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Procter & Gamble

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The Procter & Gamble Company
Ivorydale Technical Center
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ORIGINAL

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(Attention: Section 8(e) Coordinator)
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, S. W.
Washington, D. C. 20460



INIT 09/28/95

Contains No CBI

RE: TSCA Section 8(e) Submission for N-Ethylnitrosourea

ATTN: TSCA Section 8(e) Coordinator

This submission is made in accordance with TSCA Section 8(e) requirements and discharges any TSCA Section 8(e) responsibilities that exist for our Company regarding the information described herein.

This submission provides information from an in vivo micronucleus assay in peripheral blood of transgenic mice with N-Ethylnitrosourea (CAS # 759-73-9), a known carcinogen and mutagen. The testing of this material was solely for basic research to establish the predictive ability and utility of this test method. The test compounds induced changes which are indicative of a mutagen.

We have handled and will continue to handle these materials with appropriate caution in keeping with our standard practice for handling all chemical substances.

If you wish further information, please contact me.

Very truly yours,

THE PROCTER AND GAMBLE COMPANY

W. E. Bishop, Ph. D.
Manager Risk, Policy & Regulatory Sciences
Telephone: 513/627-6145



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MATERIALS AND METHODS

Animals

Male B6C3F1 Big Blue transgenic mice were obtained from Stratagene (La Jolla, CA). The mice were housed individually in cages on hardwood chip bedding. A 12 hour light/dark cycle was maintained and Purina Lab Chow and water were available *ad libitum*.

Animal Treatments

ENU was administered by a single intraperitoneal injection.

Analysis of Micronuclei

Preparation of AO-coated slides Acridine orange (AO; Sigma Chemical, St. Louis, MO; 1 mg/ml) was dissolved in distilled water. Microscope slides were heated to 70 C then a 10 ul aliquot of AO solution was placed on a warm slide and spread by rolling a glass rod back and forth across the slide. Alternatively, slides were prepared by placing 20 ul of AO solution on one slide, then another slide was placed upside down on the AO solution and pulled in the opposite direction; this makes two well spread slides. The slides were air dried and stored in a closed slide box until they were analyzed.

Peripheral blood smears Peripheral blood was obtained by carefully slicing the tip of the tail using a sharp scalpel. When the tail began to bleed one drop was placed in the center area of the slide, a 20 x 50 coverslip was placed on the drop of blood, and pressure was applied to the coverslip to spread the blood evenly. The slides were placed in a slide box inside a plastic bag and stored in the refrigerator until they were analyzed.

Fluorescence microscopy The slides were scored under fluorescence using a blue (450-490 nm) excitation filter and a yellow to orange (520nm) long pass barrier filter. This combination resulted in normal chromatic cells (NCE) that appear dark green, reticulum in the reticulocytes appear red and the micronuclei, when present, appear yellow to green. Cells were scored at 250- 1000X magnification.

Scoring MN Micronuclei were scored in intact reticulocytes. The percent micronuclei refers to the number of micronuclei in intact reticulocytes per total number of intact reticulocytes. The percent reticulocytes out of total red cells was used as an indicator of toxicity.

RESULTS

Analysis of Micronuclei in Peripheral Blood

Treatment	Exposure	Dose (mg/kg)	Micronuclei/RETs	% MnRETs	% RET/Total Cells
ENU	single i.p. dose	0	8/5354	0.15	4.96
	"	40	146/4514	3.2*	2.98

* Significant $p < 0.05$ (Kastenbaum et al. 1970)

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