

REPORT PREPARED FOR COMMITTEE ON MUTAGENICITY OF
CHEMICALS IN FOOD CONSUMER PRODUCTS AND THE ENVIRONMENT
(COM).

ACTIVITY OF METHYLGLUCAMINE IN THE MOUSE BONE MARROW
MICRONUCLEUS ASSAY

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This report presents the view of the author only. It does not necessarily represent the views of the COM, the Department of Health or the Food Standards Agency.

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INTRODUCTION

N-Methyl D-glucamine (meglumine) is used in the treatment of leishmaniasis, particularly in dogs. Conflicting reports exist concerning the in vivo genetic toxicity of this compound in mice. For example, a significant increase in micronucleated polychromatic erythrocytes (MPEs) in mouse bone marrow 6h after the second of 2 doses (spaced 24h apart) has been reported in one study. In this study activity was observed at doses ((1g/kg and 500mg/kg) which were toxic to the mice. In contrast, a second independent study reported an absence of micronucleus induction in mouse bone marrow 24h after the second of two doses. In this study, toxicity was again observed at the top dose (600mg/kg), but not at the lower dose level (75mg/kg). The observations from both of these studies are detailed in Table 1. Both studies used a double dose regime with intraperitoneal injection (ip) as the route of exposure. In addition, there was agreement in the observation of toxicity at 600mg/kg (decreased spontaneous activity, temporary loss of coordination, hypersensitivity after the first dose). Toxicity at 500mg/kg was also noted in the second study. There were several differences between the two studies (Table 1), the most notable of which was the sampling time (6h after the last dose in the first study and 24h after the last dose in the second one). Other differences include the vehicle used for meglumine (0.25% methyl cellulose in the first study and distilled water in the second) and the mouse strain (BS1 and CD1 for studies 1 & 2 respectively).

Following consideration of the available mutagenicity data by the COM, a number of experiments were undertaken to further elucidate whether meglumine has mutagenic potential. Meglumine had been referred to the COM by the Food Standards Agency. A series of experiments are described in the following report, which were designed to determine whether meglumine is a micronucleus-inducing agent. Dose levels, route of exposure, dosing regime and sampling times were based on the observations described in Table 1. In addition, two strains of mice were used (AP and CBA). Cyclophosphamide was used as a positive control in all experiments. The results were considered by the COM at its 25 April 2002 and 10 October 2002 meetings

MATERIALS AND METHODS

Chemicals Meglumine (MEG) and cyclophosphamide (CP) were purchased from the Sigma Chemical Company (Poole, Dorset). MEG was stored at room temperature and CP was stored at +4°C until used. Both compounds were dissolved in distilled water to give the appropriate dose levels. Doses of up to 1g/10ml were prepared for MEG and a dose of 30mg/10ml was used for CP based on previously published data (Krishna et al, 1995). All dosing solutions were left at room temperature for the duration of the study.

Animals and Dosing 6-8 week old male Alpk:Ap;CD-1 mice were obtained from a barriered animal breeding unit (Astrazeneca, Macclesfield) and 6-8 week old male CBA mice were purchased from Harlan-Olac (UK). The animals were housed up to 5/cage in solid bottom cages and allowed at least 24h to

acclimatize. They were allowed Rat and Mouse No 1(RM1; Special Diet Services Ltd., Witham, Essex, UK) diet and water ad libitum for the duration of the study.

As toxicity was observed in both original studies (Table 1), dose-ranging investigations were performed on both of the mouse strains used in the experiments described herein. Thus, animals (2/group) received 2 doses of MEG at the appropriate dose level by intraperitoneal (ip) injection. The doses were spaced 24h apart and a dosing volume of 10ml/kg body weight was used. Animals were exposed to increasing doses of MEG (up to 1g/kg) in a step-wise fashion and were observed for signs of toxicity for up to a maximum of 4 days after the last injection. A top dose of 1g/kg was used in the main micronucleus studies for both mouse strains based on the absence of toxicity in the dose-ranging studies.

Three independent studies were performed to determine the micronucleus-inducing capability of MEG. In the first experiment, male AP mice were exposed to 2 ip injections of 1g/kg MEG and were sacrificed 6h and 24h after the last dose, which encompassed both the positive and negative observations of earlier reports (Table 1). A second study, using male CBA mice allowed a more rigorous investigation of the activity of MEG and observations were recorded 6h and 24h after both a single and double exposure to the compound. The final experiment attempted to repeat the positive observations of the first study (in AP mice).

Termination Animals were terminated either 6h or 24h after one or two exposures to the appropriate compound (Table 2) by an overdose of fluothane followed by cervical dislocation. Bone marrow smears were prepared, stained and analysed for the presence of MPE (among 200PE) as well as the ratio of PE:NE (based on the assessment of 100 cells) as described previously (Tinwell and Ashby 1989). Data were assessed for statistical significance using a one-sided Student t-test.

RESULTS

In contrast to earlier reports, the dose ranging experiments performed in both AP and CBA mice indicated that the animals could tolerate doses up to 1g/kg without any adverse effects. Thus, this dose level was used as the top dose in the main experiments. One AP mouse in the first main experiment exhibited symptoms similar to those described in the original experiments and was removed from study early (Table 2).

MEG was investigated in three independent experiments and these data are presented in Table 2. In the first experiment, AP mice received 2 injections of MEG (1g/kg) and were sacrificed either 6h or 24h after the last dose. As with the earlier experiments, a significant increase in the MPE population was observed at the early timepoint (6h) but not at the later time point (24h). The increase in MPE was coupled with a significant decrease in the PE/NE ratio, which, again, was not observed at the later time point. CP gave the expected significant increase in MPE at both sample times.

As it was unlikely that the positive response observed at the early time point was due to the second injection and was more likely to be in response to the first exposure, a second experiment was conducted to investigate more fully the activity of MEG. Thus, mice were exposed to one or two injections of MEG and were sacrificed 6h or 24h after the first or second dose (Table 2). CBA mice, rather than AP mice, were used in this experiment, as the control MPE levels in AP mice were unusually high. In contrast to Experiment 1, a significant increase in the MPE population was not observed at any of the timepoints (Table 2). However, a significant reduction in PE/NE ratio was observed 6h after 2 doses MEG (1g/kg) similar to that observed in Experiment 1. The positive control, CP, gave the expected responses 24h after the first injection as well as at both time points following 2 exposures. The inactivity of CP 6h after a single dose confirmed that the two dose protocol in which termination is 6h after the second dose is based on practice and not data.

The disparity between Experiments 1 and 2 led to the conduct of a third study in which AP mice were exposed to 2 doses of MEG and sacrificed 6h after the last dose (as in our first positive repeat study). In this experiment (Table 2), a marginal positive response ($p < 0.05$) was observed following exposure to 500mg/kg MEG. However, the significant increase in MPE observed in Experiment 1 following exposure to 1g/kg could not be repeated although a significant reduction in the PE/NE ratio was again observed. CP gave the usual positive response.

DISCUSSION

The activity of MEG as an *in vivo* genotoxin is still unclear, despite the extensive investigations described herein. Thus, our original positive response, as was originally reported by others, was observed in AP mice 6h after 2 injections of 1g/kg MEG (Expt 1, Table 2). However, this effect could neither be repeated in an independent study using the same mouse strain (Expt 3, Table 2), nor in a second mouse strain (Expt 2, Table 2). However, three aspects of the data described herein are worthy of noting. First, there was a wide variation in response to MEG, particularly in the AP mice, with some animals clearly responding to this compound whilst others had only control-like MPE levels. For example the range of MPE values 6h after 2 doses of 1g/kg MEG was 5-16.5 in Experiment 1 and 4-15 in Experiment 3. Closer inspection of the original positive study (see table 1) indicated similar occurrences (MPE levels ranged from 18-50 for 1g/kg and 14-71 for 500mg/kg with a mean control value of 23 ± 5.8). Unfortunately, no such individual animal data were available for the original negative study (see table 1). Second, although MEG was marginally toxic to the bone marrow, as evidenced by a significant reduction in the PE/NE ratio (Expts 1-3, Table 2), there was an overall absence of clinical signs of toxicity (one animal in Expt 1 exhibited severe toxicity). This was somewhat surprising given that both of the earlier studies (using two different strains of mouse) reported severe signs of toxicity at similar and lower doses to those used here (Table 1). Thus, one possibility is that any genotoxic response to MEG may be highly dependent on its overt toxicity to the individual animal, but this is not an invariable or established correlation. This observation has been previously

observed for the liver carcinogen DMN (Morrison and Ashby 1994). In that report, DMN was clearly positive at lethal doses but at non-lethal doses it was, at best, only marginally active. Finally, the possibility that the activity of MEG, observed 6h after a second injection only, was due to the final injection is unfounded, as cell cycle kinetics would be unable to sustain such an immediate response. The absence of a response 6h after a single injection of CP further supports this argument.

In conclusion, MEG remains of uncertain genetic toxicity. Its chemical structure does not suggest genetic toxicity apart from the low chance of formaldehyde formation from the N-Me group. Given the totality of the available data it cannot be concluded that this agent presents a potential carcinogenic or mutagenic hazard to humans. Equally, it cannot be described as being without genetic toxicity.

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Study	Mouse Strain	Dose Regimen		Compound	Vehicle	Dose Ranging			Main Study		
		# Doses ^A	Sample Time ^B			Dose (/kg)	# Animals	Comments	Dose (/kg)	# Animals	Comments
1	BS1	2	6h	N-methyl D-glucamine	0.25% methyl cellulose	5g 2.5g 1.75g 1g 600mg 300mg	6 6 6 6 6 6	Death Death Death Toxicity but no deaths Toxicity but no deaths No toxicity	1g 500mg	8 8	Positive at both doses. Positive in repeat 1g/kg. 1000PE/animal analysed
2	CD-1	2	24h & 48h	N-methyl D-glucamine	Distilled water	700mg 600mg 500mg 300mg	14 mice Used in Total	Toxicity Toxicity; 1 death Toxicity No toxicity	600mg 75mg	8 8	Negative at both doses. 500PE/animal analysed

Table 1. Data provided from previous studies. A: animals received 2 ip injections spaced 24h apart. B: Sample time refers to time after the final dose.

Expt. No (Mouse Strain)	Dose (/kg)	No. of Doses	Sample Time ^A	No. of Animals	MPE/1000PE based on 2000PE assessed		PE/NE ± SD
					Individual animal data	Group Mean ± SD	
1 (AP)	DW	2	6h	9	4, 7.5, 6.5, 4, 3.5, 4.5, 4, 5.5, 5	4.9 ± 1.3	0.9 ± 0.1
	MEG (1g)	2		10	14.5, 9.5, 6.5, 12, 10, 5, 10, 16.5, 6.5, 15 ^C	10.6 ± 3.9**	0.7 ± 0.2**
	CP (30mg)	2		5	50, 47.5, 54, 68.5, 37	51.4 ± 11.4**	0.9 ± 0.2
	DW	2	24h	9	3.5, 5.5, 3, 8, 2, 4, 3, 4.5, 6.5	4.4 ± 1.9	0.9 ± 0.2
	MEG (1g)	2		8	3.5, 5.5, 5.5, 3, 5, 5, 5, 4.5	4.6 ± 0.9	0.8 ± 0.1
	CP (30mg)	2		5	47.5, 61.5, 58, 52, 51	54.0 ± 5.6**	0.6 ± 0.2*
2 (CBA)	DW	1	6h	8	1.5, 2.5, 1.5, 3.5, 2, 2.5, 2.5, 2	2.3 ± 0.7	1.1 ± 0.2
	MEG (1g)	1		8	4, 5.5, 3.5, 4.5, 5, 1, 1.5, 2	3.4 ± 1.7	1.1 ± 0.1
	CP (30mg)	1		8	5, 3, 2.5, 3.5, 2.5, 3, 2, 4	3.2 ± 1.0	1.2 ± 0.1
	DW	1	24h	9	3, 2, 3, 3, 2.5, 2.5, 2, 2, 2.5	2.5 ± 0.4	1.4 ± 0.2
	MEG (1g)	1		9	2, 2.5, 2, 4.5, 0.5, 5, 3, 3, 4	2.9 ± 1.4	1.2 ± 0.1
	CP (30mg)	1		3	29, 27, 37.5	31.2 ± 5.6**	1.1 ± 0.1
	DW	2	6h	9	3, 2.5, 4.5, 2, 7, 3, 2.5, 3, 2	3.3 ± 1.6	1.2 ± 0.1
	MEG (500mg)	2		9	2, 2, 2.5, 6, 1, 2.5, 2, 2, 3	2.6 ± 1.4	1.1 ± 0.2
	MEG (1g)	2		9	4.5, 5, 3.5, 1, 2.5, 5, 1, 5, 2.5	3.3 ± 1.7	0.9 ± 0.3**
	CP (30mg)	2	3	46, 28, 47	40.3 ± 10.7**	1.0 ± 0.1	
	DW	2	24h	9	1, 1.5, 4, 3.5, 6.5, 3, 1.5, 4, 4	3.2 ± 1.7	1.2 ± 0.1
	MEG (1g)	2		7 ^D	2.5, 1.5, 3.5, 3.5, 4.5, 1.5, 3	2.9 ± 1.1	1.1 ± 0.3
	CP (30mg)	2		3	47.5, 47, 34.5	43 ± 7.4**	0.8 ± 0.2**
3 (AP)	DW	2	6h	9	6.5, 7.5, 6, 8, 5, 7.5, 1.5, 5.5, 5.5	5.9 ± 1.9	1.0 ± 0.2
	MEG (500mg)	2		9	7, 12.5, 7.5, 6.5, 11, 2.5, 5.5, 11.5, 12	8.4 ± 3.5*	1.0 ± 0.2
	MEG (1g)	2		9	5.5, 15, 9, 8.5, 4, 9, 4.5, 5, 5	7.3 ± 3.5	0.7 ± 0.3*
	CP (30mg)	2		3	27, 44, 42	37.7 ± 9.3**	0.7 ± 0.1*

Table 2. Activity of meglumine in the bone marrow of male mice. A: Sample time is time after last injection. B: numbers in parentheses refer to the number of animals whose slides have been scored. C: This animal showed extreme signs of toxicity (hunched, piloerection, cold to touch); this was reflected in the PE/NE ratio of 0.31. D: one animal from this group was found dead in cage and a

second was removed from study due to toxicity (both cases were misdosed). Data assessed for statistical significance using a one-sided Student t-test; *: $p < 0.05$; **: $p < 0.01$.