Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Fenoxycarb

EC number: 276-696-7
CAS number: 72490-01-8

ECHA/RAC/CLH-O-0000001884-67-03/A2

Adopted

14 September 2012

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON FENOXYCARB

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

Substance name: fenoxycarb
CAS number: 72490-01-8
EC number: 276-696-7

General comments

Date	Country / Organisa-tion / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
12/09/2011	Spain / MSCA	We are in agreement with the classification proposal submitted by DE.	Thank you for the support.	Noted
30/09/2011	United Kingdom / MSCA	For many of the hazard classes we do not consider that the CLH report contains enough information to allow the reader to form an opinion on the classification of this substance. Although, we are aware that detailed information is provided in the DAR/CAR, we consider that the CLH report should be a stand-alone document and believe that the reader would benefit from additional information (e.g. whether the observed effects are significant, their magnitude/severity, the relevant dose(s) and the number of animals affected). We suggest this information is provided in the response to comments table to aid the rapporteur. Page 6 – Proposed labelling. Whilst precautionary statements have been included in the proposal, we note that these will not be included in the Annex VI entry and that the final choice of P statements is at the discretion of the supplier.	We see little difference whether the additional details are provided in the "response to comments" or the DAR/CAR. For future dossiers, we will include more details in the report.	OK. but the answer is not sufficient for this report There is insufficient detail for different endpoints, specifically for repeated dose and reprotoxicity.
30/09/2011	Portugal / Portuguese Environmen t Agency	Considering the present proposal, we agree to establish a harmonised classification & labelling for FENOXYCARB. The proposed environmental classification fulfils the criteria established in CLP Regulation (2nd ATP) for the M-factor, therefore, we support this proposal.	Thank you for the support.	Noted

Carcinogenicity

Date	Country / Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
29/09/2011	Denmark / Danish Environment Protection Agency	Denmark agrees with the proposed classification regarding carcinogenicity (Carc 2; H351). Based on the findings of increased rates of lung carcinoma and/or adenoma in mice (male and female) and hepatocellular carcinoma/benign hepatoma in liver in mice (male) we believe that there is evidence of a carcinogenic effect and that a comparison with the criteria justifies a classification as carcinogenic in hazard category 2. It is also noted that Fenoxycarb has previously been evaluated under the review programme of Regulation (EC) No. 1490/2002. In a peer review of the Draft Assessment Report for Fenoxycarb, the European Food Safety Authority (EFSA) has likewise concluded that the classification Carc3; R40 (Dir. 67/548/EEC) is justified (EFSA, 2010). The proposed harmonised classification for carcinogenicity is thus in accordance with EFSAs conclusions. General comments to section 5.8 – Carcinogenicity (page 18-23): A second carcinogenicity study in mice (Everett et al. 1987) is referred to in the section "Other information" (page 29). Apparently this study also reports increased incidence of lung adenoma and carcinoma as well as liver tumors – but the study is not described under section 5.8 (Carcinogenicity). However, the findings in this study could strengthen the argumentation for classification. The argumentation for the suggested classification could be improved and made clearer by introducing a paragraph on "comparison with criteria" (which is also part of the CLP report template).	Thank you for the support. Thank you for the suggestion to include a paragraph "comparison with the criteria"; for the next dossiers to be prepared, we will use the most up to date template. The study by Everett is described in the back part of this document.	You will notice that the CLP criteria have been modified compared to the previous 67/548/CEE directive Therefore RAC proposes a classification as Carc. 1B.
30/09/2011	United Kingdom / MSCA	Page 18- oral studies Please state the method of administration (e.g. diet or gavage) and whether the study was conducted according to a guideline or GLP. We consider that non-neoplastic information should be provided in this section of the CLH report to allow the reader to establish the maximum tolerated dose (MTD). Please provide this information in the response to	Details on the carcinogenicity studies (Goodyer, Bachmann, Everett) are reported in the back part of this document. The incidences of	Noted

Date	Country / Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		comments table. We note in the DAR that reference is made to an 80-week study (Everett et al,1987) conducted with CD-1 mice, but is not included in the CLH report. Please include the details of this study in the response to comments table or state why you consider that this study is not relevant for classification. Page 29- other information In the section of the CLH report entitled 'other information', you state that Harderian gland tumours were observed in mice, however, these tumours are not discussed in the carcinogenicity section of the CLH report. Please include the incidence rate of this tumour type and a discussion of their relevance to humans in the response to comments table.	harderian gland tumours in males were 7/50 vs. 10/50 vs. 7/50 vs. 13/50 in control group vs. 30 ppm vs. 110 ppm vs. 420 ppm.	

Mutagenicity

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment

Toxicity to reproduction

Date	Country / Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment

<table>
<tr><th>Date</th><th>Country / Organisation/ MSCA</th><th>Comment</th><th>Dossier submitter's response to comment</th><th>RAC's response to comment</th></tr>
<tr><td>29/09/2011</td><td>Denmark / Danish Environment Protection Agency</td><td>No classification is proposed for reproductive toxicity by the dossier submitter. Fenoxycarb has previously been evaluated under the review programme of Regulation (EC) No. 1490/2002. In EFSAs peer review of the Draft Assessment Report for Fenoxycarb (EFSA 2010) a proposal for classification for reproductive toxicity (Repr3, R63) was agreed by the majority of the experts. EFSA's proposal is based on a prenatal developmental study with rabbits showing an increased incidence of spina bifida at 300 mg/kg bw/day, reported to be above historical data. In the CLH report the same study (Hummler and McKinney 1984) was evaluated and it was concluded that the observations in the rabbit study were considered unrelated to the test substance for a number of reasons. -E.g. that the incidence of the two malformations was within the range reported for the historical control data and that the findings were not reproducible. In order to make a conclusion on this endpoint, Denmark encourages the dossier submitter to clarify the different interpretations of rabbit study with respect to whether the findings are within the range of historical control data, taking into account the recent conclusions of EFSA.</td><td>The applicant in the PPP procedure did several surveys in the testing laboratory for historical control data (including a harmonisation of the terminology) and submitted the results in several versions. The historical control data used in this dossier could not be taken into account in the PPP procedure due to procedural restrictions to introduce new data at a late procedural stage.

In summary, the submitted dossier uses different (i.e., more complete) historical control data than those that were available at EFSA.</td><td>It was concluded by RAC, in agreement with the DS, that classification for toxicity to reproduction is not warranted. The reasoning is elaborated in the opinion document.</td></tr>
<tr><td>30/09/2011</td><td>United Kingdom / MSCA</td><td>Please state, in the response to comments table, the incidence of spina bifida and tail reduction defects observed in the Hummler and McKinney (1984) study and the relevant historical control values.</td><td>Following information is cited from the additional report:<table><tr><td>Dose (mg/kg bw/d)</td><td>0</td><td>30</td><td>100</td><td>300</td></tr><tr><td>Dams examined</td><td>18</td><td>20</td><td>17</td><td>20</td></tr><tr><td>Dams with live foetus</td><td>17</td><td>17</td><td>17</td><td>18</td></tr><tr><td>No of</td><td>12</td><td>10</td><td>10</td><td>12</td></tr></table></td><td>These data were not reported in the submitted dossier, however considered in the RAC opinion.</td></tr>
</table>

<table>
<tr><th>Date</th><th>Country / Organisation/ MSCA</th><th>Comment</th><th>Dossier submitter's response to comment</th><th>RAC's response to comment</th></tr>
<tr><td></td><td></td><td></td><td>
<table>
<tr><td>foetus</td><td>2</td><td>1</td><td>9</td><td>6</td></tr>
<tr><td>Spina bifida</td><td>0</td><td>0</td><td>1 (1)</td><td>3 (2)</td></tr>
<tr><td>Hypo-plastic tail</td><td>1 (1)</td><td>1 (1)</td><td>0</td><td>4 (3)</td></tr>
</table>
No. of affected foetuses (no. of affected litters)

<table>
<tr><td>Dose (mg/kg bw/d)</td><td>0</td><td>200</td></tr>
<tr><td>Dams examined</td><td>35</td><td>33</td></tr>
<tr><td>Dams with live foetus</td><td>35</td><td>33</td></tr>
<tr><td>No of foetus</td><td>349</td><td>234</td></tr>
<tr><td>Spina bifida</td><td>0</td><td>0</td></tr>
<tr><td>Hypo-plastic tail</td><td>1 (1)</td><td>0</td></tr>
</table>
No. of affected foetuses (no. of affected litters)

Following historical control data is cited from Moxon: Fenoxycarb – evaluation of potential developmental toxicity in the rabbit (dated 26 May 2010).

Historical incidences of spina bifida:
0 in 34 studies,</td><td></td></tr>
</table>

Date	Country / Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
			1 (0.8%-1.1%) in 4 studies, 2 (1.9%) in 1 study, 3 (3.4%) in 1 study. Historical incidences of tail malformations: 0 in 30 studies, 1 (0.4%-1%) in 9 studies, 3 (3.4%) in 1 study.	
30/09/2011	United Kingdom / Syngenta	P. 24 The Conclusion on Developmental Toxicity - Although proposed by EFSA, Syngenta believe that no classification for developmental toxicity is required. In the rat, no embryotoxicity was observed up to and including dose levels where maternal toxicity occurred. In the rabbit, spina bifida and tail malformations observed are within historical control data and thus, not treatment-related. Furthermore, these findings were not seen in a supplementary study using a larger group size of animals at a dose level bridging the dose levels used in the main study. *ECHA comment: The document attached "Fenoxycarb Classification - Syngenta Response to EChA Annex VI Report (Sept 2011).docx" is provide separately.*	Noted.	Noted

Respiratory sensitisation

Date	Country / Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment

Other hazards and endpoints

Date	Country / Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
19/09/2011	Belgium / MSCA	Environment Based on the results of the aquatic toxicity test (EC50 all trophic levels<1mg/l, 21dNOEC Daphnia magna = 0.0000016 mg/l) the fact that	Thank you.	Agreement noted

Date	Country / Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		the substance is considered as not rapidly degradable it is justified to classify, following the classification criteria of the 2nd ATP, as Aquatic Acute 1, H400 and Aquatic chronic 1,H410. Furthermore, the substance shows potential to bioaccumulate (BCF >500), In view of the proposed classification and toxicity band for acute toxicity between 0.1 and 1mg/l, an M-factor for acute toxicity of 1could be assigned, and an M-factor for chronic toxicity of 10 000 (non rapidly degradable substance and toxicity band between 0.000001 and 0.00001 mg/l). In conclusion : we support the proposed adaptation of the environmental classification to the 2nd ATP.		
30/09/2011	United Kingdom / MSCA	Page 14 – repeated dose toxicity We consider that this section of the CLH report should contain additional information (e.g. whether the observed effects are significant, their magnitude/severity, the relevant dose(s) and the number of animals affected). For completeness, please consider providing this information in the response to comments table. You state that liver effects are observed at 45 mg/kg bw/day in the 90 day rat study. These effects occur within the range for classification in STOT-RE 2 (10<C≤100 mg/kg bw/day), however, without more information on the severity of these effects the reader cannot form an opinion on whether they are relevant for classification. Please provide, in the response to comments table, an indication of the severity of these effects and a justification as to why you did not consider these effects relevant for classification. In section 5.8.5, you state that fenoxycarb is a peroxisome proliferator type enzyme inducer; please consider this information in your discussions on the relevance of observed liver effects to humans.	The requested additional information is included in the DAR. Thank you for the suggestion how to enhance our future dossiers. Considering the received comments, other MSCA seemed content with the way the information was presented. In the 90-d rat study by Bachmann (1993) larger liver was seen in 2/10 females and centrilobular hypertrophy in 8/10 females at a dose level of 750 ppm (49.6 mg/kg bw/d). No effects on liver were observed in males of	The severity effects are provided and discussed in the RAC opinion.

Date	Country / Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
			this dose level. These findings are considered not sufficient to propose classification with STOT-RE 2 In the 28-d rat study by Suter (1986), peroxisome proliferation was observed at a dose level of 790 mg/kg bw/d, but not at 138 mg/kg bw/d.	

ATTACHMENTS RECEIVED:

Toxicity to reproduction

Fenoxycarb classification - Syngenta response to ECHA Annex VI Report (Sept 2011) submitted by the United Kingdom on behalf of Syngenta.

Following pages:
Details on the carcinogenicity studies with fenoxycarb, taken from the revised additional report (March 2010)

B.6.5 LONG-TERM TOXICITY AND CARCINOGENICITY STUDIES (ANNEX IIA 5.5)

B.6.5.1 Chronic toxicity and carcinogenicity

STUDY 1

Characteristics

reference	:	Goodyer, 1992 / Hardisty, 1991 (re-examination) / Goodyer, 2007, 2007a and 2007b	exposure	:	52 weeks (interim kill) 104 weeks (terminal kill)
type of study	:	104-week combined toxicity/carcinogenicity study with a 52-week interim sacrifice	doses	:	0, 200, 600, 1800 mg/kg food[1]
year of execution	:	1983-1985	vehicle	:	none
test substance	:	Ro 13-5223/000, Lot 2, Purity 96.6%	GLP statement	:	yes
route	:	oral, via the diet	guideline	:	OECD 453
species	:	Sprague Dawley Rat, Crl:CD(SD)BR	acceptability	:	see acceptability
group size	:	10/sex/group interim kill 50/sex/group terminal kill	NOAEL	:	8.1 mg/kg bw/day in males, 10.0 mg/kg bw/day in females

[1] equal to 0, 8.1, 24.7, 74.4 mg/kg bw/day in males and 0, 10.9, 33.1, 100.4 mg/kg bw/day in females

Study design

The study was performed in accordance with OECD guideline 453. Selection of dose levels was based on data from a 6-week dose range-finding study (HLE project number 161/122, not available to the reviewer). Histopathology was performed in control and high dose animals, in addition liver was microscopically examined in the mid dose groups. Furthermore histopathological examination was performed on all animals which died or were killed in extremis during the study.

The following deviations from OECD guideline 453 were observed:

- Group size: the interim kill groups contained 10 animals/sex (all groups), while OECD guideline requests 20 animals/sex in the high dose group.
- Haematology (incl. white blood cell differential count): performed on 10 males and 10 females groups 1 and 4, while 20 animals/sex are requested for all groups. No measurement was performed in week 13. No blood sample was collected for animals killed intercurrently for differential leukocyte counts.
- Urinalysis: performed on groups 1 and 4, while this is requested for all groups. No measurement was performed in week 13. The same animals should have been selected as for clinical chemistry, this was not the case.
- Clinical chemistry: performed on groups 1 and 4, while this is requested for all groups.
- Pathology: additional organs were weighed: heart, lungs, spleen, thyroids.

In 1991, re-examination of the pituitary gland, thyroid gland and liver from male rats was performed to verify the incidence of potential treatment-related findings and the address concerns raised by the Environmental Protection Agency (Hardisty, 1991). Also the pituitary individual animal data are provided (Goodyer, 2007b). Summary tables for urinalysis and macroscopic findings are provided in a statement by Goodyer, 2007. A statistical analysis of all the microscopic data, including neoplastic findings, is provided in a statement by Goodyer, 2007a.

Results

The results are summarised in tables 6.5.1.1 – 6.5.1.3.

Table 6.5.1.1

Dose (mg/kg food)	0		200		600		1800		dr
	m	f	m	f	m	f	m	f	
Mortality									
-interim kill	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	
-terminal kill	12/50	12/50	17/50	11/50	16/50	21/50	13/50	18/50	f
Clinical signs	No treatment related changes								
Body weight (gain)									
-week 1-52							dc	dc	
-week 1-104							normal	normal	
Food consumption							dc	dc	
Ophthalmoscopy	no treatment related changes								
Haematology									
-wk25: red blood cells							dc		
-wk25: haemoglobin							dc		
-wk25: platelets							dc		
Urinalysis	no treatment related changes								
Clinical chemistry									
-ALP (wk 25-102)							ic	ic[1]	
-AGAT, ALAT (wk 78)							ic		
-AGAT, ALAT (wk 102)					ic		ic		
-Albumin (wk 25-102)							ic[2]		
-Albumin (wk 78)								ic	
-A/G ratio (wk 25-102)							ic		
-A/G ratio (wk 51-102)								ic	
Organ weights									
-liver (wk 52)					i	i	ic[3]	ic[4]	m
-liver (wk 104)					i	i	i		m
Pathology									
macroscopy	no treatment related changes								
microscopy (terminal kill + sporadics)									
neoplastic lesions									
-pituitary adenoma	24/49	29/50	14/22	12/50	17/23	23/50	24/49	30/50	
-pituitary carcinoma	1/49	14/50	0/22	14/50	0/23	10/50	1/49	9/50	
microscopy- (interim kill)									
non-neoplastic lesions									
liver:									
-hypertrophy	0/10	0/10	0/10	0/10	2/10	0/10	6/10	2/10	m
-focal necrosis	0/10	0/10	1/10	1/10	3/10	1/10	5/10	0/10	m
-pigmented hepatocytes	0/10	0/10	0/10	0/10	2/10	0/10	4/10	0/10	m
-focal fibrosis	0/10	0/10	1/10	0/10	0/10	0/10	3/10	0/10	
microscopy- (terminal kill)									
non-neoplastic lesions									
liver:									
-hypertrophy	0/50	0/38	0/50	0/39	9/50	0/29	18/50	8/32	m
-focal necrosis	1/50	1/38	3/50	0/39	14/50	2/29	14/50	0/32	m
-focal fibrosis	4/50	0/38	5/50	0/39	15/50	0/29	19/50	0/32	m

dr dose related

oc/ic statistically significantly decreased/increased compared to the controls
di/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative
1 increase in ALP was not statistically significant in week 78
2 increase in albumin was not statistically significant in week 78

Table 6.5.1.2 Absolute and relative liver weight

	Dietary concentration of fenoxycarb (ppm)							
	Males				Females			
Study period	0	200	600	1800	0	200	600	1800
Interim kill: Adjusted (g)	-	-	-	-	8.094	8.816	8.943	10.390***
Interim kill: Relative (%)	2.388	2.208	2.774	2.688 #	-	-	-	-
Terminal kill: Adjusted (g)	15.287	14.577	17.009	16.213	9.078	9.754	11.268	12.743***

*** Statistically significant difference from control group mean, p<0.001
# significant using the dose response test
Adjusted – to terminal bodyweight (g) Relative – organ/bodyweight ratio (%)

Table 6.5.1.3 Alkaline phosphatase, ASAT and ALAT

	Dietary concentration of fenoxycarb (ppm)							
	Males				Females			
Week:Parameter	0	200	600	1800	0	200	600	1800
25: Alkaline phosphatase	143	-	-	200**	73	-	-	107*
51: Alkaline phosphatase	158	-	-	243***	60	-	-	119**
78: Alkaline phosphatase	143	-	-	272**	84	-	-	131
102: Alkaline phosphatase	136	163	202	286***	72	74	94	104*
102: ASAT	78	90	197**	153*	85	79	107	104
102: ALAT	29	30	82**	73*	37	34	41	34

* Statistically significant difference from control group mean, p<0.05
** Statistically significant difference from control group mean, p<0.01
*** Statistically significant difference from control group mean, p<0.001

Acceptability

The study is considered acceptable, despite a few shortcomings:

No data were presented in the study report on clinical signs, palpable tumours, ophthalmoscopy, and summary tables were missing for urinalysis and macroscopic findings. These data were later provided by the notifier in a separate summary, showing no treatement related changes in these parameters.

Since an effect on mortality was seen in females at the mid and high dose level, all female groups should have been examined in histopathology, as recommended in the OECD guideline.

It was apparent that survival in this study was much higher than in historical control data. In the present study, survival was 76% for both males and females of the control group, whereas historical control data from the test facility indicate survival in the range of 30-58%. ~~Furthermore, statistical analysis of neoplastic data was not performed, which should be provided by the notifier.~~

It was remarkable, that in the present study, the incidences of pituitary carcinoma in females of all groups including controls (28, 28, 20, 18%) were much higher than in historical control data, where incidences ranged between 0-4% for females. In the study report, no explanation was given for this phenomenon.

Conclusions

Treatment of rats for 24 months with the test substance did not affect mortality in males, but higher mortality rates were noted for females at 600 and 1800 mg/kg food. Mortality rates in females of groups 1, 2, 3 and 4 respectively were 12/50, 11/50, 21/50 and 18/50.

Body weight gain was decreased over the first year in males and females at 1800 mg/kg food (91% and 92% of controls) and comparable to controls over the second year. Final body weights were 97% of control weights for both sexes. At 1800 mg/kg food, food consumption was slightly decreased (approximately 94 to 99% of controls) throughout the study in males, and up to week 64 in females.

Red blood cell indices were decreased in males at 1800 mg/kg food in week 25 only (96% of controls). In the absence of further findings at other time points, or in the other sex, they were considered of no toxicological relevance.

Clinical biochemistry investigations revealed moderate effects in the mid and high dose group. At 1800 mg/kg food, effects became apparent from week 25 onwards, and these comprised of increased values for ASAT and ALAT (males only) and ALP, albumin and albumin/globulin ratio (both sexes). Enzyme activities were increased up to 250% of controls, and protein indices up to 130% of controls. Changes in males at 600 mg/kg food were confined to increased ASAT and ALAT values in week 102 only (maximally 283% of controls).

At the interim kill in week 52, liver weights or liver:body weight ratios were increased in males and females at 600 mg/kg food (116% and 110% of controls) and in males and females at 1800 mg/kg food (113% and 126% of controls). Statistical significance was reached at the high dose only. At the terminal kill, a similar trend was observed: liver weights were increased at 600 mg/kg food for both sexes (111% and 113% of controls), and at 1800 mg/kg food for both sexes (106% and 128% of controls). The microscopic correlates for these higher liver weights were centrilobular hypertrophy and focal necrosis, associated with repair processes such as fibrosis and foci of pigmented hepatocytes. Hepatic centrilobular hypertrophy was seen at the interim and terminal kill in males at 600 mg/kg food, and in males and females at 1800 mg/kg food. Focal necrosis in the liver was increased at the interim and terminal kill in males at 800 and 1600 mg/kg food. Focal fibrosis was noted in males at 1800 mg/kg food, both at the interim and terminal kill, while pigmented hepatocytes was seen in the high dose males after 52 weeks only. The incidences and nature of histological findings in the liver in animals at 200 mg/kg food, were comparable to controls and the background data.

It was also remarkable that incidences of pituitary carcinoma in females of all groups (28, 28, 20, 18%) were much higher than in historical control data, where incidences ranged between 0-4% for females. However, the effect was not dose-dependent and is not considered treatment related. The incidence of pituitary carcinoma in males has been changed compared to the original study report, based on a re-examination. This re-read established that several of the pituitary tumours previously diagnosed as carcinoma should more accurately be diagnosed as adenoma. The results of this re-read show that there is no effect of fenoxycarb treatment on pituitary adenoma or carcinoma incidence in males.

Based on the above considerations, a NOAEL was set at 200 mg/kg food, equivalent to 8.1 and 10.9 mg/kg bw/day in males and females respectively).

STUDY 2

Characteristics

reference	:	Bachmann M., 1995	exposure	:	18 months
type of study	:	78-week carcinogenicity study	doses	:	0, 10, 50, 500, 2000 mg/kg food[1]
year of execution	:	1992-1994	Vehicle	:	none
test substance	:	CGA 114597 tech. batch 139044, purity 97.6%	GLP statement	:	yes
route	:	oral, via the diet	Guideline	:	OECD 451
species	:	Albino mouse, Tif:MAGf (inbred)	Acceptability	:	acceptable
group size	:	50 males, 50 females (Main) 10 males, 10 females (Satellite[2])	NOAEL	:	5.75 mg/kg bw/day in males, 5.33 mg/kg bw/day in females

[1] equal to 0, 1.10, 5.75, 55.4, 222 mg/kg bw/day in males and 0, 1.04, 5.33, 51.5, 201 mg/kg bw/day in females
[2] satellite animals were used for haematology investigations.

Study design

The study was performed in accordance with OECD 451.

In addition, organ weights were determined at terminal necropsy, and histopathology was performed on Main animals of all groups. Selection of dose levels was based on a previous 80-week carcinogenicity/toxicity study in mice (Study 3, Everett, 1987).

Results

The results are summarised in table 6.5.1.4 and 6.5.1.5.

Table 6.5.1.4

Dose (mg/kg food)	0		10		50		600		2000		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality, n=60 (incl. Satellites, n=60)	11 (15)	2[5] (4)	12 (13)	11 (13)	11 (15)	12 (14)	8 (8)	13 (14)	11 (14)	10 (11)	
Clinical signs	no treatment-related findings										
Body weight (gain)								d/dc		d/dc	f
Food consumption							ic	i[2]	ic [1]	i[2]	m,f
Haematology	no treatment-related findings										
Organ weights											
- liver							ic [4]	ic [1]	ic [4]	ic [4]	m,f
- kidney										ic [1]	
- adrenal							ic [4]		ic [4]	dc [4]	m
Pathology											
macroscopy											
- lungs: nodules	7/60	1/60	8/60	4/60	3/60	6/59	12/60	4/60	17/60	8/60	m
- lungs: masses	0/60	1/60	3/60	3/60	3/60	1/59	4/60	2/60	4/60	4/60	m
- liver: enlarged	2/60	4/60	1/60	3/60	4/60	6/59	6/60	5/60	16/60	14/60	
- liver: masses	18/60	4/60	13/60	2/60	20/60	6/59	26/60	5/60	38/60	5/60	m
- spleen: enlarged	8/60	34/60	12/60	31/60	19/60	29/59	24/60	33/60	26/60	32/60	m
microscopy											
neoplastic lesions											
- pulmonary adenoma	8/50	1/50	8/50	5/50	4/50	4/49	14/50	6/50	16/50	11/50	m
- pulmonary carcinoma	1/50	2/50	3/50	2/50	1/50	2/49	10/50	3/50	10/50	9/50	m
- pulmonary pooled[3]	9/50	3/50	11/50	7/50	5/50	6/49	21/50[4]	9/50[4]	22/50[4]	20/50[4]	m,f
- benign hepatoma	11/50	2/50	12/50	2/50	9/50	3/49	13/50	1/50	16/50	3/50	
- hepatocell. carcinoma	8/50	1/50	4/50	0/50	12/50	2/49	17/50	0/50	21/50	2/50	m
- hepatic tumours pooled[3]	16/50	3/50	13/50	2/50	17/50	5/49	25/50	1/50	29/50[4]	4/50	m
microscopy											
non-neoplastic lesions											
- liver: foci of cellular change	5/50	2/50	1/50	4/50	7/50	3/49	2/50	3/50	9/50	7/50	
- liver: fatty change	39/50	42/50	33/50	34/50	39/50	28/49	26/50	15/50	30/50	8/50	m,f
- adrenal cortex: atrophy	33/50	0/50	25/50	0/50	19/50	0/50	26/50	0/50	15/50	0/50	
- bone marrow: fatty atrophy	0/50	36/50	0/50	30/50	0/50	26/49	0/50	23/50	0/50	16/50	f
- pancreas: fatty atrophy	0/50	6/50	0/50	3/50	1/50	2/49	0/50	2/50	0/50	0/50	
- ovaries: atrophy		19/50		18/50		22/49		26/50		31/50	

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative
1 also relative food consumption
2 relative food consumption only, from week 62 onwards
3 shows the number of animals bearing at least one benign or malignant tumour of indicated type
4 multiplicity also increased
5 mortality in control females was exceptionally low, while mortality in treated females was within the normal range.

Table 6.5.1.5 Absolute (g) and relative liver weights (%) and between brackets % change compared to control

Dose (mg/kg food)	0		10		50		500		2000	
	m	f	m	f	m	f	m	f	m	f
Abs. liver weights	2.97	2.48	2.89	2.30	2.90	2.26	3.30*	2.41	4.37*	2.97*
(% change)			(97)	(93)	(98)	(91)	(111)	(97)	(147)	(120)
Rel. liver weights	55.67	50.24	56.98	46.75	57.35	49.03	64.80*	53.05*	90.77*	70.70*
(% change)			(95)	(93)	(96)	(98)	(109)	(106)	(152)	(141)

* statistically significantly increased compared to the control group mean, p<0.01

Acceptability

The study is considered acceptable.

Although no clinical chemistry and haematology were performed, it is considered justified to establish a NOAEL in the present study, considering the effects observed in the semichronic and chronic toxicity studies with different species.

Conclusions

Treatment of mice for 78 weeks with concentrations of 0, 10, 50, 500, 2000 mg/kg in the diet did not affect mortality, clinical signs, and haematology parameters. Body weights of treated males were unaffected, while a decrease was seen in females treated with 500 or 2000 mg/kg food (final body weights were 91 and 87% of controls). Over the last 6 months of treatment, a higher food intake was recorded for males at 500 and 2000 mg/kg food, and food consumption ratios were elevated for females at 500 mg/kg, and for both sexes at 2000 mg/kg food.

Higher liver weights and/or liver:body weight ratios were observed for males and females at 500 and 2000 mg/kg food. The increased (relative) liver weight is not critical for the derivation of the NOAEL, because the increase at 500 ppm is 11% or lower and there was no histopathology in the liver at this dose level.

Kidney:body weight ratios were increased in females at 2000 mg/kg food. Adrenal weights and/or adrenal:body weight ratios were increased for males at 500 and 2000 mg/kg food, while a decrease was seen for females at 2000 mg/kg food.

Necropsy revealed an increased incidence of nodules/masses in the lungs of males at 500 and 2000 mg/kg food, and in females at 2000 mg/kg food. Also, an increased incidence of hepatic masses was observed macroscopically in males at 500 and 2000 mg/kg food. Enlargement of the liver, noted in high dose males and females, correlated with a variety of microscopic findings which were not dose related, and was therefore considered of no toxicological relevance. Splenic enlargement in treated males was considered not to represent a direct effect, and was considered related to the enhancement of haematopoiesis by the presence of neoplasia.

Higher incidences of pulmonary tumours were confirmed microscopically in both sexes at 500 and 2000 mg/kg food, and higher incidences of hepatocellular tumours were recorded for males of these dose groups. A treatment-related increase in multiplicity of these tumour types was demonstrated. Also, the non-neoplastic finding of hepatic foci of cellular change was increased in males and females at 2000 mg/kg food.

Further dose-related non-neoplastic findings were considered to represent a non-specific reaction to stress (males) or were considered secondary to body weight suppression (females). In males these included: decreased incidences of hepatic fatty change and decreased incidences of atrophy in the adrenal cortex. In females these included: lower incidences for fatty atrophy in the bone marrow, for hepatic fatty change and for pancreatic fatty atrophy, and increased incidences of ovarian atrophy.

Based on the increased incidences of lung and liver tumours ~~above considerations~~, the NOAEL was set at 50 mg/kg food (equivalent to 5.75 and 5.33 mg/kg bw/day in males and females respectively). Fenoxycarb exhibited an oncogenic potential in mice, based on higher incidences of pulmonary tumours in males and females and hepatocellular tumours in males at 500 and 2000 mg/kg food.

See also B.6.8 for mechanistic studies and further assessment of the observed tumour incidences.

STUDY 3

Characteristics

reference	:	Everett, 1987	exposure	:	80 weeks (terminal kill) 52 weeks (interim kill)
type of study	:	80-week combined toxicity/carcinogenicity study with a 52-week interim sacrifice	doses	:	0, 30, 110, 420 mg/kg food for males, 0, 20, 80, 320 mg/kg food for females[1]
year of execution	:	1984-1985	vehicle	:	none
test substance	:	Ro 13-5223/000 Batch 83, Purity 96.6%	GLP statement	:	yes
route	:	oral, via the diet	guideline	:	OECD 453
species	:	CD-1 mouse	acceptability	:	yes
group size	:	50/sex/group terminal kill 10/sex/group pretrial bleed 10/sex/group interim bleed wk26 10/sex/group interim kill wk52 10/sex group 1+4 Recovery wk59	LOAEL	:	≤ 5.3 mg/kg bw/day in males and ≤ 4.4 mg/kg bw/day in females)

1 equal to 0, 5.3, 19.3, 73.9 mg/kg bw/day in males and 0, 4.4, 16.9, 72.2 mg/kg bw/day in females for the terminal kill, and equal to 0, 6.0, 21.7, 81.8 mg/kg bw/day in males and 0, 4.8, 18.2, 71.6 mg/kg bw/day in females for the interim kill

Study design

The study was performed in accordance with OECD guideline 453. Selection of dose levels was based on preliminary studies (IRI Project 430561) and data from the sponsor.

The following deviations from OECD guideline 453 were observed:

- Dose level selection: the chosen levels for the high dose groups were considered to be too low, to represent a Maximum Tolerated Dose.
- Group size: the interim kill groups contained 10 animals/sex (all groups) plus 10 Recovery animals (groups 1+4), while OECD guideline requests 20 animals/sex in the high dose group.
- Haematology: performed on 10 animals/sex/group, while 20 animals/sex are requested. No measurement was performed in week 13.
- Urinalysis: no measurement was performed in week 13.
- Pathology: additional sectioning of liver, Harderian glands and lungs was performed. Only a limited set of tissues was examined for the animals of the interim kill.

Results

The results are summarised in table 6.5.1.6 – 6.5.1.8.

Table 6.5.1.6

Dose (mg/kg food)	0	0	30	20	110	80	420	320	dr
	m	f	m	f	m	f	m	f	
Mortality (n=60)	8	9	5	11	7	7	12	7	
Clinical signs	no data available in study report								
Body weight (gain)	no treatment-related findings								
Food consumption	no treatment-related findings								
Haematology	no treatment-related findings								
Urinalysis	no treatment-related findings								
Clinical chemistry									
- AST (wk 26)					i		ic		m
- ALP (wk 52)							ic [1]		
- LDH (wk 80)							ic		
Organ weights									
-liver (wk 52)					i[2]		i[2] / ic[3]		m
-liver (wk 80)							i[2]		
Pathology									
macroscopy	no treatment-related findings								
microscopy (terminal kill + sporadics)									
neoplastic lesions									
-lungs: alveolar/bronchiolar tumours									
malignant	2/50		6/50		6/50		7/50		
benign and malignant combined	7/50		13/50		13/50		20/50		m
after additional sectioning:									
malignant	2/50		6/50		7/50		7/50		
benign and malignant combined	11/50		16/50		18/50		25/50 [4]		m
microscopy- (interim kill)									
non-neoplastic lesions[5]	no treatment-related findings								
microscopy- (terminal kill)									
non-neoplastic lesions	no treatment-related findings								

dr	dose related
dc/ic	statistically significantly decreased/increased compared to the controls
d/i	decreased/increased, but not statistically significantly compared to the controls
a/r	absolute/relative
1	ALP no longer increased after 6-week recovery period
2	liver weights at end of recovery did not differ from controls
3	after deletion of liver tumours
4	multiplicity also increased
5	Results of additional sectioning of livers are presented in a Supplement to the report. There were no findings noted.

Table 6.5.1.7 Absolute (g) liver weights in males and between brackets % change compared to control

Dose (mg/kg food)	0	30	110	420
Abs. liver weights at 52 weeks	2.07	2.19	2.27	2.44
(% change)		(106)	(110)	(118)
Abs. liver weights at 80 weeks	2.38	2.46	2.26	2.76
(% change)		(103)	(95)	(116)

Table 6.5.1.8 Absolute (g) liver weights in females and between brackets % change compared to control

Dose (mg/kg food)	0	20	80	320
Abs. liver weights at 52 weeks (% change)	1.64	1.51 (92)	1.55 (95)	1.88 (115)
Abs. liver weights at 80 weeks (% change)	1.68	1.53 (91)	1.71 (102)	1.88 (112)

Acceptability

The study is considered acceptable, despite a few shortcomings:

- The chosen levels for the high dose groups were considered to be too low, to represent a Maximum Tolerated Dose.
- No data were presented on clinical signs, and statistical evaluations were limited (i.e. no statistics on survival data, no age-adjusted test on tumour incidences, no statistics on benign lung tumours, and no statistical analysis on multiplicity).
- The additional investigation on lung tumours by Dr. Roe was not performed blindly, and no individual data were presented.
- Comparison of lung tumour incidences in the present study with historical data is no longer possible, since lungs were exhaustively sectioned.
- The errata contained in the back of the study file were not signed, and did not fully match with the errata up front.

Conclusions

Toxicity study

Treatment of mice for 52 weeks with concentrations of 0, 30, 110, 420 mg/kg food for males, and 0, 20, 80, 320 mg/kg food for females did not affect mortality, clinical signs, body weights and food consumption, haematology parameters, and macroscopic and microscopic pathology.

The only changes that were noted at the high dose were increased ASAT levels after 26 weeks (132% of controls) and ALP levels after 52 weeks (165% of controls) in high dose males, and associated higher liver weights. No such findings were present after 6 weeks Recovery. Higher liver weights in females of the high dose were not accompanied by enzyme changes nor histopathology. At the intermediate dose, only 2 males showed increased ASAT levels after 26 weeks, and liver weights were marginally higher after 52 weeks.

Carcinogenicity study

After 80 weeks of treatment with fenoxycarb, no effects were noted on mortality, clinical signs, body weights and food consumption, and haematology parameters.

At the high dose, LDH levels were increased in males after 80 weeks (142% of controls), and liver weights were increased. Histology of livers from all animals did not reveal any morphological changes.

Neoplastic lesions were found in the lungs. A statistically significant trend ($p<0.01$) was found for higher incidences of alveolar/bronchiolar tumours (benign and malignant combined) in males of all treated groups.

Malignant tumour incidences were not statistically different to controls for any dose. Multiplicity was also increased. All other findings were found to be within the range of normal background pathology, or were typical age-related degenerative changes in mice.

An expert opinion on the findings in the lungs was included in the study file, and arguments were presented to question the biological relevance of higher tumour incidences in the lungs in this study. However, this examination was not performed blindly, and no individual data were presented. And although full sectioning of the lungs may seem advantageous to detect undiagnosed tumours, comparison with historical data is no longer possible, which is an essential part of the evaluation of carcinogenicity study outcome. Also, proper statistical tests were lacking (age-adjusted test on tumour incidences, statistics on benign lung tumours, and statistical analysis on multiplicity).

Based on the above considerations, it was concluded that fenoxycarb exhibited an oncogenic potential in mice, based on higher incidences of alveolar/bronchiolar tumours in the lungs of males of all treated groups.

Based on above consideration, a NOAEL could not be established, since an increased incidence of lung tumours in males in all dose groups. A LOAEL of ≤ 30 and ≤ 20 mg/kg food for males and females, equivalent to ≤ 5.3 and ≤ 4.8 mg/kg bw/day for males and females respectively.

B.6.5.2 Summary

The results of the chronic toxicity and carcinogenicity studies are summarised in tables 6.5.2.1.

Table 6.5.2.1 Chronic toxicity and carcinogenicity studies with fenoxycarb

Duration (months)	Species	Route	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day	Critical effects	Reference
24 months	rat	oral (diet)	8.1 (M) 10.9 (F)	24.7 (M) 33.1 (F)	clinical biochemistry changes, liver weights + hypertrophy +focal necrosis	Goodyer, 1992
18 months	mouse	oral (diet)	5.8 (M) 5.3 (F)	55.4 (M) 51.5 (F)	pulmonary and hepatocellular tumours, hepatic foci of cellular change	Bachmann M., 1995
18 months	mouse	oral (diet)	≤ 5.3 (M) ≤ 4.4 (F)	- -	alveolar/bronchiolar tumours (M)	Everett, 1987

M = Males, F = Females

In a 24-months combined chronic/carcinogenicity study in rats, dose levels of 0, 200, 600, 1800 mg/kg food were tested. Decreased body weight gain and food consumption were found in males and females at 1800 mg/kg food. Mild clinical biochemistry effects were found in mid and high dose groups and included higher levels of ASAT, ALAT, and/or ALP, albumin and albumin/globulin ratio.

Liver weights were increased in males and females of the mid and high dose, after 52 weeks as well as after 104 weeks. Microscopic correlates in the liver were hepatic centrilobular hypertrophy and focal necrosis, associated with repair processes such as fibrosis and foci of pigmented hepatocytes. Histological findings in the liver in animals at 200 mg/kg food, were comparable to controls and the background data.