No Evidence of Toxicity or Carcinogenicity of Pentaerythritol Tetranitrate Given in the Diet to F344 Rats and B6C3F1 Mice for up to Two years

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Key words: pentaerythritol tetranitrate (PETN); vasodilator; explosive; toxicity; carcinogenicity; rats; mice.

Toxicology and carcinogenesis studies of pentaerythritol tetranitrate (PETN), an organic nitrate used in explosives and as a therapeutic agent for angina pectoris, were conducted by administering diets containing PETN,NF (National Formulary Grade, a 1:4 mixture of PETN and lactose) to both sexes of F344 rats and B6C3F1 mice in 14-day, 13-week and 2-year studies. PETN was found to be essentially non-toxic in 14-day and 13-week studies at dietary concentrations as high as $10^5$ ppm; the weight gain of female rats was lower than that of controls at 5000 and $10^5$ ppm in the 13-week study. In the 13-week studies, one in ten high-dose female rats had an adenoma of the Zymbal gland and one in ten high-dose female mice had a hepatocellular adenoma. Dietary concentrations chosen for the 2-year studies were 5000 and $10^5$ ppm for male rats and male and female mice, and 1240 and 2500 ppm for female rats. In the 2-year studies, there were no adverse effects on survival or body weight gains in either sex of rats or mice. No neoplastic or non-neoplastic lesions were considered to be related clearly to PETN administration. Neoplasms of the Zymbal gland occurred at low incidences in PETN-exposed groups of both sexes of rats in the 2-year study.

INTRODUCTION

Pentaerythritol tetranitrate (PETN; CAS no. 78-11-5), a white crystalline material first prepared by Vignon and Gerin in 1901, is an explosive used as an admixture with TNT in small caliber projectiles and grenades and finds limited use in detonating fuses, boosters and detonators. PETN is also one of a number of organic nitrates used therapeutically in the treatment of angina pectoris. For this purpose PETN is formulated with an inert ingredient, usually lactose, to decrease the potential explosion hazard.

$$\text{CH}_3\text{ONO}_2$$

$$\text{O}_2\text{NOCH}_2-\text{C}-\text{CH}_2\text{ONO}_2$$

$$\text{CH}_2\text{ONO}_2$$

McConnell et al. reviewed the experience with occupational disease and industrial hygiene in government-owned ordnance explosives plants in the USA during World War II. An apparent increased number of sudden deaths among workers was observed, but in nearly one million worker-years of exposure to the various organic nitrates, no fatalities were attributed to the aliphatic nitrates. An undetermined number of episodes of mild illness or dermatitis was attributed to exposure to PETN. Workers involved in the production of nitroglycerin and other organic nitrates that are readily absorbed through the skin suffered at times from severe headaches, dizziness and postural weakness. The risk of developing these symptoms while working with PETN is not considered to be high because of the relatively poor dermal absorption of the chemical and because it is usually processed as a wet slurry or precipitate.

Early studies to evaluate the therapeutic usefulness of PETN were performed by Takeshita, who demonstrated the blood pressure lowering ability in rabbits. Dunning reviewed the large number of subsequent studies performed from 1943 to 1969 that led to the establishment of PETN as an antianginal agent. PETN is currently prescribed to decrease the number, intensity and duration of angina attacks, and to reduce the necessity for the use of nitroglycerin in the relief of acute attacks of angina. The currently recommended dosage for adults is one 40 mg tablet four times per day, or ca. 2.3 mg kg$^{-1}$day$^{-1}$. These doses are higher than those recommended in the 1960s and early 1970s prior to recognition that degradation of the liver was sufficient to inactivate rapidly the lower doses.

DiCarlo et al. demonstrated the absorption of labeled PETN from the rat gastrointestinal tract. PETN was found to be stable in stomach acid. It binds to both plasma proteins and erythrocytes, and denitrification...
reactions (the major metabolic pathway) occur with both blood components in vitro and in subfractions from other tissues. Denitrations appear to be most rapid with the higher nitrated metabolites, resulting in accumulation of the mono- and dinitrated forms. The reaction requires reduced glutathione and the enzyme glutathione–organic nitrate reductase. Removal of one or more nitro groups allows the resulting alcohol to form glucuronide conjugates of pentaerythritol mono-, di- and trinitrate which were isolated from the bile of Wistar rats given \(^{14}\text{C}\)-labeled pentaerythritol trinitrate by intravenous injection. Pentaerythritol was determined to be the final metabolite in the rat. Crew et al. found that urinary excretion of label from \(^{14}\text{C}\)-pentaerythritol trinitrate was reduced by 60% in biliary cannulated Wistar rats compared to non-cannulated rats, suggesting that glucuronidated metabolites undergo enterohepatic circulation. Studies with mice have indicated a basic similarity in metabolism with the rat.

Pharmacokinetic studies with PETN following oral or intra-arterial administration in Sprague-Dawley rats have shown that PETN is extracted from the blood and metabolized in the blood vessel wall. Studies in humans have indicated absorption of at least 60% of an oral dose of \(^{14}\text{C}\)-PETN. Label appeared in the blood within 15 min of administration, and only the mono- and dinitrated forms were found. The predominant forms in the urine were mononitrate and the dinitrated pentaerythritol.

Information on the toxicity of PETN is scant and information on its potential carcinogenicity has not been reported in the literature. For this reason, the National Toxicology Program performed 14-day, 13-week and 2-year studies with PETN. The results of these studies form the basis of this report.

**EXPERIMENTAL**

**Chemicals**

National Formulary Grade pentaerythritol tetrinitrate (PETN,NF), a 1:4 mixture of PETN and D-lactose monohydrate, was obtained from ICI America, Inc. for the 14-day studies and from R. W. Greeff and Co. for the 13-week and 2-year studies. The identity of PETN was confirmed by infrared and nuclear resonance spectroscopy. The purity was determined to be >99% by elemental analysis, Karl Fischer water analysis and thin-layer chromatography, with specific rotation measurements to determine the lactose content (see Ref. 17). PETN was found to be stable for at least 2 weeks when mixed in feed and stored in the dark at temperatures up to 25°C. A 6% loss was detected from feed held for 2 weeks at 45°C. During the animal studies, dosed feed mixtures were held for no longer than 2 weeks at 0-5°C. More than 98% of the formulated diets analyzed during the studies were within ±10% of the target concentrations. Diets contained up to 50000 ppm of the PETN–lactose mixture (10000 ppm PETN).

**Experimental design**

Four- to six-week-old male and female F344 rats and B6C3F1 mice were obtained from Charles River Breeding Laboratories (Portage, MI) for all studies. Animals were housed five per polycarbonate cage with heat-treated hardwood chip bedding. Feed (NIH-07, Zeigler Bros., Gardeners, PA) and water were available ad libitum. After 2- to 4-week acclimation periods, animals were distributed to weight classes and then assigned to cages according to a table of random numbers. Numbers of animals per dose group and PETN concentrations in the feed are given in Table 1. Body weights and clinical signs were recorded throughout the studies. Methemoglobin concentrations were determined on blood collected from the jugular vein of all animals at the end of the 13-week study. Animals were killed with carbon dioxide, necropsies were performed and tissues were examined for gross lesions. Approximately 40 tissues per animal were preserved in 10% neutral buffered formalin, trimmed, embedded in paraffin, sectioned at a thickness of 6 μm, stained with hematoxylin and eosin and examined microscopically. Histopathological examination of tissues from the 2-year studies was performed according to an ‘inverse pyramid’ design. Other details of pathology procedures and review processes have been reported.

**Statistical analyses**

Differences in survival were analyzed by life table methods. For analyses of tumor incidence data, three procedures were used to assess dose–response trends and pairwise differences between dosed groups and controls:

(i) life table analyses (for neoplasms considered to be potentially fatal);
(ii) logistic regression test (for tumors observed in animals dying from an unrelated cause);
(iii) Fisher Exact/Cochran-Armitage Trend Analyses.

For further discussion of these statistical methods, see Haseman and NTP.

**RESULTS**

**Prechronic studies**

In 14-day studies, all rats and mice lived to the end of the studies. The final mean body weight of female
mice that received feed containing 10,000 ppm was 13% lower than that of controls; mean body weights of other groups of rats and mice were comparable to those of controls. No compound-related clinical signs or histopathological lesions were noted.

In 13-week studies, all rats and mice lived to the end of the studies. Body weights of dosed and control groups were similar except for female rats receiving diets containing 5000 or 10,000 ppm, which were 6–7% lower than those of controls at the end of the study. Kidney-to-body-weight ratios of female rats and female mice receiving diets containing 10,000 ppm PETN were marginally higher than those of controls (6–8%), but no compound-related microscopic lesions were detected in these organs. Weights of other organs (brain, liver, thymus, heart, lung) were not affected by PETN feeding. Methemoglobin levels were <1% for all groups of male and female rats and mice sampled at the end of the study. No treatment-related microscopic lesions were found in any tissue of either sex of rats or mice. An adenoma of the Zymbal gland was observed in 1/10 high-dose female rats and a hepatocellular adenoma was observed in 1/10 high-dose female mice.

Two-year studies

Rats. No differences in survival were observed for either sex of rats (final survival: males—control 22/50, low dose 29/50, high dose 29/50; females—33/50, 33/50, 31/50). Mean body weights of male rats receiving diets containing 10,000 ppm PETN were ca. 2–5% lower than those of controls during the study. Mean body weights of low-dose male rats and female rats were similar to controls. The average amount of PETN consumed per day was ca. 240 or 490 mg kg⁻¹ for males and 80 or 165 mg kg⁻¹ for females. No compound-related clinical signs were observed.

Adenomas or carcinomas of the Zymbal gland occurred in dosed male and female rats (Table 2). The Zymbal gland is a specialized auditory sebaceous gland, 3–5 mm in diameter, anterioventral to the orifice of the external ear. Carcinomas were visible grossly as ulcerated masses on the side of the head below the ear, and consisted of irregular acini and solid sheets of cells showing sebaceous and/or squamous differentiation. There was invasion of the surrounding soft tissues and ear canal. Adenomas were smaller, better circumscribed and demonstrated more uniform sebaceous differentiation than the carcinomas. Special efforts were taken to collect and microscopically evaluate the paired Zymbal glands from each animal, but because of the small size, sampling was incomplete in several groups.

Follicular cell adenomas or carcinomas of the thyroid gland occurred in female rats, with a positive trend identified by the logistic regression test (Table 2), although the incidence in the high-dose group (3/50) was not statistically different from that in controls (0/50). Mononuclear cell leukemia in male rats occurred with a negative trend; the incidence in the high-dose group was also statistically lower than that in the controls (Table 2). No non-neoplastic lesions appeared to be related to PETN.

Mice. Survival of dosed male mice was greater than that of controls: survival controls 26/49 (one animal was mis-sexed), low dose 38/50, high dose 38/50; survival of female mice did not differ among groups (38/50, 30/50, 38/50). Body weights of treated and control male and female mice were similar throughout the 2-year studies. The average daily amount of PETN consumed by male mice was ca. 810 or 1620 mg kg⁻¹ and by female mice was 1020 or 1936 mg kg⁻¹. No compound-related clinical signs were observed.

No increases in neoplastic or non-neoplastic lesions were considered to be related to the consumption of diets containing PETN by male or female mice. Negative trends were observed in the incidences of tumors of the subcutaneous tissues (fibromas,
DISCUSSION

The PETN formulation used in these studies contained lactose as a stabilizing agent (1:4, PETN to lactose), which is the typical formulation used in therapeutics. The dietary concentrations used in these studies ranged up to 5% by weight of the PETN–lactose mixture, thus the maximum PETN concentration was ca. 1% (10 000 ppm) and that of lactose was 4%. Lactose is also present in the NIH-07 diet at a normal concentration of ca. 1%.

The PETN–lactose mixture was found to be non-toxic to both rats and mice in 14-day and 13-week studies. No non-neoplastic lesions were attributed to PETN in rats or mice in the 13-week studies. No neoplastic lesions were considered to be attributed clearly to PETN in rats or mice. Neoplasms of the Zymbal gland were observed in three low- and two high-dose male rats and in one low- and three high-dose female rats. The incidences of these uncommonly occurring neoplasms in the treated groups were not statistically different compared to the concurrent controls and were within the upper range of historical control incidences for these neoplasms in both sexes (males: mean incidence 1%, range 0-8%; females: mean incidence 0.6%, range 0-6%).

The incidence of hyperplasia did not suggest an increase in proliferative lesions of the Zymbal gland. Nonetheless, the occurrence of nine neoplasms in treated rats compared to none in the controls, coupled with the observation of a Zymbal gland tumor in a high-dose female rat in the 13-week study, suggests that Zymbal gland neoplasms may be related to PETN administration.

Follicular cell adenomas or carcinomas of the thyroid gland occurred in three of the 10 000 ppm female rats; none was observed in the control or low-dose group in the 2-year study. The historical control incidence for these tumors is 0.8% at the study laboratory and 1% in the overall experience of the program. Follicular cell hyperplasia was not increased in dosed groups, nor was the incidence of follicular cell tumors increased in male rats. Although no more than two follicular cell tumors have been observed previously in any control group of female rats in other NTP studies, this marginal increase was not considered to be related to PETN administration.

Tumors of the subcutaneous tissues (primarily fibromas and fibrosarcomas) occurred with a negative trend in male mice. However, these tumors occurred in the control group at a rate nearly five times that of the mixture because higher doses resulted in a lower weight gain (−17 to −19%) than controls in the 13-week study, although final body weights in the 2-year studies were within 7% of controls.

In the 2-year studies, the only PETN-treated group to show an apparently chemical-related effect on body weight was the 10 000 ppm male rats, which were as much as 9% lighter than controls. Survival of treated male rats and male mice was higher than that of controls. No neoplastic or non-neoplastic lesions were considered to be attributed clearly to PETN in rats or mice. Neoplasms of the Zymbal gland were observed in three low- and two high-dose male rats and in one low- and three high-dose female rats. The incidences of these uncommonly occurring neoplasms in the treated groups were not statistically different compared to the concurrent controls and were within the upper range of historical control incidences for these neoplasms in both sexes (males: mean incidence 1%, range 0-8%; females: mean incidence 0.6%, range 0-6%).

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Table 3. Number of mice with selected lesions in the 2-year pentaerythritol tetranitrate studies

<table>
<thead>
<tr>
<th>Site/lesion</th>
<th>Dose (ppm)</th>
<th></th>
<th></th>
<th>Males</th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>5000</td>
<td>10 000</td>
<td>0</td>
<td>5000</td>
<td>10 000</td>
</tr>
<tr>
<td>Subcutaneous tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroma or fibrosarcoma</td>
<td>19/49*</td>
<td>4/50</td>
<td>9/50</td>
<td>0/49</td>
<td>2/50</td>
<td>1/46</td>
</tr>
<tr>
<td>Sarcoma or neurosarcoma</td>
<td>2/49</td>
<td>3/50</td>
<td>0/50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined skin tumors</td>
<td>20/49</td>
<td>7/50</td>
<td>9/50</td>
<td>0/49</td>
<td>2/50</td>
<td>1/46</td>
</tr>
<tr>
<td>Log. regres. 16</td>
<td>0.006N</td>
<td>0.002N</td>
<td>0.012N</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Denominator reflects animals examined microscopically.
16 Results of logistic regression tests; P values for the trend statistic appear under the control column; P values from pairwise comparisons appear under the respective dose group columns. N refers to a decreasing trend in tumor rates.
seen in historical control male mice (mean incidence 8.7%). The reasons for this increase are not clear, but the presence of these neoplasms accounted in part for the higher number of sacrifices of animals in moribund condition in the control group compared to the dosed groups, and was a major reason for the overall lower survival of the control group.

No reports of long-term studies with other organic nitrates were found in the literature. PETN was found to be negative in the Salmonella mutagenicity assay with and without metabolic activation and did not induce chromosomal aberrations in Chinese hamster ovary cells. PETN did produce a small increase in sister chromatid exchange in CHO cells, but the response was not clearly dose related.13 Thus, it would appear from the collected genetic toxicity and animal data that PETN has a low potential for toxic and carcinogenic activity.

Acknowledgement

These studies were performed under contract to the National Toxicology Program at EG&G Mason Research Institute, MA, USA.

REFERENCES