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Newark, Delaware 19714-0050

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March 19, 1993

DUPONT CENTRAL RESEARCH AND DEVELOPMENT

**EXPRESS MAIL - RETURN RECEIPT REQUESTED**



FYI-93-000878  
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Document Processing Center (TS-790)  
Attention: FYI Coordinator  
Office of Pollution Prevention and Toxics  
U.S. Environmental Protection Agency  
401 M Street SW  
Washington, DC 20460



84930000016

Dear Coordinator:

**O-NITROTOLUENE (CAS# 88-72-2)**

This letter is to inform you of preliminary information concerning a supplementary toxicity study on o-nitrotoluene (CAS No. 88-72-2) and o-toluidine hydrochloride (CAS No. 636-21-5) conducted by the National Toxicology Program. This information was provided to DuPont in the form of a draft study report abstract, the contents and conclusions of which have not been reviewed by the NTP staff nor are they available to DuPont. A copy of the draft abstract is attached.

Groups of rats were fed control diets or diets containing the test compounds at a concentration of 5000 ppm. Additional groups of rats with reduced gut microflora were fed control or test diets containing o-nitrotoluene at the same concentration. Diets containing the test compounds were administered for 13 weeks or for 13 weeks followed by a 13 week period on control diet.

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Treatment-related changes included those observed in a previous 90-day feeding study with o-nitrotoluene conducted by NTP, and those observed in studies reported in the literature with o-toluidine hydrochloride. In addition to those effects, proliferative hepatic lesions were observed in male rats fed the diets containing o-nitrotoluene that appeared to progress as cholangiocarcinomas during the 13-week compound-free period.

The draft abstract does not contain incidence data for the finding of cholangiocarcinoma. Furthermore, the report has not been reviewed completely by NTP and is subject to modification. For these reasons it is not possible for DuPont to render an independent judgment of the significance of these findings with respect to human health risk.

Sincerely,

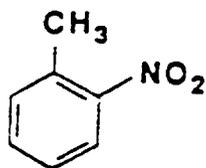
*Charles F. Reinhardt*

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Director

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11/11/93

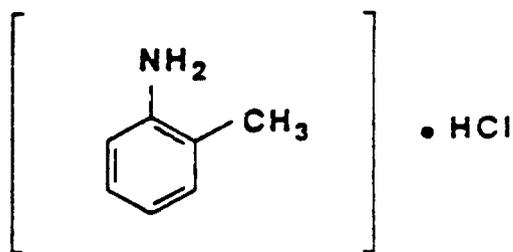
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*o*-NitrotolueneMolecular Formula: C<sub>7</sub>H<sub>7</sub>NO<sub>2</sub>

CAS Number: 88-72-2

Molecular Weight: 137.13

Synonyms: 2-nitrotoluene,  
2-, *o*-methylnitrobenzene, nitrotoluol,  
nitrophenylmethane

*o*-Toluidine HydrochlorideMolecular Formula: C<sub>7</sub>H<sub>9</sub>N.HCl

CAS Number: 636-21-5

Molecular Weight: 143.62

Synonyms: 2-toluidine hydrochloride,  
2-, *o*-aminotoluene hydrochloride  
methylaniline

### ABSTRACT

*o*-Nitrotoluene and *o*-toluidine hydrochloride are high-production, structurally-related chemicals that are suspected and demonstrated animal carcinogens, respectively. This study was conducted in order to 1) confirm that a 13-week treatment with *o*-nitrotoluene by the dosed-feed route results in the induction of mesotheliomas in the male Fischer 344 rat, 2) study the progression of proliferative mesothelial and hepatic lesions for a period of 13 weeks after the administration of *o*-nitrotoluene has been discontinued, 3) determine the effect of an alteration in normal bacterial flora on the toxicity/tumorigenicity of *o*-nitrotoluene, and 4) compare the target organ toxicities of *o*-nitrotoluene and *o*-toluidine hydrochloride.

*o*-Nitrotoluene was administered in dosed-feed at a concentration of 5000 ppm to conventional flora male Fischer 344 rats (normal endogenous gastrointestinal tract bacterial flora) and to altered flora male Fischer 344 rats that were pretreated with an antibiotic mixture containing tetracycline hydrochloride, neomycin sulfate, and nystatin. *o*-Toluidine hydrochloride was administered in dosed-feed at a concentration of 5000 ppm to conventional flora male Fischer 344 rats only. There were also control groups for both conventional and altered flora male Fischer 344 rats that received untreated feed.

A total of 200 male Fischer 344 rats were included in the experimental design for this study. There were 20 rats in each control group (conventional and altered flora) and 60 rats in each of the

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conventional flora/*o*-nitrotoluene and *o*-toluidine hydrochloride dose groups. In addition, there were 40 rats in the altered flora/*o*-nitrotoluene dose group. Following approximately 13 weeks of treatment, 10 control rats from each of the conventional flora and altered flora groups and 20 rats each from the conventional flora/*o*-nitrotoluene, altered flora/*o*-nitrotoluene, and *o*-toluidine hydrochloride groups were terminated for interim pathology evaluation. At this point, serum was collected from 10 control rats in both the conventional and altered flora treatment groups (total of 20) and 10 rats from each *o*-nitrotoluene and *o*-toluidine hydrochloride treatment group (total of 30) and were frozen for possible chemical/metabolite analysis at a later date (tests to be performed at NIEHS). Also at the interim necropsy, cecal specimens were collected from 5 randomly selected animals in each of the conventional flora/control, altered flora/control, conventional flora/*o*-nitrotoluene, and the altered flora/*o*-nitrotoluene groups. Cecal specimens were subsequently cultured and enumerated for total microbiological count according to standard microbiological techniques.

The remaining animals in the conventional flora control group were maintained on control diet until the end of the study. Antibiotic treatment and/or chemical administration was discontinued for the 10 remaining rats in the altered flora/control group and 20 rats from each of the *o*-nitrotoluene (conventional and altered flora) and *o*-toluidine hydrochloride groups; these rats were maintained on the control diet for approximately an additional 13 weeks. The remaining 20 rats from each conventional flora treatment group were maintained on test chemical until the end of the study at approximately 26 weeks. At the interim and study terminations, animals were necropsied, and a limited number of tissues (liver, testis, epididymis, kidney, urinary bladder, spleen) were processed for histopathological evaluation.

Average daily chemical exposure indices were similar for *o*-nitrotoluene and *o*-toluidine hydrochloride and were estimated to range from 285 to 304 mg chemical/kg body weight/day. All animals survived until the scheduled interim and terminal necropsies and no treatment-related signs of toxicity were observed over the course of this study.

Irrespective of the length of the treatment regimen, status of the gastrointestinal flora (conventional or altered), or the inclusion of a recovery period, the administration of *o*-nitrotoluene and *o*-toluidine hydrochloride in the dosed-feed resulted in statistically significant depressions in in-life and terminal group mean body weights relative to respective control groups. In general, reductions in group mean body weight values were of a greater magnitude in *o*-nitrotoluene dose groups than in *o*-toluidine hydrochloride dose groups.

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The administration of *o*-nitrotoluene in the dosed-feed resulted in significant reductions in overall group mean food consumption values, irrespective of the duration of treatment, the status of gastrointestinal flora, or the inclusion of a recovery group. In contrast, overall group mean food consumption values for comparable *o*-toluidine hydrochloride treatment groups were similar to respective control group values.

As with the previous 13-week dosed-feed study of *o*-nitrotoluene sponsored by the National Toxicology Program, the administration of this chemical in the dosed-feed for a period of 13 weeks resulted in the induction of mesotheliomas in the male Fischer 344 rats that were only detected microscopically. Mesothelial hyperplasia and mesotheliomas were evident in both conventional and altered flora *o*-nitrotoluene groups; lesions were found on the epididymis at 13 weeks and on the epididymis and testes at 26 weeks. All *o*-nitrotoluene-induced mesotheliomas of the testes were interpreted to be metastatic tumors of epididymal origin.

Proliferative mesothelial and hepatic lesions appeared to progress in male Fischer 344 rats during the 13-week period after the initial 13-week exposure to *o*-nitrotoluene was discontinued. Mesothelial hyperplasia and mesotheliomas of the epididymides were found at a higher incidence and greater severity in both altered and conventional flora *o*-nitrotoluene treatment groups 13 weeks after treatment with this chemical was discontinued than in the comparable treatment groups that were administered *o*-nitrotoluene for 13 weeks and then terminated. Mesothelial hyperplasia and metastatic mesothelioma of the testes were evident in the altered flora *o*-nitrotoluene treatment group 13 weeks after chemical administration was discontinued, but not in any treatment groups at the interim termination. Proliferative hepatic lesions also progressed as cholangiocarcinomas and were evident in the conventional flora *o*-nitrotoluene group 13 weeks after chemical administration was discontinued, but were not present in any *o*-nitrotoluene animals at the interim termination.

When compared to conventional flora control groups, the administration of the antibiotic mixture did not result in any adverse clinical signs or evidence of gross or microscopic alterations. The quantitation of bacterial flora at the interim necropsy suggested that the administration of the antibiotic mixture decreased the level of intestinal flora in both control and *o*-nitrotoluene-treated rats. However, the reduction in bacterial flora did not appear to affect the toxicity or the tumorigenicity of *o*-nitrotoluene. In general, the incidence and severity of microscopic lesions observed in the liver, kidney, urinary bladder, testis, epididymis, and spleen did not appear to differ significantly between conventional and altered flora *o*-nitrotoluene groups at the interim necropsy or at study termination.

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Histopathologic lesions attributed to *o*-nitrotoluene administration were identified in all six organs examined: epididymis, testis, liver, spleen, urinary bladder, and kidney. The administration of *o*-toluidine hydrochloride resulted in the formation of lesions in all organs examined except the testis. Although the number of organs affected by the administration of *o*-nitrotoluene and *o*-toluidine hydrochloride was similar, the nature and/or severity of the lesions in these organs differed for each of these chemicals. With the exception of the kidney, chemically-induced changes in absolute and relative organ weights generally appeared to correlate with gross and microscopic alterations at the interim and/or terminal sacrifices.

The results with *o*-nitrotoluene suggest strong carcinogenic potential. Administration of this chemical resulted in tumors of the epididymis, testis, and liver. In the case of the epididymis, evidence of tumorigenicity in the form of mesothelioma was apparent by 13 weeks. Numerous mesotheliomas of the epididymis and testes (metastatic) were also apparent in *o*-nitrotoluene dose groups at study termination. *o*-Nitrotoluene administration was also associated with many mesothelial hyperplasias of the epididymis and testis. *o*-Toluidine hydrochloride resulted in only a few lesions of the mesothelium in these organs. In addition to mesotheliomas, *o*-nitrotoluene induced cholangiocarcinoma formation in the liver at study termination.

In addition to the neoplastic changes, *o*-nitrotoluene caused testicular degeneration in all animals on study. *o*-Nitrotoluene also stimulated hepatic oval cells at both interim and final termination and produced a significant hepatotoxic effect at study termination in the form of moderate vacuolar changes. *o*-Nitrotoluene probably stimulated the production of alpha 2- $\mu$  globulin as evidenced by hyaline droplet formation that was observed in the kidneys of animals exposed to chemical for 13 or 26 weeks.

*o*-Toluidine hydrochloride administration caused significant congestion, capsular fibrosis and lymphatic angiectasis in the spleen and significant urothelial hyperplasia of the urinary bladder. The administration of *o*-nitrotoluene resulted in only minor focal capsular fibrosis of the spleen and a minor urinary bladder hyperplasia. Both chemicals, however, were found to increase erythropoiesis and pigment deposition in the spleen. *o*-Toluidine hydrochloride-induced capsular-cell stimulation of the spleen and urothelial cell hyperplasia of the urinary bladder were interpreted to be preneoplastic changes.

**This report has been prepared by a laboratory that conducted these studies under the direction and support of the National Toxicology Program. The contents and conclusions have not been reviewed by the NTP staff at this time and therefore do not necessarily represent the position of the NTP.**

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