenicity NMRI mice studies from July 1981 to August 1988 which covers the time of the [redacted], 1986b study (Bomhard, 1993). In the ovaries, the incidence of Theca/granulosa cell tumors was highest at 150 ppm, but not at the highest dose so that no dose response is seen, a statistical test for a positive trend was negative; furthermore they are within published HCD for NMRI mice (Bomhard, 1993). The incidence of ovarian Sertoli cell tumors was higher in control animals than in the treated groups, so that there is no dose- or treatment relationship. The ovarian luteoma incidences are not clearly dose-dependent, and furthermore within the published HCD, so that they are also not treatment-related. The other tumors occurred isolated without any dose-relationship and thus are due to variation. Appropriate statistical evaluations were performed as stated in the report. They were conducted for neoplastic lesions, for which the incidences appeared increased in the treated groups when compared with the controls. The statistical evaluations testing for a positive trend with respect to dose rate were performed according to PETO et al., 1980. Thus, appropriate statistics were applied on the tumor data from the animals scheduled for terminal kill (KO), however, for the discussed ovarian and uterine tumors discussed before, also for the Theca/granulosa cell tumors, no statistical significance is noted.

Also the CLH report states that ovarian tubular adenomas are common spontaneous tumor in aged mice, that there was no clear dose-response in the incidences of ovarian tubular adenoma, that the increases in incidences in desmedipham treated mice were not statistically significant, that no preneoplastic findings in ovaries were reported and that no indications for oncogenicity in ovaries were observed in other mice study or in rat studies with desmedipham. Furthermore, ovarian tubular adenomas do not occur in humans (Alison and Morgan 1987).

Therefore we agree with the CLH conclusion that this finding does not warrant classification.

Lung adenoma (Husband et al, 1994)

The RMS concluded that in the mouse oncogenicity study from [redacted], 1994 the incidence of benign lung tumors in females was regarded as slightly increased at the highest dose. An overview of the lung findings is given in the following table:

Organ	Doses (ppm)							
	Males				Females			
	0	400	1000	2500	0	400	1000	2500

Lung								
No. animals examined	49	49	49	50	50	49	49	49
Pulmonary adenoma	13	10	11	10	3	5	6	9
Pulmonary adenoma (%)					6	10	12	18
Pulmonary adenocarcinoma	4	3	8	4	1	3	5	1

This overview shows that the incidences of lung tumors in females are only slightly increased at 2500 ppm and that the lung adenocarcinoma incidences are not affected by the treatment. In males no effect was seen. Therefore, a lung tumorigenic potential is unlikely since in such case, also the incidences in males would be assumed to be affected and furthermore, also the incidence of lung adenocarcinomas would be expected to be increased at the highest dose. Since the MTD was exceeded at 1000 and 2500 ppm (see liver tumor discussion), it is anyway questionable whether these doses can be used for the evaluation of a tumorigenic potential.
A comparison with published HCD from [redacted] (Spontaneous neoplastic lesions in the Crl:CD-1 BR mouse, March 1995) supports this view. An overview is given in the following table:

HCD Lung adenoma (%)	Study duration	Reference
Females		
0 – 15.38	18 months	Charles River, 1995
4.00 – 18.37	24 months	Charles River, 1995

It can be seen that the HCD of the 18-month studies almost cover the lung adenoma incidences and the HCD for the 24-month studies cover them. Therefore, since the slight increase of lung adenoma incidences in females only, but not in males occurred only at doses which clearly exceeded the MTD and since the incidences are covered by published HCD for this strain from studies around the time of the study conduct, the slight increases are not regarded as evidence of a lung tumorigenic potential so that this is of no human relevance.

We agree with the statement in the CLH report that "Crl:CD-1 BR mice have variable spontaneous incidence of lung adenoma. There were no differences in tumour incidences in males or in adenocarcinoma incidences in females, and in this study the maximum tolerated dose was exceeded in high dose females. There were no indications of oncogenicity in lungs in other studies with desmedipham." and with the CLH conclusion that this finding does not warrant classification.

Overall, therefore we agree with the CLH conclusion that a carcinogenicity classification is not warranted.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment DMP_ECHA commenting_task force_sanitized.pdf

Dossier Submitter's Response

Thank you for your comments.
We agree that the increases in incidences of pituitary adenomas of the pars distalis in

Wistar KFM Han male rats are marginal. With regard to your statement that "in the other long-term rat studies no effect on pituitary tumor incidences were seen", we note that other long-term rat studies with desmedipham (as well as with phenmedipham) were performed using Sprague-Dawley rats which have very high spontaneous incidence of pituitary adenomas (generally >50%). This may mask the carcinogenic effect and thus it is not valid to use this statement to overrule the pituitary adenoma findings in Wistar KFM Han rats.
RAC's response
Thank you for the analysis. RAC agrees that classification is not warranted.

Date	Country	Organisation	Type of Organisation	Comment number
13.02.2019	Netherlands		MemberState	7
Comment received				
Multiple tumours have been observed in rat and mouse. These are all benign types. The increase in pituitary adenoma of the pars distalis in male Wistar rats (24-month study) was not statistically significant, not dose-related and the incidences were within the range of the historical control data (32.8%, 28.6%, 47.1% and 40% at control, low, mid and high dose group, respectively; HCD: 19.6-54.2%). Hence, this observation is considered not relevant for classification. It is therefore not clear why on page 28 of the CLH-report (section 10.9.2, final alinea) it is stated that "However, based on pituitary and lung adenomas classification of desmedipham for category 2 carcinogenicity could be argued". The increase in ovarian tubular adenoma in female NMRI mice (24-month study) was not statistically significant and not dose-related (2%, 13%, 15%, 8% at control, low, mid and high dose group, respectively). Historical control data (0-4.4%) showed that the incidences were outside the HCD-range. It is noted that the HCD-data have limitations, as these are not derived from the performing laboratory but from another laboratory. No increase in carcinoma was noticed. The increase in lung adenoma in female Crl:CD-1 mice (80-week study) was statistically significant and dose-related and slightly outside de HCD-range for the high dose group (6%, 10%, 12% and 18% at control, low, mid and high dose; HCD-range 0-15.38%). No increase in lung adenocarcinoma was noticed. The increase in incidences of hepatocellular adenomas and total liver tumours in (male/female) Crl:CD-1 mice (80-week study) was statistically significant. However, it is agreed that this is more likely to be related to the excessive liver toxicity. No increase in malignant type liver tumours was noticed. NL agrees with the Dossier Submitter that this is a borderline case. Given the limitations concerning the ovarian tubular adenoma and lung adenoma, a 'no classification' might be considered based on the data of desmedipham.				
Dossier Submitter's Response				
Thank you for your comments and support. We agree that the increases in incidences of pituitary adenoma of the pars distalis in desmedipham treated male Wistar rats are marginal. As elaborated in the clh report (page 24.) we however consider this finding possibly treatment-related and thus relevant for classification due to following reasons. The study with the structurally and toxicologically related substance, phenmedipham, with the same rat strain (Wistar Han) revealed dose-dependent statistically significant (at high dose) increase in the incidence of adenomas of pituitary pars distalis in males only. The increase was most pronounced in prematurely decedent males and the adenomas were reported to cause mortality. There are some indications that the pituitary tumors appeared earlier also in desmedipham treated Wistar Han males compared to controls. For example during the last year of the study enlarged pituitary was more frequently observed in desmedipham treated males compared to controls (incidences 5%, 15%, 12% and 20%, at 0, 3.2, 15.7				

and 80 mg/kg bw/day, respectively) being more common in the decedent animals. With regard to lung adenomas, the study report or RAR does not state that the slightly increased incidences would be statistically significant compared to concurrent controls. Please see also response to comment number 5.
RAC's response
RAC agrees that no classification is appropriate.

Date	Country	Organisation	Type of Organisation	Comment number
12.02.2019	Denmark		MemberState	8
Comment received				
Regarding pituitary adenomas the results from the closely related substance phenmedipham could also be taken into consideration. Phenmedipham demonstrated increased incidence of pituitary adenomas in male wistar rats. For Desmedipham the effect was (like phenmedipham) only observed in male rats. However, we acknowledge that the pituitary adenomas from the desmedipham study are within HCD and not a clear dosis-response relationship was established. Regarding the benign pulmonary adenomas in females, we consider them treatment related and should be considered for classification. The ovaian tubular adenomas in female mice (104 week study) we also consider treatment related, although, we acknowledge that the high dose does not fit in to the dose-response relationship. Overall, desmedipham should be considered carc 2.				
Dossier Submitter's Response				
Thank you for your comments.				
RAC's response				
The increase in pulmonary adenomas (B.6.5/04) was not statistically significant on pairwise comparison and was not accompanied by non-neoplastic findings. There was no increase in pulmonary adenomas in males. Therefore, this finding is not considered to warrant classification. As to the ovarian tubular adenomas (B.6.5/05), there was no significant increase at the top dose (750 ppm) and no dose-response relationship. In addition, there was no increase in ovarian tumours at 2 500 ppm in the other mouse carcinogenicity study. Therefore, RAC considers the ovarian adenomas unlikely to be treatment-related. Regarding the pituitary adenomas (B.6.5/03), RAC does not see any clear increase above concurrent or historical controls. Overall, RAC agrees with the DS's proposal of no classification.				

Date	Country	Organisation	Type of Organisation	Comment number
15.02.2019	France		MemberState	9
Comment received				
FR, page 27-28: We agree with the borderline evidence regarding the carcinogenicity potential of desmedipham, highlighted by the MSCA. However, we support a classification Carc. 2 H351				

rather than no classification. This proposal is based on the increased ovarian tubular adenoma observed in all dosed females in the 2-year mouse study (above concurrent controls and literature historical control values) and on the slight dose-dependent increased incidence of pulmonary adenomas in females observed in the 18-month mouse study. In addition, the MCSA noted that there are some indications in the desmedipham database that this active substance may induce hormonal imbalance affecting ovaries (increased ovarian weights, increased incidences of ovarian cysts). It should be also noted that a classification Carc. Cat.2 H351 has been proposed for phenmedipham, an active substance with similar chemical structure, chemical properties, breakdown products and toxicological profiles.
Dossier Submitter's Response
Thank you for your comments. We note that ovarian tubular adenomas are common spontaneous tumour in aged mice, there weas no clear dose-response in the incidences of ovarian tubular adenomas and the increases in incidences in desmedipham treated mice were not statistically significant. No preneoplastic findings in ovaries were reported and no indications for oncogenicity in ovaries were observed in other mice study or in rat studies with desmedipham. Moreover, ovarian tubular adenomas do not occur in humans, hence, this finding has low relevance for humans. Overall, we consider the reported data on lung adenomas and pituitary adenomas of the pars distalis more relevant findings for carcinogenicity classification of desmedipham than ovarian tubular adenomas.
RAC's response
Please see the response to comment no. 8.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
11.02.2019	Germany		MemberState	10
Comment received				
Classification with Repr. 2 is supported. 10.10.6 The statement at the end of the fourth paragraph that "no classification is considered appropriate" for the skeletal effects is somewhat confusing. Perhaps it would be better to state that no classification based on skeletal effects is considered appropriate.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
RAC agrees that a Repr. 2 classification for adverse effects on development is warranted.				

Date	Country	Organisation	Type of Organisation	Comment number
13.02.2019	Netherlands		MemberState	11
Comment received				
NL agrees with the 'no classification' for effects on sexual function and fertility. With respect to the effects on sperm count as noted in the key 2-generation study, the Dossier Submitters considerations are supported. We also agree with the rationale of the Dossier Submitter related to the adverse effects on parameters of onset of puberty in the F-1 generation of the key 2-generation study.				

With respect to the proposed classification for effects on development, the uncertainties are noticed. Overall, we support the cat. 2 classification for adverse effects on development.
Dossier Submitter's Response
Thank you for your support.
RAC's response
RAC agrees that no classification for fertility, and Category 2 for development is appropriate.

Date	Country	Organisation	Type of Organisation	Comment number
12.02.2019	Denmark		MemberState	12
Comment received				
Development: We agree that desmedipham should be classified as Repr. 2: H361d based on the treatment related effects observed in the rat developmental studies: palatoschisis / micrognathia and agnathia / open eyes incidences in the study 1985a (RAR B.6.6.2) and the occurrence of cleft palate and visceral effects in study 1991 (RAR B.6.6.2/05). In addition, we think that fertility should also be considered. Fertility: We do not agree with non-classification for fertility. Effects on sperm parameters in both the parent and the F1 generation was observed in the 2-generation study from 2003. The decreased total cauda epididymal sperm number in both generations should be considered an adverse effect on male reproduction. Absence of effect on fertility index or other sperm parameters or histopathology cannot override effects on sperm counts. This is stated in OECD 43 guidance on assessment of reprotoxicity: "Again, information on the other sperm parameters and histopatholgy should be considered in the overall interpretation. Testicular lesions of sufficient magnitude will be reflected in the sperm counts, but changes in sperm counts should not be discounted in the absence of histological lesions." Furthermore, according to the same guidance (OECD 43), the fertility index cannot be used as a measure of unaffected sperm: "A reduction in sperm count may not result in reduced fertility, particularly in rodent studies. This is due to the fact that rats and mice have a tremendous excess of spermatozoa in their ejaculates, and as such sperm counts have to be reduced by as much as 90% to affect fertility." Effects observed on sperm parameters suggests desmedipham adversely affects the male reproductive system. Therefore, we would propose to classify desmedipham on this basis as Repr. 2: H361f.				
Dossier Submitter's Response				
Thank you for your support regarding developmental toxicity classification. With regards to classification for fertility based on epididymal sperm count, we maintain our view on non classification but agree that this might be a borderline case. It should be discussed whether the observed changes in the sperm number in the caudal epididymis at the two highest doses in F1 generation are treatment related, and if yes, further discuss the relevance of the magnitude of the changes to humans taking into account the large standard deviations including the control. In the P generation there was a decrease in the caudal sperm number only at the highest dose but again with a large standard deviation including control. Based on the results in the homogenization-resistant testis spermatid numbers it seems that there were no treatment-related changes in the sperm production. As stated in the CLH report no changes were observed in the reproductive organ weights or histopathology.				

There are xenobiotics which have shown to affect epididymal sperm count without an effect on the testis sperm production. The change in epididymal sperm count could be e.g. due to altered sperm transit time through the epididymis. Alterations in the transit time may affect the sperm maturation process which is modulated by androgens and contractile activity of the epidymal smooth muscle layer. Some chemicals such as diethylstilbestrol, a synthetic estrogen, or hydroxyflutamide, a synthetic antiandrogen, have been shown to reduce the sperm transit time in the epididymis and thus accelerating the transit. This results in reduced caudal sperm number, and due to impaired maturation process also impaired sperm quality and impaired fertility. These effects can at least partially be restored by testosterone supplementation. Other chemicals such as guanethidine slows down the transit by abolishing peripheral sympathetic nerves innervating the smooth muscle thus increasing the sperm reserve in the epididymis. A non-selective serotonin-norepinephrine reuptake inhibitor sibutramine accelerates the transit due to its sympathomimetic effects. (E.g. Kempinas and Klinefelter, 2014, 2018; Dal Bianco Fernandez et al. 2007; Goyal et al. 2001; Borges et al. 2013). If DMP was agreed to have an effect on the epididymal sperm reserve, the mode of action is not known. DMP is not known to have estrogenic or antiandrogenic effects. Testosterone levels were not determined in the studies however at least there was no effect on the mating index suggesting that there was no effect of DMP on sexual behaviour or on reproductive organ development in the F1 generation offspring.

Goyal et al. 2001. Diethylstilbestrol-treated adult rats with altered epididymal sperm numbers and sperm motility parameters, but without alterations in sperm production and sperm motility. Biology of Reproduction, 64: 927-934

Dal Bianco Fernandez et al. 2007. Effects of altered epididymal sperm transit time on sperm quality. International Journal of Andrology, 31: 427-437

Borges et al. 2013. Slimmer or Fertile? Pharmacological mechanisms involved in reduced sperm quality and fertility in rats exposed to the anorexigen sibutramine. Plos One, 8: e66091

Kempinas and Klinefelter 2014. Intepreting histopathology in the epididymis. Spermatogenesis, 4:2, e979114

Kempinas and Klinefelter, 2018. The Epididymis as a target for Toxicants. Comprehensive Toxicology, third edition. Vol 4, pp. 112-127

RAC's response

RAC agrees that Category 2 is warranted for development.

As to fertility, RAC concludes that the observed reduction in epidymal sperm count by 10 % in the absence of biologically significant changes in other sperm parameters (motility, morphology, testicular sperm count) or in male reproductive organs (histopathology, weight) is not sufficient for classification.

Date	Country	Organisation	Type of Organisation	Comment number
15.02.2019	France		MemberState	13
Comment received				
FR, page 45: The proposed classification as Reprotoxic category 2 H361d is supported. However, based on the dose-response decrease of sperm count observed in P and F1-generation in the 2G rat study (above 10 % in the highest dose), we are of the opinion that a classification for fertility (H361f) would be also justified.				
Dossier Submitter's Response				
Thank you for your support regarding developmental toxicity classification. Regarding fertility and the caudal sperm count we disagree that there is a dose-response in the P generation sperm counts. For other points, please see our response to comment number 12.				
RAC's response				
Please see the response to comment no. 12.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
11.02.2019	Germany		MemberState	14
Comment received				
Classification with STOT-RE 2 is supported. In addition to the blood, consideration may also be given to the lungs, ovaries and liver as target organs due to the increased inflammatory reaction seen in the lungs of rats (RAR B.6.5/03) and mice (RAR B.6.5/05), the increased incidences of ovarian cysts in rats (RAR B.6.5/03) and mice (RAR B.6.5/05) and the clear liver toxicity evident in the long-term studies.				
Dossier Submitter's Response				
Thank you for your support. We note that relevant dose levels for STOT RE cat 2 classification are ≤ 60 ppm (Cat 2: 1.25 < C ≤ 12.5 mg/kg bw/day, i.e 24-month oral carcinogenicity study). In the 24-month oral carcinogenicity study in rats (RAR B.6.5/03, 1986a) perivascular cuffing (lymphoid cell infiltration) and macrophage accumulation in lungs was increased in all treated males and females in 1500 ppm (see Tables below). Pulmonal inflammation in lungs was slightly increased in both sexes at 1500 ppm (80 - 100 mg/kg/bw/day). In the re-evaluation of the histopathological findings no clear differences in the perivascular cuffing or pulmonal inflammation were seen (dRAR B.6.5/03, 2000). Macrophage accumulation in lungs was increased at 1500 ppm in males and females. According to the study report (RAR 6.5/03, 1986a) this effect may indicate a mild phospholipidosis, a widely reported condition in laboratory rodents, which has been associated with cationic amphiphilic compounds. In ovaries, cysts were increased in females at 1500 ppm (incidences 11/70, 11/70, 14/70 and 21/70 at 0, 60, 300 and 1500 ppm, respectively). Liver weights were increased at all doses in females but not in males. Increased hematopoiesis in the liver was noted in females at 1500 ppm and increased hemosiderosis of the liver was observed in males at 1500 ppm.				

Table 151. Histopathological findings in lungs (dRAR B.6.5/03, 1986a)

Endpoint		Dose (ppm)							
		Males				Females			
		0	60	300	1500	0	60	300	1500
Lungs									
Macrophage accumulation	interim	0/10	0/10	1/10	3/10	1/10	1/10	0/10	1/10
	terminal	11/60	12/60	17/60	24/60	13/60	11/60	12/60	28/60
	total (%)	(16%)	(17%)	(26%)	(38%)	(20%)	(17%)	(17%)	(41%)
Perivascular cuffing	interim	1/10	0/10	0/10	3/10	0/10	1/10	1/10	0/10
	terminal	9/60	14/60	16/60	14/60	9/60	7/60	11/60	18/60
	total (%)	(14%)	(20%)	(23%)	(24%)	(13%)	(11%)	(17%)	(26%)
Pulmonal inflammation[1)]	interim	1/10	1/10	1/10	1/10	0/10	0/10	0/10	0/10
	terminal	8/60	9/60	5/60	19/60	11/60	7/60	9/60	15/60
	total (%)	(13%)	(14%)	(8%)	(28%)	(16%)	(10%)	(13%)	(21%)

1) Bronchitis/pneumonitis/bronchopneumonia/interst.pneumonia/pleuritis

Table: Updated histopathological findings in lungs (dRAR B.6.5/03, 2000)

Endpoint	Dose (ppm)							
	Males				Females			
	0	**60**	**300**	**1500**	**0**	**60**	**300**	**1500**
Lungs								
Focus(i) of alveolar macrophages	12	9	7	18	5	3	3	12
Interim	0/10	0/10	1/10	3/10	2/10	1/10	0/10	1/10
Terminal	17/60	12/60	10/60	30/60	16/60	13/60	14/60	29/60
Total	17/70	12/70	11/70	33/70	18/70	14/70	14/70	30/70
Perivascular cuffing	0/70	0/70	0/70	0/70	0/70	0/70	0/70	0/70
Pulmonal inflammation (incl. acute bronchopneumonia, pneumonitis)								
Interim	1/10	1/10	0/10	1/10	0/10	1/10	0/10	0/10
Terminal	0/60	1/60	1/60	0/60	11/60	5/60	7/60	11/60

No inflammatory reaction in the lungs was reported in the 24-month oral carcinogenicity study in mice (RAR B.6.5/05, 1986b). Incidences of ovarian cysts were 7(14%), 7(15%), 9(19%) and 13(27%) at 0, 30, 150, 750 ppm, respectively. No clear effect on liver was seen at the dose level (30 ppm) relevant for STOT RE Cat 2 classification.

In the 80-week mice carcinogenicity study (RAR 6.5/04, 1994) slightly increased incidences of accumulation of fluid in the thoracic cavity and an increase in alveolar macrophages at 1000 ppm (153 mg/kg bw/day) and at 4000 ppm (402 mg/kg bw/day) males were observed. There were no differences in histological findings of lungs in females in this study. Overall, no clear effects on lung, ovaries or liver were seen in rats or mice at the dose level relevant for STOT RE Cat 2 classification.

RAC's response

RAC agrees with the DS that no effects were observed in the lungs, ovaries, or the liver at doses below the guidance values for classification.

As to the haemolytic anaemia, RAC notes that none of the individual criteria according to the CLP guidance is met.
Regarding the criterion of CLP Annex I 3.9.1.4 ("generalised changes of a less severe nature involving several organs"), the CLP guidance exemplifies it by the following criteria:

- Marked increase of haemosiderosis in the spleen, liver or kidney in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at

≥ 10 %) in a 28-day study
- Significant increase in haemosiderosis in the spleen, liver or kidney in combination with microscopic effects like necrosis, fibrosis or cirrhosis

Neither of these two criteria is met for desmedipham.

Overall, RAC concluded on no classification for STOT RE.

Date	Country	Organisation	Type of Organisation	Comment number
13.02.2019	Netherlands		MemberState	15

Comment received

NL agrees with the Dossier submitter that this might be a borderline case.
The haematopoietic system, thyroid and nervous system are the target tissues.
It is agreed that the observed effect on AChE (when focusing primarily on the rat repeated dose studies RAR B.6.3.2/02 and RAR B.6.3.2/04) provides insufficient evidence for classification.
Thyroid effects have been noticed in the rat and dog repeated dose studies, though these are considered insufficient with respect to its severity.
The effects on the haematological system are also observed with the structurally related chemical phenmedipham. With respect to the effects on the haematological system, none of the individual studies fulfills the criteria for a STOT RE classification. Effects at dose levels relevant for classification were only slight to moderate, as for example the reductions in Hb levels were never ≥20%.
The Dossier submitter proposes to apply the criterion as described in CLP Annex I 3.9.1.4 ("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"). However, one can question the validity of applying this criterion as the various adverse effects concern specific effects (i.e. effects on the haematological system) and not generalized changes involving several organs.
In conclusion, although the data point towards the haematological system as the primary target, we consider the severity of the effects to be insufficient for classification for STOT RE, and the Dossier Submitters proposal for a cat 2 is not supported.

Dossier Submitter's Response

Thank you for your comment. We agree that the severity of the haemotoxic effects is not high and represents a borderline case for classification.

RAC's response

RAC agrees with no classification for STOT RE.

Regarding the criterion of CLP Annex I 3.9.1.4 ("generalised changes of a less severe nature involving several organs"), the CLP guidance exemplifies it by the following criteria:
- Marked increase of haemosiderosis in the spleen, liver or kidney in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥ 10 %) in a 28-day study
- Significant increase in haemosiderosis in the spleen, liver or kidney in combination with microscopic effects like necrosis, fibrosis or cirrhosis

Neither of these two criteria is met for desmedipham.

Date	Country	Organisation	Type of Organisation	Comment number
12.02.2019	Denmark		MemberState	16
Comment received				
We agree that desmedipham affects blood parameters in such an extent that classification STOT RE 2 (blood) is warranted.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Please see the response to comment no. 14.				

Date	Country	Organisation	Type of Organisation	Comment number
15.02.2019	France		MemberState	17
Comment received				
FR, page 74: The proposed classification as STOT RE H373 (blood) is supported.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Please see the response to comment no. 14.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
11.02.2019	Germany		MemberState	18
Comment received				
page 3, point 2.1 Proposed harmonised classification and labelling (Table 6): We support the proposal of classification for environmental hazards as Aquatic acute 1 (H400), Aquatic chronic 1 (H410) and acute/chronic M-factor of 10.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you.				

Date	Country	Organisation	Type of Organisation	Comment number
15.02.2019	United Kingdom		MemberState	19
Comment received				
Desmedipham (EC: 237-198-5; CAS: 13684-56-5) Algal growth inhibition study: The presented algal ErC50 is estimated from the ratio of initial measured 0.178 mg/l treatment to the ErC50 initial measured concentrations and applying this ratio to the geometric mean measured concentration for the 0.178 mg/l (im) treatment.				

We would prefer to see a statistically derived endpoints based on dose-response curves using mean measured treatments. We note that the CLH report states that 'plotting the percentage inhibition against geomean concentrations gives appr. Same ErC50 value with observation' and anticipate that a statistical ErC50 could still lie in the 0.1 to 0.1 mg/l classification range. This is supported by a using the same method to estimate the ErC50 using the 0.619 mg/l (im) treatment ratio from the ErC50 (im) which results in an estimated ErC50 (gmm) of 0.04 mg/l.

Given the pH was lowered for the study and increased more than the test guideline recommendation of 1.5 units over the study period, we wonder whether it would be useful to compare control data to validity criteria in the current OECD TG 201 to confirm the adaption did not impact study controls.

Additional algal growth inhibition studies:
We note additional algal data are included in the RAR (2017) but not presented in the CLH as 'intermediate' analytical measurements were not included. While this may be a requirement for review under PPP, it is not required for hazard assessment. This means valid, GLP studies (e.g. XXXX (2005) and XXXX (2011)) should be presented as part of the CLH process. We note each of these studies include valid NOErCs based on geometric mean measured data. While these would not impact the chronic classification proposal which is based on M. spicatum, they are relevant as the current algal study could not generate a true NOErC (i.e. effects were observed at all concentrations and a ≤ value was presented).

Myriophyllum spicatum study:
Given the rapid loss of test item over the semi-static renewal periods, we note that it would be more appropriate to determine the geometric mean for each renewal period, and calculate the mean exposure over the whole exposure period calculated from these data. We anticipate this would result in an updated study NOEC in the same classification range.

Chironomus riparius studies:
Significant and rapid loss of the test item was observed in both studies meaning that 28-day NOECs based on initial measured concentrations may not be appropriate. Full details of analytical support (including if sediment samples were analysed) are not available in the CLH report. Please can you consider if a time-weighted average endpoint would be more appropriate?

Dossier Submitter's Response

Thank you for your comment. Please find below our response to the items you have raised.

<u>Algal growth inhibition study:</u>
We agree that it would be useful to run statistical analysis with geometric mean measured concentrations to confirm the estimated E_rC_{50} value (i.e. to confirm if a statistical E_rC_{50} could lie in the 0.01 to 0.1 mg/l classification range). Unfortunately, we do not have an appropriate statistical program to run such analysis. In case you have already conducted such an analysis on this data we would appreciate your contribution. Regarding the validity of the study the control data was compared to the criteria set in OECD guideline 201 (2011), and the study fulfilled them. This has been stated in the CLH dossier.

<u>Additional algal growth inhibition studies:</u>
There were four other algae study presented in the RAR which were not included in the CLH dossier. Two of them were rejected not only because desmedipham was lost from the test media and no intermediate analyses were presented, but because of other identified deficienses e.g. the validity criteria set in the test guideline was not fulfilled, solvent

concentrations were higher than recommended, details on the test substance purity or certificate of analysis were not presented.

Two other studies (XXXX (2005) and XXXX (2011)) fulfilled the validity criteria set in OECD 201 test guideline (for the control growth and the CV for section-by-section specific growth rate and average specific growth rates during the whole test period in control) and were conducted in compliance with the GLP. These studies were, however, considered not valid in the RAR due to severe loss of the test item during the test period. We agree that these studies should have been presented in the CLH dossier even though they do not change the proposed classification of desmedipham. We acknowledge that regarding unstable substances the CLP guidance says that where measured data are available for the start and end of test, the L(E)C50, for classification purposes, may be calculated based on the geometric mean concentration of the start and end of test and where concentrations at the end of test are below the analytical detection limit, such concentrations shall be considered to be half that detection limit.

The static test performed in **2005 with *Desmodesmus subspicatus*** provided a NOEC value of 1.34 mg/L .Test concentrations were <LOD at the end of the test and results were based on initial measured concentration. During the PPP renewal assessment process E_rC_{10} value of 0.0128 mg/L based on geometric mean measured concentrations was provided and presented in the RAR. The temperature difference was within ± 2 °C in the test and the pH value in the control replicates increased not higher than 1.5 units.

The other study performed in **2011 with *Pseudokirchneriella subcapitata*** had also difficulties to maintain the test substance concentrations. Measurements were carried out in test media samples containing algae, in test media without algae and in test media after centrifugation. This procedure was carried out to verify whether the adsorption of desmedipham could have an impact on the outcome of the study and to investigate possible problems due to the solubility of the test item. In the test report an E_rC_{10} value of 0.0649 mg a.s./L based on time weighted mean test concentrations of desmedipham from centrifuged test media was provided for desmedipham.

During the PPP renewal assessment process endpoints were also recalculated to be based on geometric mean measured concentrations. LOQ/2 were used when measurements were < LOQ resulting: 0.0021, 0.0026, 0.0033, 0.0060, 0.012, 0.025 and 0.007 mg a.s/L. The recalculations of the geometric measured concentration of desmedipham were initially performed on the media with algae. However, the highest concentration was found to be lower than the 2 next lower concentrations. This fact was not consistent with the concentrations measured in media without algae or in centrifuged media. It was also not consistent with the level of effects observed at the highest exposure concentrations showing the highest inhibition. Concequently, EC_{50}, EC_{20} and EC_{10} were recalculated on the basis of the centrifuged media concentrations as they were very similar to those in the media with algae (disregarding the highest concentration) resulting >0.32 mg/L, 0.0164 mg/L and 0.0080 mg/L, respectively (for growth rate).

<u>*Myriophyllum spicatum* study:</u>
The test results of this study used for classification purpose are based on geometric mean measured concentration and samples from each renewal period have been already taken into account in the calculations.
It is noted that OECD Guidance document on aqueous-phase aquatic toxicity testing of difficult test chemicals Nr 23 (second edition) says that in situation when measured concentrations do not remain within 80-120 % of nominal effects concentrations may be determined and expressed relative to the time-weighted mean measured concentrations for

static-renewal systems. However, we have not done that but this could be considered.

Chironomus riparius studies:

In the ***Chironomus riparius*** **study performed in 2002** samples were taken from the water column only on day 0, 7 and 21 and were analysed for the concentrations of desmedipham and its hydrolysis metabolite, EHPC. On day 0 samples were taken only from the lowest, the middle and the highest concentrations, and from all concentrations on days 7 and 21 (nominal concentrations were 0.125, 0.250, 0.500, 1.0 and 2.0 mg/L). Test item analysis and validation of the method have been described in the test report. The results from the sample analysis for desmedipham are provided in the following table.

DMP [mg/L]							
	contr.	contr.+					
nomin. cont.	0.000	0.000	0.125	0.250	0.500	1.000	2.000
nomin. a.i.	0.000	0.000	0.123	0.246	0.491	0.982	1.964
day 0	0.000	0.000	0.000	*	0.210	*	0.254
day 7	0.000	0.000	0.000	0.000	0.000	0.000	0.000
day 21	0.000	0.000	0.000	0.000	0.000	0.000	0.000

* no sample available

Sum of DMP and EHPC

[DMP + EHPC (converted into equivalents of DMP)] [mg/L]							
	contr.	contr.+					
nomin. cont.	0.000	0.000	0.125	0.250	0.500	1.000	2.000
nomin. a.i.	0.000	0.000	0.123	0.246	0.491	0.982	1.964
day 0	0.000	0.000	0.138	*	0.546	*	0.688
day 7	0.000	0.000	0.092	0.181	0.393	0.781	1.552
day 21	0.000	0.000	0.030	0.000	0.000	0.220	0.655
mean a.i.	0.000	0.000	0.087	0.091	0.313	0.501	0.965
variability			4.60	**	**	3.54	2.37

* no sample available

** no value for the variability, because one result is 0

Desmedipham was detected only from the middle and highest test concentrations (nominal 0.500 mg/L and 2.00 mg/L, respectively) on day 0. When the concentrations of parent and metabolite were combined it was seen that initial mean measured concentrations at 0.125 and 0.5 mg/L were within 80-120 % of the nominal. Therefore, in the test report the results were based on initial nominal desmedipham concentrations which were the sum of desmedipham and EHPC (converted into desmedipham by multiplying with 1.65725 [ratio of molar masses]). LOD for desmedipham was 10.52 µg/L and LOQ 17.53 µg/L, and for EHPC 8.01 µg/L and 13.34 µg/L, respectively. NOEC (emergence ratio) was established at the nominal initial concentrations of 1.0 mg/L.

During the PPP renewal assessment process it was requested that endpoint should be based on geometric mean measured initial concentrations of desmedipham. Following table is provided in the RAR:

Table B.9.2.5.3-3: Geomean measured initial concentrations of desmedipham

Nominal concentration mg a.s./L	Initially measured DMP concentration	
	mg a.s./L	% of nominal
Control	< LOQ	-
Solvent control	< LOQ	-
0.125	< LOQ	5%
0.250	n.d.	-
0.500	0.210	42%
1.000	n.d.	-
2.000	0.254	13%
Arithmetic mean recovery:		20%
Geometric mean recovery:		14%

LOQ: Limit of Quantification = 13.34 µg/L
n.d.: not determined

In the RAR it is stated that NOEC level was recalculated using geomean of initially measured concentration and the geometric mean recovery 14 %, i.e. 1.0 mg a.s./L * 14 % = 0.14 mg a.s./L. According to test report this 1.0 mg a.s./L seems to be a nominal value for day 0 as there was no measurements taken on that day. As no analytical results are available for day 0 we do not think that reliable NOEC could be recalculated based on time-weighted average concentrations.

No sediment samples were taken during the study, and exposure via the sediment cannot be ruled out. However, the results from the other available Chironomie study indicate that this might happen in some level.

In the ***Chironomus riparius* study performed in 2005** the concentrations of desmedipham and EHPC were taken from the overlying water, pore water and from the sediment. The measured concentrations of desmedipham (0.44, 1.8 and 7.0 mg a.i./L) in the overlying water after one hour of dosing were 66.6, 78.3 and 47.7 % of nominal, respectively. After four hours of application they were dropped to 28.9, 8.6 and 21.4 % of nominal, respectively, and after 1 day they were less than 0.3 % of nominal indicating that desmedipham disappeared very quickly from the overlying water. Desmedipham was found in higher concentrations in the sediment than in the overlying water towards the end of the study (see table below). Low concentrations of desmedipham were found in pore water. Desmedipham concentration peaked in one sediment sample (hour 24) which was considered to be an outlier. Based on these results it was concluded in the test report that desmedipham degraded rapidly from the overlying water to EHPC which began to partitioning between overlying water and sediment within 1 hour of application.

No treatment-related effects were observed on mean emergence ratios, development times and development rates, therefore, the NOEC was 7.0 mg a.s./L based on nominal concentrations (sum of desmedipham and EHPC). It is noted that in the CLH report Table 55 it is mistakenly stated that the NOEC value of 7.0 mg a.s./L is based on initial measured concentrations of the sum of desmedipham and EHPC.

During the PPP renewal assessment process endpoints were requested to be based on geometric mean measured initial concentrations. Following table was presented in the RAR:

Table B.9.2.5.3-6: Geomean measured initial concentrations of desmedipham

Nominal concentration mg a.s./L	Initially measured DMP concentration	
	mg a.s./L	% of nominal
Control	< LOQ	-
Solvent	< LOQ	-
0.44	0.293	67%
0.88	n.d.	-
1.8	1.410	78%
3.5	n.d.	-
7.0	3.340	48%

LOQ: Limit of Quantification (0.133 µg a.s./L)
n.d.: not determined

Initial measured desmedipham concentration 3.340 mg a.s./L corresponds to the NOEC 7.0 mg a.s./L (nominal) and this value was used in the RAR. As no treatment-related effects were observed during the study we calculated TWA concentration in overlying water only for the highest concentration tested with the formula: (Conc 0-Conc1/(ln(conc0)-ln(conc1))*days. This resulted the NOEC of 0.0246 mg a.i./L based on TWA. Measured concentrations of desmedipham and EHPC in overlying water, sediment and pore water are provided in the following tables.

Table 1 Measured concentrations of desmedipham in overlying water, sediment and pore water samples

Nominal test concentrations mg a.i./L	**Sampling Interval (Day)**	**Measured desmedipham concentration (µg a.i./L) in overlying water (1)**	**Measured desmedipham concentration (µg a.i./L) in sediment (2)**	**Measured desmedipham concentration (µg a.i./L) in pore water (3)**
0.44	Hour 1	293	62.5	0.779
	Hour 4	127	106	0.573
	Hour 24	0.252	28.4	0.193
	Day 7	< LOQ	13.9	< LOQ
	Day 28	<LOQ	3.46	< LOQ
0.88		n.d.	n.d.	n.d.
1.8	Hour 1	1410	263	5.77
	Hour 4	155	264	2.98
	Hour 24	1.93	72.8	0.853
	Day 7	< LOQ	17.7	< LOQ
	Day 28	< LOQ	44.0	0.319
3.5		n.d.	n.d.	n.d.
7.0	Hour 1	3340	144	205
	Hour 4	1500	159	39.3
	Hour 24	16.6	3012	17.4
	Day 7	0.417	193	2.27
	Day 28	0.170	250	0.662

* n.d. not determined
(1) LOQ 0.133 µg a.i./L

(2) LOQ 4.00 µg a.i./L
(3) LOQ 0.133 µg a.i./L

Table 2 Measured concentrations of EHPC in overlying water, sediment and pore water samples

Nominal test concentrations mg a.i./L	Sampling Interval (Day)	Measured EHPC concentration (µg a.i./L) in overlying water (1)	Measured EHPC concentration (µg a.i./L) in sediment (2)	Measured EHPC concentration (µg a.i./L) in pore water (3)
0.44	Hour 1	123	12.4	86.1
	Hour 4	238	22.7	88.0
	Hour 24	283	110	51.1
	Day 7	237	317	140
	Day 28	101	269	279
0.88		n.d.	n.d.	n.d.
1.8	Hour 1	467	22.3	69.4
	Hour 4	1220	155	15.2
	Hour 24	1508	260	12.3
	Day 7	907	604	521
	Day 28	550	3103	514
3.5		n.d.	n.d.	n.d.
7.0	Hour 1	1590	8391	16.9
	Hour 4	3000	4610	18.9
	Hour 24	4703	1448	52.8
	Day 7	3670	2224	1400
	Day 28	2090	3857	1750

* n.d. not determined
(1) LOQ 0.133 µg a.i./L
(2) LOQ 4.00 µg a.i./L
(3) LOQ 0.133 µg a.i./L

RAC's response

Algae growth inhibition study

RAC considers the study valid since it fulfils validity criteria. RAC agrees that statistically derived endpoints are preferred and has calculated them based on geometric mean measured concentrations and growth rate (see next two tables).

Nominal, measured and geomean concentrations of desmedipham

Nominal	Conc (0h)	Conc (24h)	Conc (48h)	Conc (72h)	Geomean (48h)	Geomean (72h)
0.065	0.053	Lost	0.005	0.005	0.016	0.016
0.108	0.084	0.01	0.005	0.005	0.020	0.020
0.18	0.141	0.021	0.013	0.005	0.043	0.027
0.3	0.178	0.041	0.064	0.013	0.107	0.048
0.5	0.619	Lost	0.041	0.02	0.159	0.111

Where the concentration was not detected LOD/2 was considered as proposed in the CLH.

Geomean concentations, cells density, growth rates and inhibition %.

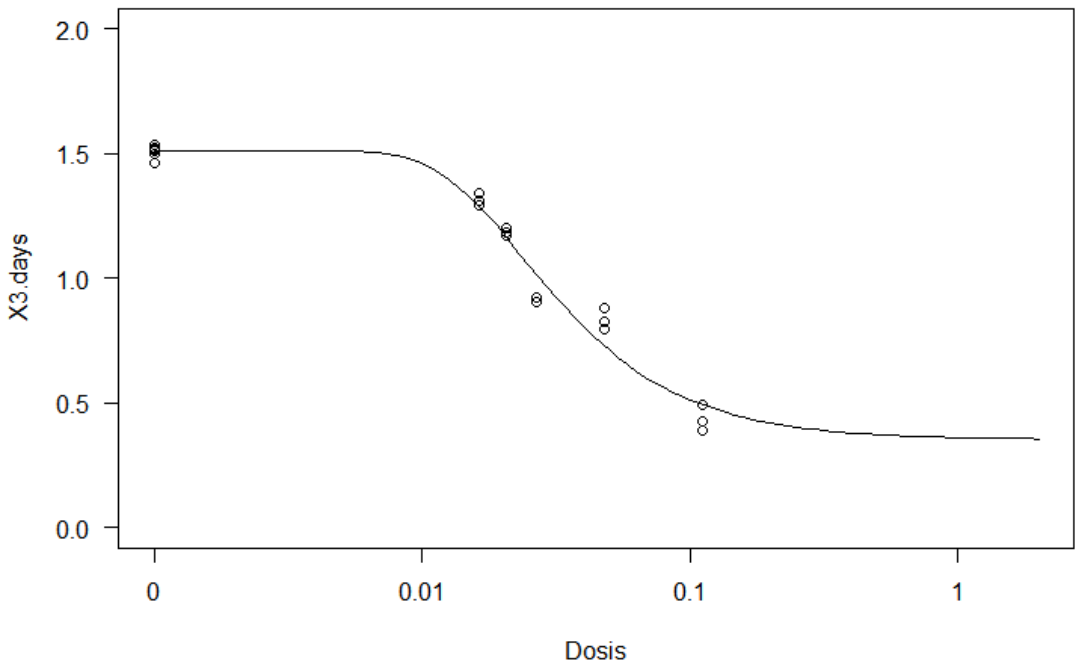
Conc 48h	Conc 72h	0h	24h	48h	72h	Growth 2 days	Inhib %	Growth 3 days	Inhib%
control	control	10 000	50 000	150 000	924 000	1.35		1.51	
control	control	10 000	40 000	140 000	906 000	1.32		1.50	
control	control	10 000	24 000	150 000	810 000	1.35		1.46	
control	control	10 000	30 000	150 000	994 000	1.35		1.53	
control	control	10 000	36 000	150 000	966 000	1.35		1.52	
control	control	10 000	24 000	140 000	952 000	1.32		1.52	

0.016	0.016	10 000	24 000	100 000	564 000	1.15	16.1	1.34	12.7
0.016	0.016	10 000	20 000	72 000	514 000	0.99		1.31	
0.016	0.016	10 000	26 000	120 000	482 000	1.24		1.29	
0.020	0.020	10 000	8 000	26 000	366 000	0.48	53.2	1.20	21.4
0.020	0.020	10 000	10 000	38 000	352 000	0.67		1.19	
0.020	0.020	10 000	10 000	44 000	334 000	0.74		1.17	
0.043	0.027	10 000	8 000	24 000	160 000	0.44	56.7	0.92	39.2
0.043	0.027	10 000	8 000	40 000	150 000	0.69		0.90	
0.043	0.027	10 000	10 000	34 000	160 000	0.61		0.92	
0.107	0.048	10 000	10 000	32 000	110 000	0.58	67.1	0.80	44.6
0.107	0.048	10 000	6 000	22 000	140 000	0.39		0.88	
0.107	0.048	10 000	10 000	20 000	120 000	0.35		0.83	
0.159	0.111	10 000	10 000	8 000	44 000	-0.11	117	0.49	71.1
0.159	0.111	10 000	8 000	8 000	36 000	-0.11		0.43	
0.159	0.111	10 000	10 000	4 000	32 000	-0.46		0.39	

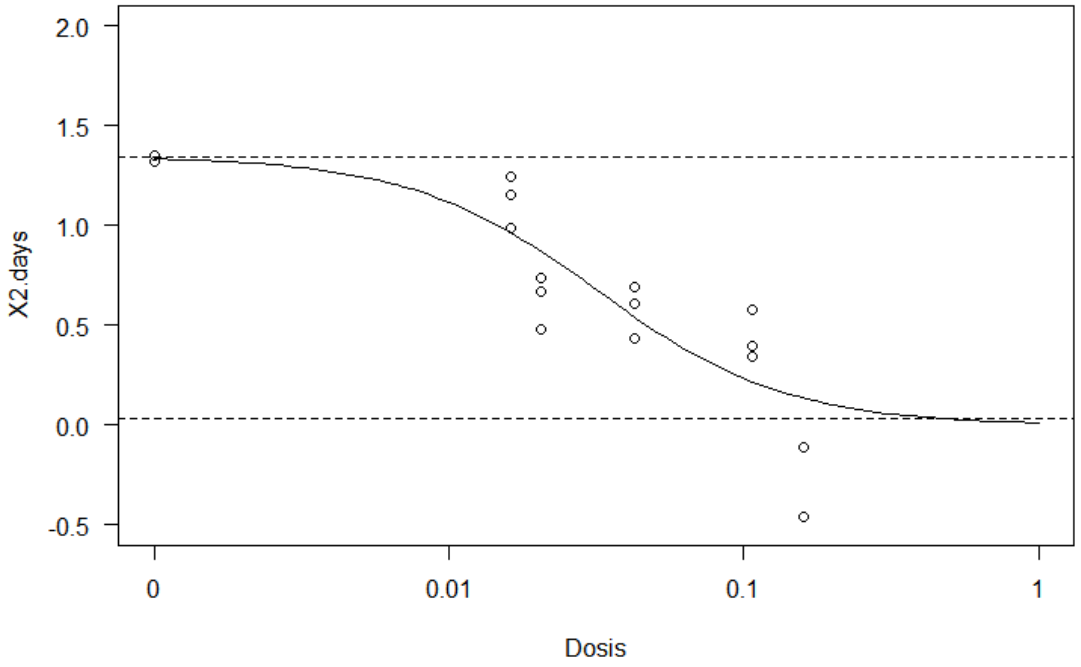
From the graphs below it can be seen that at 72h the model adjusts better than at 48h: E_rC_{50} (72h) = 0.045 mg/L ± 2 × 0.01353855; EC_{10} (72h) = 0.014 mg/L ± 2 × 0.00098038 mg/L.

However, looking at the above table we see that the EC_{50} (72h) value should be in between concentrations 0.048 mg/L (44.6 % inhibition) and 0.11 mg/L (71 % inhibition). The modelled EC_{50} is a little bit below this value probably because of the concentration range selected with all concentrations but one producing less than 50 % inhibition. The EC_{10} has a better adjustment. Nevertheless the results proof that with statistically derived endpoints the same classification outcome would be obtained.

Model adjusted for 72 hours based on geomean concentrations



Model adjusted for 48 hours based on geomean concentrations



Additional algal growth inhibition studies:
RAC agrees and considers that the lack of intermediate measurements do not invalidate the studies. The tests fulfil validity criteria of OECD TG 201. For the first test RAR 9.2.6.1/04 endpoints based on time weighted mean are E_rC_{50} > 0.059 mg/L and E_rC_{10} = 0.0128 mg/L. For the second RAR 9.2.6.1/05, EC_{50} > 0.032 mg/L, and an EC_{10} = 0.0080 mg/L were obtained.

Myriophyllum spicatum study
RAC agrees with the DS response and considers calculations based on geomean measured concentrations appropriate. This is a common approach. In addition, CLP Guidance mentions regarding unstable substances that where measured data are available at the start and end of media renewal periods (as may be available for the semi-static tests), the geometric mean for each renewal period should be calculated, and the mean exposure over the whole exposure period calculated from these data.

Chironomus riparius:
For the first study RAC considers that using TWA would result in unrealistically low effect values in a static test of 28 days duration where the substance disappears so fast. Furthermore, and as the DS indicates there are neither reliable analytical results for day 0 as to properly apply TWA or to calculate geometric mean measured concentrations.

In this test no sediment samples were taken during the study and exposure via the sediment cannot be ruled out (the results from the other available Chironomus study indicate that this might happen; in addition, the substance has high K_{oc} > 4 000 mL/g) indicating adsorption potential. Since the substance disappears and there are no reliable initial measured concentrations, *Chironomus* is a non-target species and CLP is about hazards in the aquatic environment, the value provided based on initial measured concentration NOEC = 0.14 mg/L will be used as supporting information.

In the second study it is not clear for RAC how a NOEC could be calculated when in the test is stated that no treatment related effects were observed. If no statistically related effects were observed at all, the NOEC should be higher than and not equal to the highest concentration tested. As in the previous *Chironomus* test, RAC will consider the results as

supporting information for classification and labelling since exposure via sediment cannot be excluded. Nevertheless, the values obtained would not change the classification outcome.

Date	Country	Organisation	Type of Organisation	Comment number
15.02.2019	France		MemberState	20
Comment received				
FR agrees with the proposal of classification for environmental hazards and the proposals of acute and chronic M factors.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you.				

PUBLIC ATTACHMENTS
1. DMP_ECHA commenting_task force_sanitized.pdf [Please refer to comment No. 2, 6]