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General Electric Company
One Plastics Avenue, Pittsfield, MA 01201-3697
413-448-7110

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The enclosed manuscript titled *Uterotrophic Activity of Bisphenol-A to the Immature Rat* by J. Ashby and H. Tinwell is submitted (FYI) to the US Environmental Protection Agency as supplemental to 8EHQ-0296-13581 identified in TSCATS by Accession Number 57803, Record Number 444262.

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GE Plastics
One Plastics Avenue
Pittsfield MA 01201



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Sincerely,

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UTEROTROPHIC ACTIVITY OF BISPHENOL-A TO THE IMMATURE RAT

by

J Ashby* and H Tinwell

Zeneca Central Toxicology Laboratory
Alderley Park, Cheshire SK10 4TJ, UK

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* Correspondence to fax (0) (44) 1538388266
BPA-EHP.doc
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SUMMARY

Bisphenol-A (BPA) is shown to be active in immature AP rat uterotrophic assays when evaluated using either the oral or the subcutaneous (sc) injection routes of exposure (three daily administrations of 400-800 mg/kg BPA). Premature vaginal opening was seen for eight of fourteen animals exposed to 600 and 800 mg/kg BPA by sc injection. Vaginal opening was not produced by BPA in the gavage studies. These results are consistent with those of Dodds and Lawson (1936) who found that BPA induces persistent vaginal cornification in ovariectomised rats exposed to three twice daily injections of ~85 mg/kg BPA (total daily dose ~170 mg/kg) but they conflict with the reported inactivity of BPA in the immature mouse uterotrophic assay. The uterotrophic activity of DES in the rat is also established (0.04 mg/kg/day for three days).

INTRODUCTION

Despite extensive interest in the endocrine toxicity of bisphenol-A (BPA) to mammals (1-5), only one report of its activity in the rodent uterotrophic assay exists (4). In that study Coldham et al (4) exposed immature (18 day old) CFLP mice to 0.05, 0.5 and 5 mg BPA per mouse by subcutaneous (sc) injection on three successive days. The 5 mg dose of BPA was toxic and the treated mice were withdrawn from the study. No uterotrophic activity was observed for the two lower dose levels, and it was concluded that BPA was inactive in the mouse uterotrophic assay. This conclusion is important given that reliance is currently placed on the rat/mouse uterotrophic assay as a primary screen for estrogens. We therefore decided to evaluate this chemical in the immature rat uterotrophic assay with a view to establishing if it is inactive in this assay using both species of rodent.

MATERIALS AND METHODS

Chemicals. Bisphenol-A (BPA) was obtained from Aldrich Chemical Company and diethylstilboestrol (DES) from Sigma chemical Company. The vehicle, arachis oil, was as previously described (6).

Animals. Immature female Alpk:AP rats (21-22 days old) with body weights in the range 38-48g were obtained from the breeding unit at Zeneca, Alderley Park. Animals were housed in wire mesh cages with solid bottoms. Humidity was controlled and a 12h/12h light dark cycle was maintained. Animals were weaned on R&M No.3 diet at 19-21 days old and maintained on R&M No.3 diet from 21 days onwards (both diets obtained from Special Diet Services Ltd, Witham, Essex, UK). Diet and water were available *ad libitum*. All animals were acclimatised for 24h before being dosed.

Uterotrophic assays. The uterotrophic activities of BPA and DES were evaluated in three separate experiments using the test protocol described earlier (6). The test agents were dissolved (DES) or homogeneously suspended (6) (BPA) in arachis oil and dosed by either oral gavage or subcutaneous (sc) injection. The dosing volume for both routes of exposure was 5ml/kg body weight. Animals received 3 daily doses of the test compound and were killed by an overdose of Fluothane™ (Zeneca Pharmaceuticals) 24h after the final dose. The dose levels shown in Table 1 are the daily dose levels.

Vaginal opening (or otherwise) was recorded at the time of death. Uteri were excised, trimmed free of fat, pierced and blotted to remove excess fluid. The body of the uterus was cut just above its junction with the cervix and at the junction of the uterine horns with the ovaries. The uterus was then weighed (wet weight). Uterine dry weight was also determined by drying the uteri at 70° for 24h before re-weighing (6). DES was used as positive control agent and each study was accompanied by a vehicle (negative) control group. Animal group sizes are shown in Table 1. Changes in the weight of wet and dry uteri were assessed for statistical significance using a 2-sided Student-t test.

RESULTS AND DISCUSSION

BPA gave a positive uterotrophic assay response when administered to rats by either oral gavage or sc injection (Table 1). The magnitude of these responses was similar for each route of administration and increases in both wet and dry uterus weights were observed. Prematurely opened vaginas were observed for some of the animals in the top two BPA dose level groups (sc injection), an event not encountered to date among over 450 control animals (using either route of exposure; data not shown). The present data for BPA are consistent with the report by Dodds and Lawson (1) that it induces persistent estrus in ovariectomized rats. In those studies a total dose of 100 mg of BPA was administered per rat, as twice daily subcutaneous injections for 3 days (assuming the rats weighed ~200g, the daily dose of BPA was ~170 mg/kg).

The positive control agent for the present experiments, DES, gave a positive uterotrophic response using both routes of administration, and vaginal opening was observed for some DES animals in each experiment conducted (Table 1). This activity of DES in the rat uterotrophic assay is consistent with its similar activity in the mouse uterotrophic assay (7).

The top dose levels of BPA used in the present experiments gave no clinical signs of toxicity, or effects on body weight, and higher dose levels could clearly have been administered. A detailed dose-response relationship for the activity of BPA in the rat uterotrophic assay was not established as part of the present experiments because their purpose was to evaluate the qualitative activity of this chemical in the rat uterotrophic assay. The corresponding lack of activity reported for BPA in the immature CFLP mouse uterotrophic assay (4) is probably related to its higher toxicity when subcutaneously injected into mice, as compared to into rats. Thus, Coldham et al (4) only evaluated two non-toxic dose levels of BPA (~3 and 33 mg/kg BPA), the highest (toxic) dose evaluated (330 mg/kg) being below the lowest dose level used in the present rat experiments.

These data establish the uterotrophic activity of BPA to the rat. This information is relevant to the use of the uterotrophic assay as a primary screen for endocrine disrupting chemicals (8). However, the present data were generated at much higher dose levels than those used to establish other endocrine toxicities of BPA, in particular, its effect on the developing prostate gland of male CF1 mice (3). Given the many variables between the present data and the CF1 mouse data (3) it is not possible to relate the two experimental outcomes quantitatively.

The present data for BPA and DES have confirmed (8) that premature vaginal opening in the immature rat is independent of activity in the uterotrophic assay. In particular, the similar uterotrophic activity of BPA using the sc injection and oral gavage routes of exposure is not reflected by the vaginal opening data, where only the sc injection route was effective. This route-specific response may be due to pharmacodynamic differences of BPA between different tissues of the rat when using these different routes of exposure.

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Study No.	Compound	Daily Dose (mg/kg)	Route	No. animals	No. with open vaginas	Uterus Weight (mg) ± SD	
						Wet	Dry
1	Arachis Oil	5ml/kg	p.o.	10	0	27.7 ± 7	5.0 ± 1.0
	DES	0.04		5	2	113.2 ± 10.6**	20.0 ± 1.5**
	BPA	400		7	0	36.4 ± 8.9*	7.0 ± 1.0**
	BPA	600		7	0	38.1 ± 5.8**	7.0 ± 1.0**
	Arachis Oil	5ml/kg		10	0	30.3 ± 5.03	6.0 ± 1.0
	DES	0.04		5	1	125.0 ± 13.2**	21.0 ± 2.0**
2	BPA	400	s.c.	7	0	45.3 ± 8.4**	9.0 ± 2.0**
	BPA	600		7	4	51.9 ± 10.7**	10.0 ± 2.0**
	Arachis Oil	5ml/kg		10	0	25.4 ± 4.05	Not taken
	BPA	800		8	3	54.5 ± 15.0**	Not taken
	Arachis Oil	5ml/kg		10	0	29.1 ± 5.2	4.0 ± 1.0
	BPA	800		10	0	105.4 ± 8.8**	17.0 ± 1.4**
3	DES	0.04	p.o.	5	4	63.4 ± 12.8**	10.0 ± 2.0**
	BPA	800		7	0	31.9 ± 5.6	6.0 ± 1.0
	Arachis Oil	5ml/kg		10	0	116.4 ± 15.1**	19.0 ± 1.7**
	DES	0.04		5	2	64.9 ± 12.0**	12.0 ± 2.2**
	Arachis Oil	5ml/kg		10	0	31.9 ± 5.6	6.0 ± 1.0
	BPA	800		7	4	64.9 ± 12.0**	12.0 ± 2.2**

Table 1. Activity of bisphenol A (BPA) in immature rat uterotrophic assays. Animals were exposed to the appropriate compound once daily for three consecutive days either via oral gavage (p.o.) or subcutaneous injection (s.c.). Diethylstilbestrol (DES) was used as positive control agent. * : p<0.05; ** : p<0.01.