EVALUATION OF THE HEALTH ASPECTS OF BENZOYL PEROXIDE
AS A FOOD INGREDIENT

1980

Prepared for
Bureau of Foods
Food and Drug Administration
Department of Health and Human Services
Washington, D.C.

Contract No. FDA 223-78-2100
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Life Sciences Research Office
Federation of American Societies
for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20014
NOTICE

This report, one of a series concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior-sanctioned food substances as food ingredients, is being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-78-2100 with the Food and Drug Administration (FDA), U.S. Department of Health and Human Services. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshaling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. The Select Committee's evaluations are being made independently of FDA or any other group, governmental or nongovernmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VII.

Tentative reports are made available to the public for review in the Office of the Hearing Clerk, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the reports are approved and transmitted to FDA by the Executive Director of FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

[Signature]
Kenneth D. Fisher, Ph.D., Director
Life Sciences Research Office
FASEB
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I. INTRODUCTION

This report concerns the health aspects of using benzoyl peroxide as a food ingredient. It has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (Dailey, 1978), which summarizes the world's scientific literature from 1920 through 1978. To ensure completeness and currency as of the date of this report this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, an announcement was made in the Federal Register of April 25, 1980 (45 FR 27992-27995) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information and views on the health aspects of using benzoyl peroxide as a food ingredient. The Select Committee received no requests for such a hearing.

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the premarking clearance that is required for food additives. It is stated in the Act and in the Code of Federal Regulations (Office of the Federal Register, 1980b) [21 CFR 170.3 and 170.30] that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. These sections of the Code also indicate that expert judgment is to be based on the evaluation of results of credible toxicological testing or, for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. FDA recognizes further [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

The Select Committee on GRAS Substances of LSRO reviewed and evaluated the available information on benzoyl peroxide in full recognition of the foregoing provisions. In reaching its conclusions on safety, the Committee, in accordance with FDA's guidelines, relied primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health. This report is intended for the use of FDA in
determining the future status of this substance under the Federal Food, Drug, and Cosmetic Act. The Committee anticipates that its conclusions will be reviewed as new information becomes available.
II. BACKGROUND INFORMATION

Benzoyl peroxide \((C_6H_5CO)_2O_2\), is a colorless, odorless, tasteless, rhombic crystalline solid. The technical product melts at 103-105°C, and the recrystallized compound at 106-108°C. It is soluble in nonpolar solvents, slightly soluble in alcohol, petroleum solvents, and vegetable oils, and virtually insoluble in water (Visser't Hooft, 1948). Benzoyl peroxide may decompose explosively if subjected to excessive heat, friction, or sudden shock (NIOSH, 1977). It may also decompose spontaneously when exposed to temperatures of 75-80°C for prolonged periods. It is flammable in a dry state, but does not ignite when it contains 5% or more water. Most commercial preparations contain 15-34% water.

Benzoyl peroxide is synthesized commercially by the reaction of benzoyl chloride, sodium hydroxide, and hydrogen peroxide. Traces of benzoic acid remain after usual purification procedures. Benzoyl peroxide has been produced in the United States since 1927. It is used as a bleach for certain foods, an oxidizing agent, a polymerizing initiator in the manufacture of plastics, a curing agent for silicone rubber, a constituent of ointments for skin disorders, and an ingredient in various industrial processes (NIOSH, 1977; Visser't Hooft, 1948).

Food-grade benzoyl peroxide must assay not less than 96% of \(C_{14}H_{10}O_4\) on a dry weight basis, with limits of impurities not to exceed 3 parts per million (ppm) of arsenic, 10 ppm of lead, or 40 ppm of heavy metals expressed as lead (National Research Council, 1972).

The major use of benzoyl peroxide in the food industry is as a bleaching agent for flour. A mixture of 32% benzoyl peroxide and 68% cornstarch is used for this purpose (NIOSH, 1977). The maximum amount used as a flour bleach is about 50 ppm (Joiner, 1979). It is also used at a level of 0.002% to bleach milk in the preparation of certain cheeses. These are prior-sanctioned uses incorporated into the standards of identity for flour and cheese. Benzoyl peroxide has been accorded unpublished GRAS status as a bleaching agent for lecithin and hydroxylated lecithin (Wulfsberg, 1960). Finally, it is a regulated constituent of various materials contacting food. The uses, authorizations, and limitations of benzoyl peroxide in food are summarized in Table 1.

Studies have shown that the greater part of benzoyl peroxide added to flour decomposes into benzoic acid within a few days, although traces may persist for several weeks (Knight and Kent-Jones, 1953). Decomposition is accelerated by heating (Sharratt et al., 1964).
TABLE 1. Authorized Uses of Benzoyl Peroxide in Foods

<table>
<thead>
<tr>
<th>Product</th>
<th>Use</th>
<th>Authorization</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unpublished GRAS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lecithin</td>
<td>Bleach</td>
<td>Wulfsberg, 1960</td>
<td>Absence of residual unreacted peroxide</td>
</tr>
<tr>
<td><strong>Standards of Identity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flour*</td>
<td>Bleach</td>
<td>21 CFR 137.105 (Office of the Federal Register 1980a)</td>
<td>&quot;One part by weight...mixed with more than six parts by weight of...potassium alum, calcium sulfate, magnesium carbonate, sodium aluminum sulfate, dicalcium phosphate, tricalcium phosphate, starch, calcium carbonate ...in a quantity not more than sufficient for bleaching...</td>
</tr>
<tr>
<td><strong>Cheese†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asiago fresh and soft</td>
<td></td>
<td>21 CFR 133.102</td>
<td></td>
</tr>
<tr>
<td>Asiago medium</td>
<td></td>
<td>21 CFR 133.103</td>
<td></td>
</tr>
<tr>
<td>Asiago old</td>
<td></td>
<td>21 CFR 133.104</td>
<td></td>
</tr>
<tr>
<td>Asiago blue</td>
<td></td>
<td>21 CFR 133.106</td>
<td></td>
</tr>
<tr>
<td>Caciocavallo siciliano</td>
<td></td>
<td>21 CFR 133.111</td>
<td></td>
</tr>
<tr>
<td>Gorgonzola</td>
<td></td>
<td>21 CFR 133.141</td>
<td></td>
</tr>
<tr>
<td>Parmesan and reggiano</td>
<td></td>
<td>21 CFR 133.165</td>
<td></td>
</tr>
<tr>
<td>Provolone and pasta filata</td>
<td></td>
<td>21 CFR 133.181</td>
<td></td>
</tr>
<tr>
<td>Romano</td>
<td></td>
<td>21 CFR 133.183</td>
<td></td>
</tr>
<tr>
<td>Swiss and Emmentaler</td>
<td></td>
<td>21 CFR 133.195</td>
<td></td>
</tr>
<tr>
<td><strong>Regulated Uses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxylated lecithin</td>
<td>Oxidant</td>
<td>21 CFR 172.814 (Office of the Federal Register 1980b)</td>
<td>Separated fatty acid fraction should have acetyl values of 30 to 38</td>
</tr>
<tr>
<td>Adhesive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paper and paperboard</td>
<td>Preservative</td>
<td>21 CFR 175.105</td>
<td>In contact with aqueous and fatty acids</td>
</tr>
<tr>
<td>Polyester resins, used repeatedly</td>
<td>Catalyst</td>
<td>21 CFR 176.170</td>
<td>In contact with dry foods</td>
</tr>
<tr>
<td>Rubber articles, used repeatedly</td>
<td>Catalyst</td>
<td>21 CFR 176.180</td>
<td>Total catalyst not to exceed 1.5%</td>
</tr>
<tr>
<td></td>
<td>Accelerator</td>
<td>21 CFR 177.2420</td>
<td>Total accelerator not to exceed 1.5%</td>
</tr>
</tbody>
</table>

*Also prior-sanctioned (Cassidy, 1960; 1962).
†Also prior-sanctioned (Nicholson, 1962).
III. CONSUMER EXPOSURE

A subcommittee of the National Research Council surveyed manufacturers regarding their 1970 use of benzoyl peroxide in foods (Subcommittee on Review of the GRAS List--Phase II, 1972). Benzoyl peroxide was not included among the substances resurveyed by NRC for their level of addition in 1975 (Committee on GRAS List Survey--Phase III, 1978). Based on information supplied by those manufacturers who reported adding the compound to at least one food in a category, weighted means were calculated for the normal and maximal addition to foods in the category. According to this survey, benzoyl peroxide was added to only three categories of food. The value indicated for fats and oils is believed to represent its use in the preparation of "double bleached" and "hydroxylated" lecithin. No manufacturer reported its use in cheese manufacture despite its prior sanction for this purpose. Weighted means of the usual level of addition of benzoyl peroxide to foods by category in 1970 are as follows:

<table>
<thead>
<tr>
<th>Food category</th>
<th>Weighted mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baked goods, baking mixes</td>
<td>56 mg/kg</td>
</tr>
<tr>
<td>Grain products (pasta, rice dishes, etc.)</td>
<td>41 mg/kg</td>
</tr>
<tr>
<td>Fats and oils</td>
<td>100 mg/kg</td>
</tr>
</tbody>
</table>

The level of addition shown above does not imply that any specific food contains the indicated amount of benzoyl peroxide, nor that benzoyl peroxide was added to a majority of the foods included in a listed category. Actually, benzoyl peroxide may not have been added to many of the foods included in a given category, or lower levels may have been added than indicated above. Consequently the data probably represent an overestimation of the actual average levels of added benzoyl peroxide.

Surveys by the NRC and by the Flavor and Extract Manufacturers' Association revealed that 668,000 kg of benzoyl peroxide had been added to food in 1975 (Committee on GRAS List Survey--Phase III, 1978), corresponding to a per capita level of 8.5 mg/day. However, this represents the amount added to food, and not the amount ingested. As pointed out earlier, most of the benzoyl peroxide employed as a bleach would be converted to benzoic acid during food processing, and only traces of residual peroxide would be ingested.
IV. BIOLOGICAL STUDIES

Absorption and metabolism

There are no data on the absorption of benzoyl peroxide. Very little of the compound would be expected to survive normal food processing and most of the remaining traces would be converted to benzoic acid in the intestine (Nencki and Zaleski, 1899). Should any residual benzoyl peroxide be absorbed, it would probably be destroyed by liver peroxidases (White et al., 1973). The resulting benzoic acid would be conjugated with glycine and rapidly excreted in the urine as hippuric acid. In normal subjects, more than 80% of ingested benzoic acid can be accounted for within 3 hours as urinary hippuric acid (Kingsbury, 1923) and over 99% within 24 hours (Bridges et al., 1970).

Acute toxicity

The acute toxicity of benzoyl peroxide administered by different routes is summarized in Table 2. Given orally, the compound displays a low toxicity. The LD$_{50}$ in mice was more than 2 g/kg, and in rats, more than 6 g/kg body weight (Antonyuk, 1969). When lethal doses were given by gastric intubation, the animals displayed central nervous depression within 30-40 minutes and death occurred within 24 hours. No deaths occurred during a 14-day observation period when five adult male rats (Spartan strain) were given 3.9 g/kg of the compound suspended in corn oil (Wazeter and Goldenthal, 1973). Some muscular weakness was noted among rats receiving 950 mg/kg. None of the experimental animals died at this dosage level (Sharp, 1977).

Short-term toxicity

Radomski et al. (1948) maintained three dogs for 6 weeks on a nutritionally balanced diet containing flour to which 625 mg/kg benzoyl peroxide had been added. The rations were prepared daily by cooking in live steam for 90 minutes shortly before feeding. The daily consumption of flour ranged from 30-50 g/kg body weight/day, corresponding to a daily "intake" of approximately 20-30 mg/kg of benzoyl peroxide. However, the amount of benzoyl peroxide surviving the cooking procedure was not determined. No ill-effects were noted. Arnold (1949) investigated the development of canine hysteria ("running fits") in dogs fed diets containing flour treated with various reagents. Only treatment with nitrogen chloride produced this syndrome. A mixture of ammonium persulfate, chlorine, potassium bromate, and benzoyl peroxide was added to flour, steamed for 90 minutes before feeding, and
<table>
<thead>
<tr>
<th>Animal</th>
<th>No., Sex</th>
<th>Route</th>
<th>Dosage mg/kg</th>
<th>Measurement</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>N.S.*</td>
<td>Intragastric</td>
<td>2127</td>
<td>LD₅₀</td>
<td>Antonyuk, 1969</td>
</tr>
<tr>
<td></td>
<td>N.S.*</td>
<td>Intraperitoneal</td>
<td>148</td>
<td>LD₅₀</td>
<td>Antonyuk, 1969</td>
</tr>
<tr>
<td></td>
<td>20,F</td>
<td>Intraperitoneal</td>
<td>116</td>
<td>LD₀</td>
<td>Philpot and Roody, 1959</td>
</tr>
<tr>
<td></td>
<td>75,F</td>
<td>Intraperitoneal</td>
<td>167</td>
<td>LD₅₀</td>
<td>Philpot and Roody, 1959</td>
</tr>
<tr>
<td></td>
<td>63,F</td>
<td>Intraperitoneal</td>
<td>162</td>
<td>LD₅₀</td>
<td>Horgan, et al., 1957</td>
</tr>
<tr>
<td>Rat</td>
<td>5,M</td>
<td>Oral</td>
<td>3900</td>
<td>LD₀</td>
<td>Wazeter and Goldenthal, 1973</td>
</tr>
<tr>
<td></td>
<td>N.S.*</td>
<td>Oral</td>
<td>950</td>
<td>LD₀</td>
<td>Sharp, 1977</td>
</tr>
<tr>
<td></td>
<td>N.S.*</td>
<td>Intragastric</td>
<td>6400</td>
<td>LD₅₀</td>
<td>Antonyuk, 1969</td>
</tr>
<tr>
<td></td>
<td>N.S.*</td>
<td>Intraperitoneal</td>
<td>373</td>
<td>LD₅₀</td>
<td>Antonyuk, 1969</td>
</tr>
<tr>
<td></td>
<td>10,M</td>
<td>Inhalation</td>
<td>(19.0 g/m³ 4 hr)</td>
<td>LD₀</td>
<td>Wazeter and Goldenthal, 1973</td>
</tr>
</tbody>
</table>

*Not stated.*
fed to six dogs for 21-38 days. The added benzoyl peroxide amounted to 18 mg/kg flour providing an estimated daily "intake" of about 0.5 to 1.0 mg/kg body weight. The amount of benzoyl peroxide in the flour at the time of consumption was not determined. The dogs on this regimen gained weight normally and displayed no untoward effects.

Antonyuk (1969) injected groups of ten mice or six rats (strains unknown) intraperitoneally daily for 4 months with one-tenth, one-twentieth, or one-fiftieth of the calculated LD50 dose of benzoyl peroxide (148 mg/kg for mice and 373 mg/kg for rats). Weight gains were reduced in each group, but no deaths occurred from the cumulative effects of the sublethal doses.

Wazeter and Goldenthal (1973) exposed ten male Spartan rats weighing 232-248 g for 4 hours to atmospheric concentrations of approximately 19 g/m3 benzoyl peroxide "dust". During the single exposure period, the following signs were observed: eye squint, altered respiratory rates, dyspnea, salivation, lacrimation, erythema, and temporarily increased movement followed by a decrease in motor activities. All animals appeared normal after 24 hours. On the fifth day after exposure, a "few" of the rats showed signs of corneal opacity and ulceration of the corneal surface. All rats survived a 14-day observation period with normal weight gains.

These investigators also applied 390 mg benzoyl peroxide to the shaved, abraded backs of three male and three female New Zealand white rabbits weighing 2.5-3.0 kg each. The report does not state whether the compound was added in solid form or in solution. The areas of application were wrapped in gauze bandages. After 4 hours the bandages were removed and the areas washed with tepid tap water. No skin irritation was evident at this time nor after 24 and 72 hours. In addition, a solution (solvent not identified) containing approximately 85-95 mg benzoyl peroxide was instilled into the conjunctival sacs of four male and four female rabbits. Five of the rabbits were exposed to the solution for 5 minutes and three of the animals for 24 hours. The treated eyes were gently washed. Temporary conjunctivitis and swelling were noted, but no ulcerations or opacities resulted.

Mkhitarian et al. (1974) demonstrated an in vivo destruction of α-tocopherol by benzoyl peroxide. They injected young, white rats (120-150 g) intraperitoneally with daily doses of 48 mg benzoyl peroxide/kg body weight. The brain content of α-tocopherol was reduced by 37.5% during the first 24 hours and by 53.8% after 7 days. The levels then began to rise; after 15 days, the concentration was 7.1% below normal; and after 30 days, it was 32.5% above normal. A similar initial decrease of α-tocopherol was also noted in the liver, but recovery began earlier than in the brain. The levels after 1, 7, and 15 days were 41.2, 33.9, and 27.2% below normal, respectively. After 30 days, the liver
α-tocopherol exceeded control values by 41.1%. The lipid peroxides in the brain and liver varied inversely with the α-tocopherol content.

Long-term toxicity

Sharratt et al. (1964) fed four groups of animals, each consisting of 50 rats and 50 mice, bred in their own laboratories, equally divided by sex, a diet containing 56% wheat flour, 20% dried skim milk, 11% fish meal, 2% alfalfa meal, 2% dried yeast and yeast extract, 3% cod liver oil, and 4% salt mixture. The remaining 2% was a mixture of the flour to which sufficient commercial benzoyl peroxide was added to produce diets containing 2800, 280, 28, or 0 mg/kg benzoyl peroxide, respectively. The benzoyl peroxide content of the diet at the time of consumption was not determined, nor did the report state how long prior to feeding the benzoyl peroxide had been added. Consequently, it is unknown how much had been converted to benzoic acid and how much benzoyl peroxide had actually been ingested. The maximum daily intake of benzoyl peroxide (assuming no destruction prior to consumption) was estimated to have been approximately 280, 28, 2.8, and 0 mg/kg body weight, respectively. The weight gains by female rats receiving the highest benzoyl peroxide supplement (about 280 mg/kg) and by male rats consuming the intermediate level (about 28 mg/kg) were slightly (about 10%), but significantly less after 16 months than those attained by controls. The authors speculated that these slight depressions in growth rates at the highest levels of benzoyl peroxide supplementation were due to marginal nutritional inadequacies, possibly vitamin E deficiencies, rather than to toxic effects of benzoyl peroxide. Weight gains of the mice were not reported. There were no significant differences in the mortality during the 104-week observation period between the experimental and control groups of either species. Five rats (2 males, 3 females) consuming the diet treated with the highest level of benzoyl peroxide each developed a single malignant tumor (uterine carcinoma, testicular carcinoma, thyroid adenocarcinoma, uterine sarcoma, and uterine reticulum cell sarcoma). One tumor (mammary carcinoma) was reported among the mice on this diet. Six control rats (2 males, 4 females) also developed single malignant tumors: 2 mammary gland, and one each of testicular, thyroid, and squamous cell carcinoma of the stomach, and one spindle cell sarcoma. Three control mice (sex not stated) developed tumors (one each of pulmonary carcinoma, angioma, and liver fibroma).

Bread was prepared from flour treated with 28 ppm benzoyl peroxide; the remainder of the diet remained the same (Sharratt et al., 1964). This modified diet was fed for 16 months to 200 rats and 200 mice, "strains" noted above, divided equally by sex. The only difference from the control group was a lower mean weight gain among the female rats. The investigators believed this to be of doubtful significance because the weights of all animals varied
widely, due to a chronic infection in the colony. Six malignant tumors were reported among male rats on this diet and five among the females. Five mice on this diet also developed malignancies. Six malignant tumors were detected among the control mice, and 15 among the control rats. Baking was shown to destroy benzoyl peroxide, so little, if any, would be present in the bread consumed by the rodent.

In a continuation of this study (Sharratt et al., 1964), 25 male and 25 female mice and rats received a single subcutaneous injection of freshly prepared benzoyl peroxide suspended in starch solution. Rats received 120 mg each (about 1.2 g/kg) and mice 50 mg (about 2.5 g/kg). There was no difference in the rate of weight gain between the experimental and the control animals during a 16-month observation period. Small swellings were observed after 4 days at the injection sites of 5% of the rats and 16% of the mice. Ulcerations occurred but healed within 14 days. A chronic infection in the colony made pathological findings difficult to interpret. It appeared, however, that a higher incidence of testicular atrophy occurred in rats receiving the highest dosage level of benzoyl peroxide. The investigators attributed this to a vitamin E deficiency induced by benzoyl peroxide. No tumors were reported among the mice. One kidney carcinoma and one seminoma were reported among the treated rats and a rectal carcinoma among the controls.

Carcinogenicity

Approximately 50 mg of a 50% suspension of benzoyl peroxide in flour paste were painted on the skin of 25 male and 25 female mice for 6 consecutive days (Sharratt et al., 1964). No tumors developed at the treated sites. Another group of 25 male and 25 female rats and mice received benzoyl peroxide by three different routes. The animals were fed a flour-based diet contributing a maximum of about 280 mg benzoyl peroxide/kg/day, given a single subcutaneous injection (rats 1.2, mice 2.5 g/kg), and painted with the benzoyl peroxide paste mentioned above. The weight gains of these rats were not significantly different from those of controls over a period of 16 months, except for a slight decrease at the eighth month. The mouse weights were not reported. No significant difference between control and experimental groups was found in mortality rate, nor in incidence, distribution throughout the body, or development time of tumors.

Hueper (1961) has shown that implants of incompletely cured Silastic® (silicon rubber, processed polydimethylsiloxane) were capable of inducing sarcomas in the subcutaneous tissue of rats. Because benzoyl peroxide is a polymerization catalyst in the production of Silastic®, the possibility existed that it, rather than the polymer, was the carcinogenic agent. To resolve this uncertainty, Hueper (1964) implanted fully cured Silastic®, in which all benzoyl peroxide had been destroyed, in the subcutaneous tissue of the necks of 21 male and 14 female Bethesda
black rats about 3 months old. Fifty mg benzoyl peroxide in gelatin capsules were similarly implanted in 20 male and 15 female rats. The animals were observed for 24 months.

Ten rats implanted with Silastic® developed firm neoplasms ranging from 2-4 cm in length attached to the skin at the tumor base. Four additional rats had dark-red nodular tumors of the ileoceleal lymph nodes. Three other rats had white, soft, medullary masses involving also regional peritoneal lymph nodes in the abdomen. One rat had a firm, yellowish, nodular mass attached to the wall of the bladder. In contrast, none of the rats receiving benzoyl peroxide had tumors at the site of implantation. Malignant neoplasms were found at various sites in seven rats with benzoyl peroxide implants (four round-cell sarcomas of ileoceleal lymph nodes, one mesothelioma of the peritoneum, one myxosarcoma of the anal region, and one epidermoid carcinoma of the snout). Benign tumors appeared in three additional rats (two adenofibromas of the breast and one cystic cholangioma). None of the rats had tumors at the site of implantation. Hueper (1964) concluded that the observed tumors were not causally related to the benzoyl peroxide implants. He also cited a personal communication from Shubik that subcutaneous implantation of benzoyl peroxide into rodents had been "carcinogenically inactive."

The carcinogenicity of benzoyl peroxide was also investigated by Van Duuren et al. (1963). Thirty 8-week, male Swiss-Millerton mice were painted three times weekly with 100 mg of 5% benzoyl peroxide in benzene. The animals were examined regularly for tumors for 292 days. One mouse exposed to benzoyl peroxide developed a benign tumor, but none developed carcinomas.

**Mutagenicity**

Benzoil peroxide dissolved in dimethylsulfoxide exhibited no mutagenic activity in microbial assays with or without mammalian activation preparations (Litton Bionetics Inc., 1975). Saccharomyces cerevisiae, strain D4, and Salmonella typhimurium, strains TA-1535, 1537, and 1538, were used in modified Ames assays. Activation in the assay was provided by tissue homogenates from mice, rats, and monkeys.

Epstein et al. (1972) tested benzoil peroxide for dominant lethal mutations in ICR/Ha Swiss mice. Benzoil peroxide in tricaprylin at doses of 52 and 64 mg/kg was injected intraperitoneally into seven and nine male mice, respectively. Each animal was then caged for 1 week with three untreated virgin females, which were replaced each week for 8 consecutive weeks. All females were sacrificed 13 days after the midweek of their caging and presumptive mating. At necropsy, each female was scored for pregnancy, total implants, and for early and late fetal deaths. Late fetal deaths were very rare. The results obtained in the treated mice did not differ significantly from those in the con-
trol mice. Benzoyl peroxide in the dosage range employed did not produce dominant lethal mutations in this strain of mouse, since the only effect noted was a reduction in the pregnancy rate. These results do not preclude the possibility of effects with higher dosages or with other species or strains.

Other effects

Christner et al. (1970) found that benzoyl peroxide inhibited purified glucuronidase reversibly. A 50% inhibition of the enzyme was produced at a benzoyl peroxide concentration of 12.7 mg/liter.

Human studies

The major concerns from occupational exposure to benzoyl peroxide are the hazards arising from its flammability and explosive properties as well as irritation of the eyes and skin (NIOSH, 1977). There are a number of reports of allergic responses to benzoyl peroxide resulting from occupational exposure or clinical treatments. Baird (1945) reported a case of a young, male baker working with flour treated with benzoyl peroxide, who suffered for a year with asthmatic wheezing and severe dermatitis of the face, neck, shoulders, and arms. When the baker substituted "unimproved" wheat flour for that treated with benzoyl peroxide, the allergic reactions disappeared. Two years later, when he was again exposed to benzoyl peroxide-treated flour, he promptly developed dermatitis. A patch test with benzoic acid was positive and the author concluded this to be the allergen. However, no tests were made for residual benzoyl peroxide in the flour. Leyden and Kligman (1977) reported benzoic acid to be nonsensitizing in a series of patients who were sensitive to benzoyl peroxide.

Positive patch tests with benzoyl peroxide were reported among 38 of 400 bakers (Grosfeld, 1951; Young, 1974) and among 3 of 30 polyester processors in an aircraft plant (Malten, 1957). Jirasek and Kalensky (1962) studied 34 workers who had reported some degree of irritation upon exposure to epoxy resins, which employed benzoyl peroxide in their manufacture. Tests on these workers revealed eight who developed a "toxic reaction" to benzoyl peroxide. The nature of the tests and the reactions were not specified.

Dermatologists have also reported reactions among patients receiving various topical preparations of benzoyl peroxide for the treatment of acne. Leyden and Kligman (1977) state that benzoyl peroxide is the most frequently prescribed compound
in the United States for this condition. It has also been shown to have broad-spectrum antimicrobial activity and to be a useful agent in the treatment of chronic ulcers, tinea pedis, and tinea versicolor (Kligman et al., 1977). Colman and Roenigk (1978) found that benzoyl peroxide hastened the healing of experimentally inflicted wounds in piglets, and Hanke and Bergfeld (1978) reported the successful treatment of recurrent leg ulcers in diabetic patients with lotions containing 20% of the compound.

The reported incidence of contact sensitization to benzoyl peroxide varies widely among the various investigators. Ede (1973) conducted a double blind study on 196 acne patients. One group was treated with a placebo and three groups with different lotions each containing 5.5% benzoyl peroxide. The lotions were applied once to four times daily for 4 weeks and left on the skin for at least 3-4 hours each time. None of the patients exhibited skin sensitivity. Possible systemic effects were monitored by a series of blood and urine analyses. No significant changes could be detected at the end of the test. Clinicians with extensive experience in the use of benzoyl peroxide preparations have estimated the sensitization rate to be 1 or 2% (Pace, 1965; Fulton et al., 1974). However, Poole et al. (1970) sensitized almost 40% of adult volunteers to an ointment containing 1% sulfur and 10% benzoyl peroxide in polyethylene glycol. The investigators applied the preparation for 24 hours nine times within a 3-week period. After a 2-week interval, the preparation was applied for 24 hours to the original test sites and to untreated sites on the opposite arm. At challenge, 25 of 69 subjects exhibited severe, eczematous skin reactions on both the original and alternate sites. Six other subjects showed definite but weaker responses. Two months after the first applications, ten subjects who had exhibited moderate reactivity were rechallenged with the ointment. All reacted strongly. There was no reaction to the polyethylene glycol vehicle nor to sulfur alone.

Leyden and Kligman (1977) reported an even higher incidence of contact sensitizing capability of benzoyl peroxide. These investigators applied squares of cloth saturated with 5 or 10% benzoyl peroxide gel to 25 white, young adults for five 48-hour periods. The sensitization rate was 76% among these subjects. There was no apparent difference in reactivity between the 5 and the 10% preparations.

Secondary effects of food treatment

In evaluating the health aspects of benzoyl peroxide, the Select Committee considered not only the intrinsic toxicity of this compound, but also any secondary, possibly deleterious effects which might result from its use in foods. Three effects of
such action seem possible: 1) the formation of harmful degradation products of benzoyl peroxide; 2) the destruction of essential nutrients; and 3) the production of toxic substances from the food components.

Degradation products of benzoyl peroxide

As indicated earlier, benzoyl peroxide in food is rapidly and almost completely converted to benzoic acid during processing. This would result in an increase in the benzoic acid content of the treated food roughly equal to the benzoyl peroxide employed. Calculations in a previous section suggest that this would be approximately 8.5 mg per person per day. The direct addition of benzoic acid and sodium benzoate to food is approximately two to three times this amount (Subcommittee on Review of the GRAS List, 1972). Furthermore, benzoic acid is found in more than a score of natural foods, including fruits, spices, milk products, meats, and beverages (van Straten, 1977). A daily intake of 4-6 g of benzoic acid in man causes no toxic symptoms aside from slight gastric irritation (Goodman and Gilman, 1975). The Select Committee reviewed health aspects of benzoic acid and sodium benzoate in foods and concluded that their ingestion at present or moderately increased levels should pose no health hazard to the consumer (SCOGS, 1973). The small additional contribution from benzoyl peroxide does not alter this conclusion.

Destruction of essential nutrients

Cheese manufactured during summer months from unbleached milk exhibits a yellowish color because of a relatively high level of carotenoid pigments. Bleaching with benzoyl peroxide effectively destroys these carotenoid pigments and affords a means of controlling the color of cheese. To make up for the reduced vitamin A activity of the bleached milk, the FDA requires that "...sufficient vitamin A [be] added to the curd to compensate for the vitamin A or its precursors destroyed in the bleaching process ..." (Office of the Federal Register, 1980a). Vitamin A itself seems little affected by the normal bleaching process. Kuramoto and Jezeski (1954) reported no significant reduction of vitamin A content when 30% cream was heated at 62°C for 4 hours with 0.0009% benzoyl peroxide, a treatment which reduced the carotene content of the cream by 50%. The percentage destruction of carotene appeared to be roughly proportional to the fat content of the milk or cream. The losses of carotene under the conditions just cited were 78, 50, 38, and 15% for fat concentrations of 50, 30, 10, and 4.5%, respectively. The content of vitamin A or carotene in flour is negligible and its destruction would have little nutritional significance.
Menger (1957) reported destruction of α-tocopherol in flour after bleaching with benzoyl peroxide. Sharratt et al. (1964) observed an increased incidence of testicular atrophy among rats receiving flour treated with high levels of benzoyl peroxide. They attributed these changes to a destruction of α-tocopherol, although no analyses were performed. Thus, conventional bleaching of flour and milk may destroy some α-tocopherol. However, the α-tocopherol content of both foods is relatively small (Lampert, 1975; Ockerman, 1978), so that its destruction would seem to have little nutritional significance.

The oxidation of essential fatty acids represents another possible deleterious effect of benzoyl peroxide. Witten and Holman (1952) speculated that a prooxidant (benzoyl peroxide) might interfere with the normal metabolic conversion of linoleic and linolenic acids and that this effect could be reflected by impaired growth or development, or by other signs of essential fatty acid deficiency. They maintained rats on a fat-free diet for 3 months and then gave daily, oral supplements of 2 mg benzoyl peroxide (about 12 mg/kg body weight) together with 100 mg ethyl linoleate. Contrary to expectations, the investigators found that benzoyl peroxide stimulated growth and total fatty acid synthesis when fed with linoleate. Additional studies from the same laboratory (Holman and Greenberg, 1954) confirmed these results. Weanling, male, Sprague-Dawley rats were fed a fat-free diet until signs of moderately severe fat deficiency appeared. The diet was then supplemented with 75 mg ethyl linoleate per day alone, or together with benzoyl peroxide (about 10 mg/kg body weight). The addition of benzoyl peroxide did not reduce the effectiveness of ethyl linoleate in curing the deficiency signs, reducing water consumption, or stimulating arachidonic acid synthesis.

The amounts of unsaturated fatty acids in flour were not reduced by treatment with benzoyl peroxide. Fisher et al. (1958) added 33.3 or 333 ppm of benzoyl peroxide to flour and studied its effect on linoleic, linolenic, and arachidonic acids in flour and in dough and bread prepared from this flour. No difference from untreated flour in the concentrations of unsaturated fatty acids could be detected.

No data are available on the fate of other essential nutrients in foods bleached with benzoyl peroxide. Results with hydrogen peroxide may be relevant in this connection. Treatment of milk for 24 hours at 30°C, or for 30 minutes at 51°C with 0.3% hydrogen peroxide almost completely destroyed the small amounts of ascorbic acid and α-tocopherol present (Lück and Schillinger, 1958a,b). These treatments had no effect on thiamin, riboflavin, and pyridoxine. No reduction in methionine content was noted when fish protein concentrates were treated with 1.25% hydrogen peroxide at 50°C for 20 minutes, and only a slight reduction (8%) after treatment with 5% (Hasekh et al., 1972). It should be noted that
the concentrations of hydrogen peroxide employed in these studies were two to three orders of magnitude greater than those used in the bleaching of flour or milk with benzoyl peroxide.

Production of toxic compounds

The Select Committee recognized the possibility that benzoyl peroxide might react with various constituents in food to produce oxidation products. Such products have not been detected or identified in foods treated with benzoyl peroxide, so their existence and significance are entirely speculative at this time. Theoretically, the unsaturated fatty acids and sterols would seem the most likely candidates to undergo oxidation when treated with benzoyl peroxide. Although the possibility of such products cannot be excluded, available evidence suggests that they can be present only in very small amounts. As indicated in the previous section, the addition of 333 ppm of benzoyl peroxide caused no perceptible diminution in the concentrations of linoleic, linolenic, or arachidonic acids present in flour (Fisher et al., 1958). Witten and Holman (1952) administered daily a mixture of 100 mg ethyl myristate, 100 mg ethyl linoleate (or linolenate) and 2 mg benzoyl peroxide to rats on a fat-free diet. Rats receiving these supplements gained weight as rapidly as those receiving ethyl linoleate or linolenate alone, indicating that oxidation products of these unsaturated acids, if present, did not impair the growth response of rats. No toxic signs were reported.

Similarly, lecithin treated with benzoyl peroxide caused no impairment of growth when fed to rats. Hydrogen peroxide and benzoyl peroxide are used in the preparation of "double bleached" and "hydroxylated" lecithins (Markel, 1960; Sipos, 1973). Hydroxylated lecithin with peroxide values of 52 meq/kg was fed to weanling Sprague-Dawley rats at levels of 5 and 10% of their diets. The 10% group which received about 17 mg peroxide/kg body weight daily were maintained on this diet for 8 weeks. The rats on the 5% level (8.5 mg/kg peroxide per day) were kept for 52 weeks. Weight gains were normal for both groups and no abnormalities were detected in the vital tissues (Markel, 1960). Verrett (1975) found no increase in teratogenic effects in the developing chick embryo when 10-200 mg/kg egg weight of double bleached lecithin were administered in the air cell at 0-96 hours or via the yolk at the same time intervals. The estimated dietary intake of bleached lecithins as a component of processed foods is 4 mg daily (Subcommittee on Review of the GRAS List--Phase II, 1972) or a daily intake of less than 0.1 mg peroxide. The intake of lecithins treated with benzoyl peroxide would be less.

No data are available on the nature or likelihood of sterol oxidation products resulting from food treatment with benzoyl peroxide. Again, the production of significant amounts with current or anticipated uses of benzoyl peroxide as a bleaching agent in food seems unlikely. Smith and Kulig (1976) obtained
a yield of 0.2% cholesterol(a)oxide upon treatment of cholesterol (1 mg/ml) for 6 hours at 50°C with 0.015% hydrogen peroxide. Milk used in the production of certain cheeses is treated with 0.002% benzoyl peroxide. If this benzoyl peroxide solution were as effective as the stronger hydrogen peroxide preparation, about 0.2 mg cholesterol(a)oxide per liter of milk would be produced by this treatment. Seelkopf and Saalfelder (1962) fed 45 Holtzmann white rats of both sexes 10 mg per day of cholesterol(a)oxide (about 500 mg/kg body weight) for 68 days and 30 mg (about 1.5 g/kg) for an additional 37 days. The animals were maintained for 1.5 years before sacrificing. The incidence and kinds of tumors did not differ from the control group. Similarly, no difference from controls in tumor occurrence could be detected among 45 mice, C57B1/6 strain, subjected to the same regimen as the rats: 500 mg/kg for 68 days; 1.5 g/kg for 37 days.
V. OPINION

Benzoyl peroxide is GRAS when used as a bleaching agent in certain foods. It is used extensively for this purpose with flour and to a much lesser extent in the manufacture of some cheeses and with certain forms of lecithin. Data from food processors indicate an average per capita usage of about 8.5 mg/day. Only a small fraction of this amount would be ingested, for benzoic peroxide is rapidly degraded and little of the intact compound would survive food processing. The amount actually ingested is subjected to further destruction in the gastrointestinal tract and by tissue peroxidases.

Benzoic acid is produced by the degradation of benzoil peroxide during food processing. The amounts produced are small compared with those normally present or added to foods. There is no evidence of hazard from the ingestion of benzoic acid at these levels.

Oral administration of benzoil peroxide by animals produced toxic effects only at levels several orders of magnitude greater than the estimated consumption by man from food sources. Various tests for mutagenicity and carcinogenicity of benzoil peroxide have been negative. No tests of teratogenicity were available to the Select Committee.

Some carotene is destroyed by treatment of milk with benzoil peroxide in the manufacture of cheese. Governmental regulations require that these losses be compensated for by the addition of supplementary vitamin A. Some vitamin E and vitamin C are probably destroyed during bleaching, but their amounts in flour and milk are small and these losses would have little nutritional significance. There is no evidence that other essential nutrients are destroyed.

Oxidation products of normal food constituents are possible by the action of benzoil peroxide when it is used as a bleaching agent. These products have not been identified but could conceivably include oxidized forms of pigments, unsaturated fatty acids, and sterols in very small amounts. Possible products thus far tested have not proved hazardous when given by mouth at levels many times greater than any reasonable possible intake from food.

Benzoyl peroxide is used extensively in topical applications for skin disorders. Allergic responses have been reported among patients using these preparations as well as among individuals repeatedly exposed to the compound in the workplace. No reports have come to the attention of the Select Committee of allergic responses to benzoil peroxide in food.
In view of the above, the Select Committee concludes that:

There is no evidence in the available information on benzoyl peroxide that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used at levels that are now current or that might reasonably be expected in the future.
VI. REFERENCES CITED


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