

Proposal for Harmonised Classification and Labelling
Based on Regulation (EC) No. 1272/2008 (CLP Regulation)

Benalaxyl

(EC/List No. 275-728-7; CAS No. 71626-11-4)

Comments on Proposed Classification

Submitted by the FMC Corporation

(FMC Document No. 56999)

February 4, 2021

Proposed classification based on CLP criteria:

Current CLH in Annex VI	Aquatic Acute 1 Aquatic Chronic 1	H400 H410
Dossier submitter's (DS) proposal	Acute Tox 4 Carc. 2 STOT SE 2 Aquatic Acute 1 Aquatic Chronic 1	H302 H351 H371 (nervous system) H400 H410
Applicant's (FMC) proposal	Acute Tox 4 STOT SE 2 Aquatic Acute 1 Aquatic Chronic 1	H302 H371 (nervous system) H400 H410

Executive Summary

FMC submits the following comments for consideration by the Risk Assessment Committee (RAC) regarding the proposed Harmonised Classification and Labelling of benalaxyl.

FMC agrees with the Dossier Submitter (DS) that it is appropriate to classify benalaxyl for acute oral toxicity Category 4 (H302), specific target organ toxicity – single exposure Category 2 (H371), and for aquatic acute and chronic toxicity Category 1 (H400 and H410). However, FMC disagrees with the proposal to classify benalaxyl for carcinogenicity Category 2 (H351).

The proposal to classify benalaxyl for carcinogenicity is primarily based on the observation of a low incidence of astrocytoma in male rats in the 2-year study, in which one case was found in each of the low and mid dose groups and two cases in the high dose group. There was no incidence of astrocytoma in the females at any dose level. Historical control data (HCD) has been obtained from several publicly available sources which clearly specify that astrocytoma is a spontaneous brain tumor with high occurrence in Sprague-Dawley (SD) rats. The incidence of astrocytoma observed in the 2-year rat study with benalaxyl is within HCD ranges from these sources, and therefore, can be concluded as unrelated to benalaxyl administration. Thus, classification of benalaxyl for carcinogenicity is not warranted.

Introduction

The DS has proposed harmonised classification and labelling for benalaxyl in accordance with the CLP criteria. FMC submits the following comments in response to this proposal during the commenting period.

Physicochemical Properties and Physical Hazards

FMC agrees based on the physical and chemical properties of benalaxyl that classification for physicochemical properties and physical hazards is not required.

Evaluation of Health Hazards

Acute toxicity

Based on the available data (ATE ~ 2000mg/kg), FMC agrees with the proposal that benalaxyl be classified for acute oral toxicity (Acute Tox Category 4, H302).

Germ cell mutagenicity

Based on results of a battery of *in vitro* and *in vivo* genotoxicity studies, it can be concluded that benalaxyl is unlikely to be genotoxic and does not meet the classification criteria for germ cell mutagenicity.

Carcinogenicity

The DS has proposed to classify benalaxyl as Car. 2, H351, suspected human carcinogen based on the occurrence of a low incidence of astrocytoma in the males in the 2-year rat study.

FMC disagrees that benalaxyl meets the classification criteria for carcinogenicity.

The DS stated the following:

“astrocytoma was considered the most critical effect, and no HCD were available for the performing laboratory and that according to the literature, astrocytoma is a rare tumour in Sprague Dawley rats. In such cases the CLP guidance suggests a comparison with the historical control data. As discussed above, the observed incidence in malignancy is just a fact without a presence of performing laboratory historical data. Also, it is unclear whether the stated HC data for the Dossier studies include the results of the different periods of time. Therefore, comparison with the historical control is not considered conclusive. The data set from the Applicant Dossier, of those older historical controls (from 1977 to 1979) which were not reliable on, due to the lack of information about the protocol/techniques of preservation/microscopic examination as well as time of sacrifice of surviving animals. The large frequency and distribution in all mice groups from the studies, higher incidence in males than in female and the high mortality concluded a treatment related”.

In addition, the DS stated that there were *“19 neoplasms from 65 rats in a lifetime oral dosing studies in rats combined oncogenicity and chronic toxicity. Dose level of 100 ppm (4.42/5.64 mg/kg bw per day for male and female, respectively) is general available for the tumour’s occurrence in both sexes, with an increased incidence at 1000 ppm in males. Benalaxyl is a chemical substance which induce tumours, increase tumour incidence and/or malignancy or shorten the time to tumour occurrence.”*

FMC disagrees with the assessment conducted by the DS.

The 19 neoplasms pointed out by the DS are a sum of all types of neoplasms observed in treated and untreated groups combining both males and females. Overall, there is no treatment-related increased incidence in neither males nor females (**Tables 1&2**, metastatic neoplasms are not included). No statistical significance was found. All the incidences have been excluded as treatment related.

The DS considered astrocytoma noted in male rats to be the most critical effect. However, astrocytoma is known to be a spontaneous brain tumor with high incidence in SD rats. In this study, no incidence of astrocytoma was found in the females, and the incidences in the males were 3.7% (2 cases) in the 1000ppm high dose group, 1.9% (1 case) and 1.8% (1 case) in the low and mid- dose groups. These incidences are comparable to reported spontaneous incidence rates in SD rats (HCD provided below). Therefore, these tumors are unrelated to treatment.

The performing laboratory at which the 2-year rat study was conducted no longer exists. Thus, being unable to obtain further HCD from the performing laboratory, FMC has collected HCD from publicly available sources for the same strain of rats covering the period when the study was performed. These publications clearly conclude that astrocytoma is spontaneous in nature,

particularly in SD rats (occurring in control animals up to ~ 7%). The incidences of astrocytoma in the 2-year rat study are within the range of HCD from all the sources.

In addition, HCD shows that the incidences of astrocytoma in male and female rats are comparable. In this 2-year rat study, the incidence in the females is zero. When males and females are considered together, there is no increased incidence of astrocytoma overall.

Table 1. Data of all neoplasms in males of benalaxyl 2-year rat study

	Control		4ppm		100ppm		1000ppm	
	No. of animals: 54		No. of animals: 52		No. of animals: 57		No. of animals: 54	
	No. of cases	Rate						
adrenal	9	16.7%	7	13.5%	7	12.3%	8	14.8%
brain astrocytoma	0	0.0%	1	1.9%	1	1.8%	2	3.7%
mammary gland	1	1.9%	0	0.0%	1	1.8%	1	1.9%
liver	1	1.9%	2	3.8%	1	1.8%	0	0.0%
lung	1	1.9%	1	1.9%	0	0.0%	1	1.9%
pancreas	3	5.6%	8	15.4%	7	12.3%	6	11.1%
pituitary	11	20.4%	18	34.6%	26	45.6%	20	37.0%
salivary gland	0	0.0%	0	0.0%	0	0.0%	1	1.9%
seminal vesicles	0	0.0%	0	0.0%	0	0.0%	1	1.9%
small intestine	0	0.0%	0	0.0%	0	0.0%	1	1.9%
spleen	1	1.9%	0	0.0%	0	0.0%	0	0.0%
sternum	0	0.0%	0	0.0%	0	0.0%	2	3.7%
testes	3	5.6%	3	5.8%	3	5.3%	1	1.9%
thyroid	8	14.8%	8	15.4%	17	29.8%	4	7.4%
other mass	8	14.8%	11	21.2%	9	15.8%	7	13.0%
total no. of tumor cases	46		59		72		55	
average tumor incidence	5.7%		7.6%		8.4%		6.8%	
average no. of tumors per animal	0.85		1.13		1.26		1.02	

Table 2. All neoplasms in females of benalaxyl 2-year rat study

	Control		4ppm		100ppm		1000ppm	
	No. of animals: 54		No. of animals: 55		No. of animals: 55		No. of animals: 56	
	No. of cases	Rate						
adrenal	4	7.4%	5	9.1%	3	5.5%	1	1.8%
brain ependymoma	0	0.0%	0	0.0%	0	0.0%	1	1.8%
mammary gland	30	55.6%	27	49.1%	24	43.6%	24	42.9%
liver	3	5.6%	3	5.5%	2	3.6%	6	10.7%
lung	2	3.7%	0	0.0%	0	0.0%	0	0.0%
pancreas	1	1.9%	3	5.5%	0	0.0%	0	0.0%
pituitary	42	77.8%	40	72.7%	42	76.4%	46	82.1%
uterus	0	0.0%	3	5.5%	1	1.8%	1	1.8%
kidney	0	0.0%	0	0.0%	1	1.8%	0	0.0%
large intestine	0	0.0%	0	0.0%	0	0.0%	1	1.8%
spleen	2	3.7%	0	0.0%	0	0.0%	0	0.0%
sternum	0	0.0%	0	0.0%	1	1.8%	1	0.0%
lymph nodes	2	3.7%	1	1.8%	0	0.0%	1	1.8%
thyroid	5	9.3%	9	16.4%	4	7.3%	8	14.3%
ovary	3	5.6%	0	0.0%	1	1.8%	1	1.8%
other mass	8	14.8%	4	7.3%	3	5.5%	2	3.6%
total no. of tumor	102		95		82		93	
average tumor incidence	11.8%		10.8%		9.3%		10.3%	
average no. of tumors per animal	1.89		1.73		1.49		1.66	

HCD for astrocytoma from publicly available sources are provided here.

- A. Giknis and Clifford (2004): Compilation of Spontaneous Neoplastic Lesions and Survival in Crl:CD (SD) Rats from Control Groups, Charles River Laboratories**
<https://www.criver.com/sites/default/files/resources/CompilationofSpontaneousNeoplasticLesionsandSurvivalinCrlCD%C2%AEsDRatsFromControlGroupsMarch2013.pdf>

This compilation of spontaneous neoplastic lesions shows that astrocytoma was found in the control groups of 50% of the studies, and the spontaneous incidence rate was up to 4.29%. Data were collected from 1989 to 2002.

Neoplasms/Males-104 Weeks

LOCATION AND TUMOR	#STUDIES	TOTAL		#STUDIES USING THIS DIAGNOSIS	MINIMUM % FOUND	MAXIMUM % FOUND
		#ORGANS	PERCENT OF TOTAL			
BRAIN	30	2146				
Astrocytoma, Malignant		26	1.21	13	0.87	4.29
Ependymoma		1	0.05	1	1.43	1.43
Glioma, Malignant		3	0.14	3	0.91	1.92
Granular Cell Tumor, Benign		8	0.37	8	0.56	2.00
Granular Cell Tumor, Malignant		4	0.19	3	1.43	2.86
Hemangiosarcoma		1	0.05	1	1.92	1.92
Meningeal Sarcoma		1	0.05	1	0.87	0.87
Neuroma		1	0.05	1	0.56	0.56
Oligodendroglioma		3	0.14	3	0.56	2.00
Choroid Plexus Papilloma		1	0.05	1	1.11	1.11
SPINAL CORD	30	2146				
Astrocytoma, Malignant		3	0.14	3	0.77	1.43
Oligodendroglioma		1	0.05	1	0.56	0.56

B. Nagatani (2013): Occurrence of Spontaneous Tumors in the Central Nervous System (CNS) of F344 and SD Rats, J Toxicol. Pathol., 26(3): 263-273
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3787604/>

This publication shows the incidence for astrocytoma in the males is up to 6.7%.

The Occurrence of CNS Tumors by Study Group for CrI:CD(SD) Rats (Male)

Study ID:	#1	#2	#3	#4	#4	#5	#6	#7	#8	#9	#9	#10	#11	#13	#14	#15	#16	#17	#18	#19	#20	#21	#22	#23	#24	#25	#26	#26	To- tal	Mean (%)	Range (%)	
Year study started:	1996	1996	1998	1999	1999	2001	2001	2003	2003	2004	2004	2004	2005	2005	2005	2006	2007	2007	2007	2008	2008	2008	2008	2008	2008	2009	2009					
Route of administration:	UT	UT	FD	GA	FD	GA																										
Vehicle:			BD	MC	MC	MC	MC	MC	TG	DW	ST	MC	DW	MC	DW	MCT	BD	AG	MC	MC	MC	MC	DW	MC	MC	DW	ST					
Number of animals:	50	50	75	60	60	60	60	60	50	60	60	60	55	60	55	60	60	55	60	60	60	60	70	60	60	60	55	55	1650			
Brain																																
Astrocytoma, malignant	0	0	3	2	1	3	2	1	2	2	1	0	0	0	1	1	1	1	0	0	0	2	2	4	2	0	0	2	33	2.0	0-6.7	
Oligodendroglioma, malignant	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1	0	0	0	0	1	0	6	0.4	0-2.0	
Tumor, granular cell	0	1	2	0	0	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	1	1	1	0	1	0	0	11	0.7	0-2.7		
Meningioma, benign	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.1	0-1.7		
Meningioma, malignant	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0.1	0-1.7		
Osteosarcoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0.1	0-1.7		
Reticulosis, malignant	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.1	0-1.7		
Spinal cord																																
Astrocytoma, malignant	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.1	0-1.3		
Oligodendroglioma, malignant	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.1	0-1.7		
Brain+Spinal cord																																
Astrocytoma, malignant	0	0	4	2	1	3	2	1	2	2	1	0	0	0	1	1	1	1	0	0	0	2	2	4	2	0	0	2	34	2.1	0-6.7	

Nagatani, Kudo, Yamakawa et al.

C. Bertrand (2014): Incidence of Spontaneous Central Nervous System Tumors in CD-1 Mice and Sprague-Dawley, Han-Wistar, and Wistar Rats Used in Carcinogenicity Studies, Toxicologic Pathology, 42:1168-1173
<https://journals.sagepub.com/doi/pdf/10.1177/0192623313518114>

In this paper, it is stated that malignant astrocytoma is a predominant spontaneous tumor type in SD rats. The table below shows the average incidence for malignant astrocytoma, which was up to 1.49%. There was no data on individual studies, therefore no range of incidences reported. However, since the total number of malignant astrocytoma in the males is 5 in 4 studies, indicating at least two cases in one of the studies, the incidence was comparable to that of the 2-year rat study with benalaxyl.

—Incidence of spontaneous brain proliferative lesions in Sprague-Dawley, Han-Wistar and Wistar rats.

Findings	Incidence								
	Sprague-Dawley			Han Wistar			Wistar		
	Males (n = 335)	Females (n = 335)	Total (n = 670)	Males (n = 796)	Females (n = 796)	Total (n = 1592)	Males (n = 150)	Females (n = 150)	Total (n = 300)
Glial tumors									
Astrocytoma, malignant, low grade	1 (0.30%)	0	1 (0.15%)	0	1 (0.13%)	1 (0.06%)	1 (0.67%)	0	1 (0.33%)
Astrocytoma, malignant, high grade	4 (1.19%)	4 (1.19%)	8 (1.19%)	11 (1.38%)	1 (0.13%)	12 (0.75%)	0	0	0
Oligodendroglioma, malignant, high grade	1 (0.30%)	0	1 (0.15%)	1 (0.13%)	1 (0.13%)	2 (0.13%)	0	0	0
Glioma, mixed, malignant, high grade	0	1 (0.30%)	1 (0.15%)	0	0	0	0	0	0
Neuronal tumors									
Medulloblastoma	0	1 (0.30%)	1 (0.15%)	0	0	0	0	0	0
Meningeal tumors									
Granular cell tumor, benign	1 (0.30%)	0	1 (0.15%)	17 (2.14%)	6 (0.75%)	23 (1.45%)	2 (1.33%)	0	2 (0.67%)
Granular cell tumor, malignant	1 (0.30%)	0	1 (0.15%)	1 (0.13%)	0	1 (0.06%)	1 (0.67%)	0	1 (0.33%)
Meningioma, malignant	0	0	0	1 (0.13%)	0	1 (0.06%)	0	1 (0.67%)	1 (0.33%)
Nerve sheath tumors^a									
Schwannoma, malignant	1 (0.30%)	0	1 (0.15%)	1 (0.13%)	0	1 (0.06%)	0	0	0
Uncertain origin									
Reticulosis, malignant	0	0	0	0	0	0	1 (0.67%)	0	1 (0.33%)

^aTumors originating from the intracranial part of cranial nerves.

D. Baldrick (2005): Carcinogenicity Evaluation: Comparison of Tumor Data from Dual Control Groups in the Sprague–Dawley Rat, Toxicologic Pathology 33:283-291 <https://journals.sagepub.com/doi/pdf/10.1080/019262390908371>

This study summarized results of 13 rat carcinogenicity studies, performed between 1991 and 2002, each with 2 control groups and shows a high spontaneous incidence of astrocytoma in both control groups, 4% and 5% respectively.

TABLE 4.—Summary of major organ or common spontaneous neoplasms.

Body system	Neoplasm	Control I males		Control II males		Control I females		Control II females	
		Number (%)	% Range	Number (%)	% Range	Number (%)	% Range	Number (%)	% Range
Nervous Brain	Meningioma (B)	8(1.7)	0-3.3	6(1.3)	0-3.3	3(0.63)	0-5.0	3(0.64)	0-3.1
	Granular cell meningioma (B)	4(1.0)	0-3.3	5(1.3)	0-5.1	1(0.25)	0-2.0	3(0.77)	0-3.1
	Astrocytoma (M)	10(1.5)	0-4.0	11(1.6)	0-5.0	8(1.2)	0-5.0	3(0.44)	0-2.0
Urinary Renal	Tubular cell adenoma (B)	2(0.40)	0-1.7	1(0.20)	0-1.7	1(0.20)	0-2.0	0(0)	0
	Lipoma (B)	2(0.40)	0-1.7	1(0.20)	0-1.7	1(0.20)	0-1.7	2(0.40)	0-1.7
Cardiovascular Cardiac	Endocardial schwannoma (B)	0(0)	0	6(1.7)	0-3.3	0(0)	0	2(0.56)	0-1.7
	Endocardial schwannoma (M)	2(0.57)	0-1.7	0(0)	0	0(0)	0	1(0.28)	0-1.6
Respiratory	Bronchiolo-alveolar adenoma (B)	3(0.72)	0-2.0	0(0)	0	0(0)	0	2(0.48)	0-2.0
	Bronchiolo-alveolar carcinoma (M)	0(0)	0	3(0.75)	0-2.0	0(0)	0	1(0.25)	0-1.7

B, Benign; M, Malignant.

^aSee Table 5.

^bOnly reported in a limited number of studies in a few animals (which gave an artificially high upper range value).

E. Gopinath (1986): Spontaneous brain tumors in Sprague-Dawley rats, Food Chem. Toxic. Vol. 24, No. 2, pp. 113-120

<https://www.sciencedirect.com/science/article/abs/pii/0278691586903455>

In this paper, it is stated that astrocytoma is the most common brain tumor in rats. The incidence range observed among the studies varied from 0/100 to 3/60 in male and 0/100 to 2/50 in female rats, which is up to 5% in males and 4% in females.

Incidence of brain tumours among untreated control rats and among control and treated groups combined

Type of brain tumour	No. of brains examined . . .	No. of affected rats in:			
		Control groups only		Control and treated groups	
		Males	Females	Males	Females
		2630	2765	9490	9761
Glial tumours					
Astrocytoma		28	18	117	51
Oligodendrocytoma		1	1	4	7
Ependymoma		1	0	3	1
Meningeal tumours					
Meningioma		21*	9†	67	29
Malignant meningioma		3	0	6	0
Meningeal fibrosarcoma		2	0	4	0
Others					
Polymorphic sarcoma		0	1	0	1
Haemangioma		2	0	3	0
Choroid plexus lipoma		1	0	1	0
Pineoblastoma		—	—	2	0
Lymphosarcoma		—	—	0	1
	Total . . .	59	29	207	90

*Including eight granulocytic tumours.

†Including four granulocytic tumours.

In addition, the ages at death of rats bearing astrocytoma were recorded and the distribution showed that astrocytoma is a lesion of older rats. In the 2-year study with benalaxyl, the 4 male rats with astrocytoma were animals that survived to the end of the study.

Distribution of rats bearing astrocytomas or meningiomas according to age at death

Age at death (wk)	No. of rats* with:				Rats examined in given interval (% of total)	
	Astrocytoma		Meningioma		Males	Females
	Males	Females	Males	Females		
0-26	0	0	0	0	<1.0	<1.0
27-52	1	1	0	0	2.5	1.5
53-78	7	0	1	2	13.0	16.0
79-104	11	10	13	1	49.0	51.0
105+	9	7	7	6	35.0	31.0

*From totals of 2630 males and 2765 females examined.

- F. Solleveld, et al. (1986) Brain Tumors in Man and Animals: Report of a Workshop, Environmental Health Perspectives Vol.68, pp. 155-173**
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1474266/pdf/envhper00439-0150.pdf>

The publication summarizes the outcome of a National Toxicology Program conference that reported the incidence of astrocytoma in female SD rats at 1.3%. No information on male SD rats was provided. It was stated that brain tumors in rats are much more common than in mice. Data derived from lifetime studies show incidences of brain tumors up to 7.1% in Wistar AF/Han-EMD rats.

Brain tumor incidence and most common tumor type in various rat strains: lifetime studies.

Strain	Incidence (%) in		Most common tumor type	Reference
	Males	Females		
WAG/RIJ	2.4	3.9	Astrocytoma	Burek, 1978 (48)
AF/Han-EMD	7.1	3.3	Granular cell tumor	Sumi et al., 1976 (49)
ACI/seg HapBR	2.7	NS ¹	Granular cell tumor	Ward et al., 1983 (29)
BN/BIRLJ	2.7	5.9	Granular cell tumor	Burek, 1978 (48)
Osborne-Mendel	NS ^a	1.5	Ependymoma	Dagle et al., 1979 (50)
Sprague-Dawley	NS ^a	1.3	Astrocytoma	Dagle et al., 1979 (50)
F344	0.0	NS ^a		Coleman et al., 1977 (51)
F344/N	2.9	2.2	Astrocytoma	Solleveld et al., 1984 (52)

^a NS = not studied.

FMC agrees with the DS that the urinary bladder tumours found in 3 high dose males in the mouse oncogenicity study are not relevant to human health risk assessment. The 3 urinary bladder tumours were first considered as “transitional cell carcinoma” by the study pathologist, but a subsequent pathology working group (PWG) determined that the correct diagnosis was “submucosal mesenchymal tumour”, a lesion of non-epithelial origin, unique to the mouse urinary bladder and with no counterpart in any other species including humans. Therefore, these tumors were considered irrelevant to human risk assessment.

Specific target organ toxicity – single exposure

The DS proposed STOT-SE Category 2 for benalaxyl based on the acute neurotoxicity and range-finding acute neurotoxicity studies. It was stated in the CLH report that *“Although clinical effects observed after short term exposure were without histopathological correlations and a high mortality in the acute toxicity study, presumably caused by the neurotoxic effects. According to the CLP criteria mortalities observed within 72 hours after the first treatment can be considered an acute effect.”*

FMC does not necessarily agree that the mortality observed in the acute neurotoxicity study is specifically due to neurotoxicity. Findings in the acute neurotoxicity study are inconsistent with results from other studies where benalaxyl was administered via oral gavage. Further, there is no evidence of neurotoxicity in a 90-day subchronic neurotoxicity study. However, based on clinical and behavioural findings in the acute neurotoxicity study, FMC agrees to the proposal to classify benalaxyl STOT-SE Category 2.

Evaluation of Environmental Hazards

FMC agrees with the conclusions of retaining the classification and labelling of benalaxyl for environmental hazards – Aquatic acute and chronic toxicity Category 4 (H400 and H410).