Section A6.8.1 Teratogenicity Study

Annex Point IIA6.8.1

Developmental toxicity in the mouse

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] *(1992)* *Concurrent ingestion of lactate and aluminium can result in developmental toxicity in mice* *Res. Commun. Chem. Pathol. Pharmacol. Vol. 77, No 1, pp. 95-108* *Not GLP, Published*	
1.2	**Data protection**	No	
1.2.1	Data owner	*Purac Biochem*	X
1.2.2	Companies with letter of access	*Not applicable*	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No, not common to report in literature	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	*Aluminium hydroxide ($Al(OH)_3$) ; lactic acid; aluminium lactate.*	
3.1.1	Lot/Batch number	*Not available. Suppliers were Merck (Darmstadt) and Riedel-de Haën (Seelze).*	
3.1.2	Specification	*Not given*	
3.1.2.1	Description	*Not applicable*	
3.1.2.2	Purity	*Not given*	
3.1.2.3	Stability	*No remarks.*	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	Swiss albino (CD-1)	
3.2.3	Source	Interfauna Iberica	
3.2.4	Sex	Females (dams) exposed, sires only used for producing offspring, not exposed; offspring of both sexes.	
3.2.5	Age/weight at study initiation	28-32 g	
3.2.6	Number of animals per group	*Control: 13* *$Al(OH)_3$: 11* *$Al(OH)_3$ + lactic acid: 13* *Aluminium lactate: 10* *Lactic acid: 12*	

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3.2.7	Control animals	Yes	
3.2.8	Mating period	Not specified (until gestation).	
3.3	**Administration/ Exposure**	Oral, by gavage	
3.3.1	Duration of exposure		
		mouse: day 6-15 post mating	
3.3.2	Postexposure period	3 days (dams were killed on gestational day 18).	
		Oral	
3.3.3	Type	Gavage	
3.3.4	Concentration	Gavage 57.5..... mg aluminium/kg bw	
		$Al(OH)_3$: 166 mg/kg bw Al lactate: 627 mg/kg bw $Al(OH)_3$ + lactic acid: 166 mg/kg bw + 570 mg/kg bw Lactic acid: 570 mg/kg bw	
3.3.5	Vehicle	Not mentioned. Probably water, since control group was administered distilled water.	
3.3.6	Concentration in vehicle	Not available.	
3.3.7	Total volume applied	Not available.	
3.3.8	Controls	Distilled water.	
3.4	**Examinations**		
3.4.1	Body weight	Yes	
3.4.2	Food consumption	Yes	
3.4.3	Clinical signs	Yes (liver and kidney weights)	
3.4.4	Examination of uterine content	Gravid uterine weight	
		Number of implantations,	
3.4.5	Examination of foetuses		
3.4.5.1	General	live fetuses, resorptions, dead fetuses, post-implatation loss, sex ratio, fetal body weight	
3.4.5.2	Skelet	Yes	
3.4.5.3	Soft tissue	No (except for fetal aluminium content)	X
3.5	**Further remarks**	Note that this study was not intended to investigate the developmental toxicity of lactic acid, but of aluminium, with or without organic acid complexing agent. The effect of lactic acid on the (developmental) toxicity of aluminium is enhanced absorption of Al in the GI tract – this effect has been described for a number of carboxylic acids, including but not limited to citric acid, lactic acid and ascorbic acid.	

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		4 RESULTS AND DISCUSSION	
		We will only discuss the effects of lactic acid per se here.	
4.1	**Maternal toxic Effects**	*No effects except for a slight decrease in relative liver weight. Slight reduction in food consumption during treatment was accompanied by a larger reduction in food consumption pre-treatment, and as such is concluded not to be treatment related.*	X
4.2	**Teratogenic / embryotoxic effects**	*The only effect observed for lactic acid was a delay in parietal ossification.*	X
4.3	**Other effects**	*Decrease in aluminium content of the dam brain (possibly through complexation of native aluminium).*	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	*Study was not performed to any (given) guideline, but appears to have been well conceived and carried out.*	
5.2	**Results and discussion**	*Study was conceived to investigate the developmental toxicity of aluminium and the modifying influence of lactate on aluminium toxicokinetics – apparently spurred by concern of the use of aluminium-containing antacids in combination with complexing acids (citric acid, ascorbic acid, lactic acid) by pregnant women. While it can be concluded that lactic acid enhances the uptake of aluminium and thereby 'increases' the developmental toxicity potential of aluminium, lactic acid itself is not a developmental toxicant.*	
5.3	**Conclusion**	*Not teratogenic.*	
5.3.1	LO(A)EL maternal toxic effects	*Minor, non-relevant effects observed; dose was 570 mg/kg bw*	X
5.3.2	NO(A)EL maternal toxic effects		X
5.3.3	LO(A)EL embryotoxic / teratogenic effects	*No relevant embryotoxic or teratogenic effects*	X
5.3.4	NO(A)EL embryotoxic / teratogenic effects	*570 mg/kg bw*	
5.3.5	Reliability	*1*	X
5.3.6	Deficiencies	*No*	

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/12/19
Materials and Methods	1.2.1 Not applicable , publication 3.4.5.3 One-third of the fetuses of each group was examined for visceral anomalies.
Results and discussion	4.1 There was a statistically significant treatment-related decrease in food consumption of 15 % during treatment (see CA-table 1). Since no compensation (higher food consumption than control animals) was observed during the post-treatment period and no statistically significant decrease in weight gain it can be assumed that the lactic acid given by gavage partly covered the daily energy requirement of the dams. Thus, this finding was not considered adverse. 4.2 The delay in parietal ossification (CA-Table 3) in combination with a slightly decreased foetal weight (CA-Table 2) was not considered to represent a specific substance-related effect.
Conclusion	5.3.1. LOAEL maternal effects: > 570 mg/kg bw/d 5.3.2 NOAEL maternal effects: 570 mg/kg bw/d (only dose tested) 5.3.3 LOAEL embryotoxic / teratogenic effects: > 570 mg/kg bw/d 5.3.4 NOAEL embryotoxic / teratogenic effects: 570 mg/kg bw/d (only dose tested) Lactic acid does not exhibit a teratogenic potential under the conditions tested.
Reliability	2 (reliable with restrictions, see remarks)
Acceptability	Acceptable
Remarks	Non-guideline, non-GLP, reporting lacks some detail
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.8.1 Teratogenicity Study

Annex Point IIA6.8.1 *Developmental toxicity in the mouse*

Table A6_8-1. Table for Teratogenic effects (separate data for all dosage groups)

Maternal effects

Modify if necessary and give historical data if available

Parameter	**control data**		**low dose**	**medium dose**	**high dose**	**dose-response + / -**
	historical	study				
Number of dams examined						
Clinical findings during application of test substance						
Mortality of dams *state %*						
Abortions						
Body weight gain *day 0-x, day 0-y, day x-y, day 0-end of test,*						
Food consumption						
Water consumption *if test substance is applied with drinking water*						
Pregnancies *pregnancy rate or %*						
Necropsy findings in dams dead before end of test						

Section A6.8.1 Teratogenicity Study

Annex Point IIA6.8.1 *Developmental toxicity in the mouse*

Table A6_8-2. Table for Teratogenic effects (separate data for all dosage groups)

Litter response (Caesarean section data)

Modify if necessary and give historical data if available

Parameter	**control data**		**low dose**	**medium dose**	**high dose**	**dose-response + / -**
	historical	study				
Corpora lutea *state total/number of dams*						
Implantations *state total/number of dams*						
Resorptions *state total/number of dams*						
total number of fetuses						
pre-implantation loss *state %*						
post-implantation loss *state %*						
total number of litters						
fetuses / litter						
live fetuses / litter *state ratio*						
dead fetuses / litter *state ratio*						
fetus weight (mean) *[g]*						
placenta weight (mean) *[g]*						
crown-rump length (mean) *[mm]*						
Fetal sex ratio *[state ratio m/f]*						

Section A6.8.1 Teratogenicity Study

Annex Point IIA6.8.1 *Developmental toxicity in the mouse*

Table A6_8-3. Table for Teratogenic effects (separate data for all dosage groups)

Examination of the fetuses

Modify if necessary and give historical data if available

Parameter	control data		low dose	medium dose	high dose	dose-response + / -
	historical	study				
External malformations* *[%]*						
External anomalies* *[%]*						
Skeletal malformations* *[%]*						
Skeletal anomalies* *[%]*						
Skeletal variants* *[%]*						
Visceral malformations* *[%]*						
Visceral anomalies* *[%]*						
Variants visceral* *[%]*						

CA-Table 1

TABLE 1. Body weight change and food consumption data of mice given $Al(OH)_3$, $Al(OH)_3$ and lactic acid, aluminum lactate, or lactic acid on gestation days 6-15

	Control	$Al(OH)_3$	$Al(OH)_3$ + lactic acid	Aluminum lactate	Lactic acid
Number of dams	13	11	13	10	12
Gestational body weight change (g) on days:					
0-6 (pretreatment)	3.14±1.27	3.90±1.75	4.00±1.08	4.50±1.35	3.01±1.85
6-9	2.50±1.49	1.72±1.48	1.61±1.19	0.20±1.85***	1.25±1.71
6-12	7.92±3.83	7.18±4.44	4.92±3.68*	3.51±1.50**	6.33±2.42
6-15 (treatment)	18.79±5.88	14.18±6.74	10.77±7.21**	8.00±3.49	14.25±3.01
15-18 (posttreatment)	9.00±3.82	8.00±4.56	8.76±4.56	6.51±2.87	9.83±3.81
0-18 (gestation)	30.93±6.66	26.08±10.18	23.53±5.01*	19.01±5.78***	27.08±3.04
Food consumption (g/dam) on days:					
0-6 (pretreatment)	38.23±3.92	35.33±2.58	38.00±1.73	40.00±3.74	38.60±4.78
6-9	20.73±4.03	22.00±1.54	19.56±2.92	13.55±2.11***	16.10±2.55*
6-12	40.23±3.68	41.67±1.03	37.89±3.75	28.90±2.16***	27.00±7.14**
6-15 (treatment)	58.03±4.06	60.33±1.03	57.56±4.27	48.27±4.85*	48.60±8.73
15-18 (posttreatment)	21.83±2.03	23.33±3.06	22.33±5.02	15.91±1.97*	20.90±2.02*
0-18 (gestation)	118.09±3.97	118.99±1.54	117.89±10.41	104.18±6.23**	108.10±12.55

Results are presented as means ± SD. Asterisks indicate significantly different from controls: *$P<0.05$, **$P<0.01$, ***$P<0.001$, respectively.

CA-Table 2 Reproductive and fetal data of mice given oral $Al(OH)_3$, $Al(OH)_3$ and lactic acid, aluminum lactate, or lactic acid on gestation days 6-15

	Control	$Al(OH)_3$	$Al(OH)_3$ + lactic acid	Aluminum lactate	Lactic acid
No. of litters	13	11	13	10	12
No. of implantation sites/ litter	14.83±3.01	12.70±4.27	12.15±4.46	14.70±2.16	13.92±1.67
No. of live fetuses	14.17±3.29	11.90±4.90	10.85±4.37	13.80±2.34	13.00±1.88
No. of resorptions	0.66±0.77	0.80±1.03	1.23±1.73	0.70±0.66	0.76±1.01
No. of dead fetuses	0.00±0.00	0.00±0.00	0.07±0.27	0.20±0.63	0.16±0.38
Postimplantation loss/ litter (%)	4.45±6.53	6.29±7.92	10.69±12.91	6.12±7.24	6.61±8.13
No. of litters with dead fetuses	0	0	1	1	2
Sex ratio (M/F)	0.88±0.24	0.93±0.46	0.86±0.19	0.89±0.37	0.82±0.30
Fetal body weight/ litter (g)	1.24±0.14	1.26±0.11	1.27±0.15	1.04±0.18**	1.19±0.12

Asterisks indicate significantly differents from control, **P < 0.01.

CA-Table 3 Summary incidence of malformations and variations in fetuses from dams given oral doses of $Al(OH)_3$, $Al(OH)_3$ and lactic acid, aluminum lactate, or lactic acid on gestation days 6-15

	Control	$Al(OH)_3$	$Al(OH)_3$ + lactic acid	Aluminum lactate	Lactic acid
Internal examination					
No. of fetuses (litters) examined	54 (13)	40 (11)	50 (13)	53 (10)	47 (12)
Cleft palate	0 (0)	0 (0)	0 (0)	7 (4)*	0 (0)
Skeletal examination					
No. of fetuses (litters) examined	74 (13)	55 (11)	53 (13)	52 (10)	66 (12)
Assymetrical sternebrae	3 (2)	4 (3)	9 (6)	5 (3)	8 (5)
Dorsal hyperkiphosis	0 (0)	0 (0)	0 (0)	7 (4)*	1 (1)
Parietal, delayed ossification	0 (0)	0 (0)	0 (0)	8 (5)**	10 (4)*
Sternebrae, reduced ossification	0 (0)	0 (0)	0 (0)	7 (3)	3 (1)
Total skeletal defects	3 (2)	4 (3)	9 (6)	11 (5)	17 (6)

Asterisks indicate significantly different from control: *P < 0.05, **P < 0.01, respectively. The litter was the statistical unit of comparison.