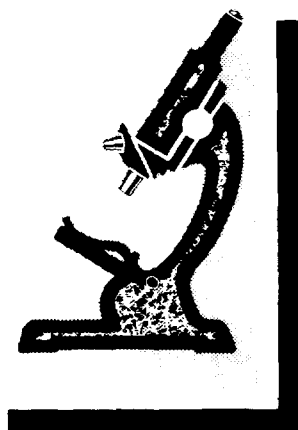
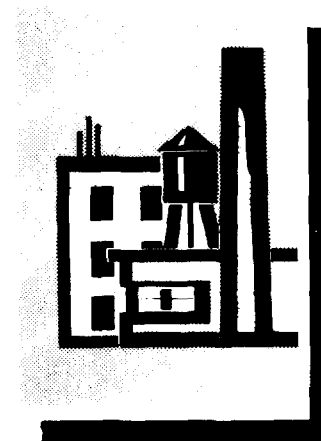


# **NIOSH**

## **SPECIAL OCCUPATIONAL HAZARD REVIEW**



### **ALTERNATIVES TO DI - 2 - ETHYLHEXYL PHTHALATE ("DOP") RESPIRATOR QUANTITATIVE FIT TESTING**

**U. S. DEPARTMENT OF HEALTH AND HUMAN SERVICES  
Public Health Service  
Centers for Disease Control  
National Institute for Occupational Safety and Health**

**SPECIAL OCCUPATIONAL  
HAZARD REVIEW**

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IN  
RESPIRATOR QUANTITATIVE FIT TESTING**

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES  
Public Health Service  
Centers for Disease Control  
National Institute for Occupational Safety and Health  
Division of Standards Development and Technology Transfer**

**March 1983**

**DISCLAIMER**

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## PREFACE

The Occupational Safety and Health Act of 1970 emphasizes the need for standards to protect the health and safety of workers exposed in the workplace. In order to fulfill its responsibilities under the Act, the National Institute for Occupational Safety and Health (NIOSH) established a program to evaluate the adverse health effects of widely used chemical and physical agents and make recommendations for preventing these adverse effects. This includes the development of documents which summarize any needed development of (or change in existing) standards. Such reports usually describe health effects including cancer, mutagenicity, teratogenicity or other effects on reproduction associated with occupational exposure to the agent or process, as well as recommending control measures, including work practices, to assist in protecting the health and well-being of workers.

This document evaluates recent information concerning di-2-ethylhexyl phthalate (DEHP) a substance widely used in the quantitative fit testing of respiratory protective devices. In 1982, the National Toxicology Program (NTP) reported positive results of tests of carcinogenicity in two species of rodents exposed to DEHP. As a result of that report, we (NIOSH) recommended several practices for reducing exposure to DEHP during the testing of respirators, and for the use of other materials in substitution for DEHP. The basis for these recommendations is provided on the following pages.

Contributions to this report by other Federal agencies or departments, external reviewers, reviewers selected by the American National Standards Institute, as well as the staff of NIOSH, are acknowledged and gratefully appreciated. The reviewers and the Federal agencies that received the report for review are listed on pages vii and viii. The conclusions and recommendations expressed in this report are those of the Director and staff of NIOSH, and not necessarily those of the reviewers or other Federal agencies. However, all comments, whether or not incorporated, have been carefully considered.



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## SYNOPSIS

Di-2-ethylhexyl phthalate (DEHP; commonly known as "DOP") is examined for occupational carcinogenic potential and overall toxicity in respirator quantitative fit testing (QNFT). A DEHP aerosol is used in QNFT to measure face seal leakage for the individual respirator wearer. Under present technology, the direct measurement of facial fit in QNFT is critical because only a limited number of respirator sizes and styles are marketed to accommodate an infinite array of human facial sizes and characteristics. NIOSH initiated this review of QNFT when the National Toxicology Program (NTP) reported that DEHP was carcinogenic in two rodent species under experimental conditions of the standard bioassay protocol. After evaluating all the information, NIOSH has concluded that a prudent course must now be followed in QNFT. Specific NIOSH recommendations for maintaining the important workplace practice of fitting respirators by QNFT are described in this report.

Although NIOSH estimates the carcinogenic risk to be minimal for the respirator wearer under normal conditions, at least the following two critical exposure factors must be considered in QNFT: (1) Exposures to the DEHP aerosol can vary for the respirator wearer being tested if QNFT is improperly conducted. (2) Field practitioners administering QNFT, especially those using portable testing equipment where aerosol ventilation is difficult to control, can be subjected to routine and varying exposures. Because such varying exposures can increase the attendant hazard, NIOSH advises that a substitute aerosol is required to replace DEHP in QNFT.

NIOSH tested several agents considered likely candidate substitutes to determine their suitability for use in existing QNFT equipment, originally made for the DEHP aerosol. The concentration and size distribution of the aerosol particles, critical factors in selecting a substitute, were determined for each candidate and compared to the aerosol characteristics of DEHP. Our experimental results indicated that (1) refined corn oil, (2) di-2-ethylhexyl sebacate (DEHS), and (3) dimethicone (Dow Corning 200 fluid, 50-centistoke) all exhibited polydisperse aerosol particle characteristics essentially equivalent to those generated with DEHP. These findings later initiated direct applications of refined corn oil and DEHS aerosols in actual respirator fitting tests. Field test results at several different facilities showed that both refined corn oil and DEHS aerosols are highly suited for conducting QNFT.

After acceptable candidates were identified by their intrinsic aerosol properties, NIOSH also reviewed reports describing the toxicity and any associated health effects for each agent, as an aid in selecting the best option. Our review revealed that extensive laboratory tests have been conducted on refined corn oil in a variety of mammalian species. All the studies evaluated indicated a low toxicity for the compound. Furthermore, refined corn oil has demonstrated a lack of carcinogenic potential during

its extensive use as a control vehicle in carcinogen bioassays and other more direct experimental applications. Although the knowledge of the toxicity of any compound is seldom complete, the low toxicity of refined corn oil is well documented, in contrast to the other candidate agents. Review of the limited available reports on DEHS and dimethicone indicated little toxicity data that is relevant to a QNFT exposure. For the moment, unresolved questions about the metabolic fate of DEHS, thought to be similar to that of DEHP, also tend to preclude this candidate from further consideration.

NIOSH aerosol test results suggested that at least three substances are suitable for use in QNFT, while the toxicity assessment indicated that only one of these agents should be considered acceptable. Since toxicity is so critical in the final selection of a feasible substitute, NIOSH concludes, from the experimental evidence examined, that refined corn oil stands apart from the other agents. For these reasons, NIOSH recommends that a refined corn oil aerosol is the best option to replace DEHP in QNFT. The appropriate maintenance requirements for using a refined corn oil aerosol in QNFT are described in this report.

## ACKNOWLEDGMENTS

The Division of Standards Development and Technology Transfer, NIOSH, had primary responsibility for the development of this Special Hazard Review on di-2-ethylhexyl phthalate (DEHP) and substitutes in respirator quantitative fit testing (QNFT). P. Jackson Schad and Rhoda J. Yarkin, the authors of this document, had NIOSH program responsibility and were either the principal investigators or project monitors for all related intramural research. P. Jackson Schad served as initial project monitor for a related extramural study conducted at the Johns Hopkins University; Jon R. May served as final project monitor. Richard L. Gross, Douglas L. Smith, and David L. West had NIOSH program management responsibility and assisted in the preparation of the document. Editorial review was performed by William N. LeVeve.

Sincere appreciation is extended to the personnel from other NIOSH divisions that provided technical research support to identify acceptable DEHP substitutes in QNFT. These include Paul A. Baron, Laurence J. Doemeny, Eugene Kennedy, Lawrence D. Reed, Jerome P. Smith, Dorothy C. Sterling (Division of Physical Sciences and Engineering), and Warren R. Myers (Division of Safety Research). In addition, Libero Ajello (Mycology Division, Bureau of Laboratories, Centers for Disease Control) examined QNFT equipment for fungal contamination from DEHP substitutes.

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Valuable and constructive comments were provided by Herbert E. Christensen, Jon R. May, and Frank L. Mitchell (Office of the Director), Paul E. Caplan (Division of Physical Sciences and Engineering), and the reviewers listed on pages vii and viii.

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## I. INTRODUCTION

Respirator design and manufacturing specifications are most effective when the respirator facepiece properly fits the face of the wearer (Pritchard, 1976). The facepiece fit of a respirator may be measured either quantitatively or qualitatively. Quantitative fit testing (QNFT) actually measures respirator facesal leakage and results in a numerical value. Qualitative tests do not directly measure facial fit, but instead rely on subjective responses of the wearer, such as odor identification of the vapor of a chemical agent like isoamyl acetate (banana oil). Positive or negative pressure tests and an irritant smoke test are other examples of common qualitative procedures. Selection of the appropriate test depends on the severity of the respiratory hazard for which the respirator is intended. QNFT is highly recommended when facesal leakage must be minimized for occupations with exposures to highly toxic atmospheres, especially those immediately dangerous to life or health. Direct measurement of facesal leakage by QNFT has become an integral part of health and safety programs designed to reduce workplace exposures to respiratory hazards.

Di-2-ethylhexyl phthalate (DEHP), used for many years to generate a polydisperse aerosol test atmosphere in QNFT, is the branched-chain isomer of di-n-octyl phthalate. Commonly known to respirator users and others as "DOP," DEHP has been considered to be well suited for QNFT because of its physical characteristics. A polydisperse aerosol consists of particles generated with a random size distribution over a narrow range, as opposed to a monodisperse aerosol, which has a uniform particle size distribution. Tests using the polydisperse DEHP aerosol have been demonstrated to be both accurate and meaningful in the evaluation of respirator fit (Hyatt et al, 1972). In QNFT, a human subject wearing a test respirator is placed in a chamber containing the DEHP challenge aerosol nebulized at a specific concentration. To determine facesal leakage, the atmosphere inside the respirator is sampled through a probe inserted into the test respirator inlet covering, and the aerosol concentration is quantified by light-scattering photometry. Leakage is expressed as the ratio between the test concentration outside the respirator and that gaining entrance inside the facepiece.

The toxicity of the phthalate esters, including DEHP, has been reviewed by several authors (Lawrence et al, 1975; Lawrence, 1978; Thomas et al, 1978; Kolesar, 1980). Although different esters of phthalic acid have been reported to exhibit varying degrees of biologic effects in laboratory tests, DEHP has been considered to have a relatively low order of toxicity. Most reports have indicated that inordinately high doses of DEHP were required to elicit toxic effects in a wide variety of mammalian species. However, continued interest in the toxicity of several phthalate esters and their extensive commercial applications prompted the National Toxicology Program (NTP) to conduct chronic bioassay testing of DEHP for possible carcinogenicity (NTP Technical Report, 1982). Under the experimental conditions of the NTP test, DEHP elicited statistically significant

increases in the incidence of hepatocellular carcinomas in both sexes of B6C3F1 mice and in female Fischer 344 rats. The data further suggested that the incidence of these liver neoplasms was dose-related.

Because of its previously reported low order of toxicity, DEHP has been considered as an acceptable substance to generate a test atmosphere for QNFT. However, the NTP's recent finding that DEHP is carcinogenic to rodents raises questions about the carcinogenic potential and human risks to the QNFT aerosol. This NIOSH report reviews both the overall toxicity and the carcinogenic potential of DEHP, and describes NIOSH test results indicating that other agents may be substituted for the DEHP aerosol in QNFT. Substitute aerosols were generated with the same equipment originally made for DEHP use. The toxicity of each potential substitute is also examined.

The thermal generation of a monodisperse DEHP aerosol, used to test filter material efficiency in respirator cartridges or other air filtration systems, is not reviewed here. This use of DEHP is different from QNFT and should constitute a separate study. Filter tests with DEHP involve a different type of exposure, and the monodisperse aerosol is generated at such a high temperature that thermal breakdown products of DEHP can result. A QNFT protocol using a polydisperse sodium chloride aerosol is also not discussed here, since this protocol uses different equipment than that for the DEHP method.

## II. TOXICITY OF DI-2-ETHYLHEXYL PHTHALATE (DEHP)

### General Toxicity

The data reviewed below suggest that DEHP has a low order of acute toxicity. Table II-1 lists LD<sub>50</sub> values in four animal species by intravenous (iv), intraperitoneal (ip), oral (per os or po), or dermal exposure routes, as adapted from Thomas et al (1978). Subacute and chronic toxic effects occurred only at relatively high doses. Rats administered 0.35% DEHP in the diet for 1 year exhibited lower body weights and increased liver and kidney weights (Nikonorow et al, 1973). No pathologic changes were found in the liver, kidneys, or spleen. At a daily oral dose of 0.2 g/kg to rats for 90 days, Shaffer et al (1945) reported no hematologic or pathologic alterations. Similar low toxicity has been reported for other phthalate plasticizers (Gaunt et al, 1968).

Lawrence et al (1975) studied cumulative effects of DEHP or di-n-octyl phthalate in male ICR mice. A series of doses of either phthalate ester was injected ip into groups of male mice 5 days/week, and an apparent LD<sub>50</sub> was calculated at the end of each week. This procedure was followed until the apparent LD<sub>50</sub> remained constant for 3 consecutive weeks, at which time it was assumed that the chronic toxicity value had been reached. Cumulative doses caused the calculated apparent LD<sub>50</sub> values to decrease as the animals were treated. The initial LD<sub>50</sub> values were 65,000 mg/kg (di-n-octyl phthalate) and 37,800 mg/kg (DEHP). At the end of 1 week, the calculated LD<sub>50</sub> dropped to 25,000 mg/kg (di-n-octyl phthalate) and 6,400 mg/kg (DEHP). The toxic response stabilized by week 10 at 3,100 mg/kg (di-n-octyl phthalate) and 1,400 mg/kg (DEHP). The authors concluded that a cumulative effect resulted from repeated exposures to DEHP or di-n-octyl phthalate. Such dramatic increases in lethality over time were interpreted by Lawrence et al to indicate a need to reconsider the element of safety assumed from the relatively high LD<sub>50</sub> values observed for these phthalate esters.

TABLE II-1

## DEHP ACUTE LETHALITY

Species	Route	LD <sub>50</sub> (g/kg)	Reference
Mouse	po	65.0*	Lawrence et al, 1975
"	ip	14.2	Calley et al, 1966
"	"	20	Mori et al, 1967
Rat	"	50*	Singh et al, 1972
"	po	26	Fassett, 1963
"	"	30.6	Shaffer et al, 1945
"	iv	13*	Miripol et al, 1975
Rabbit	po	33.9	Shaffer et al, 1945
"	iv	31	Harris et al, 1956
Guinea pig	Dermal	10*	Fassett, 1963

\*LD<sub>50</sub> given in ml/kg

Adapted from Thomas et al, 1978

#### Metabolism and Excretion

DEHP was rapidly excreted within 24 hours after either po or iv administration to male rats (Tanaka et al, 1975). Similar findings for DEHP in rats were reported by Lake et al (1975) following po administration. Daniel and Bratt (1974) examined excretion patterns following a single po administration of <sup>14</sup>C-labeled DEHP (1.8 mCi; 2.9 mg/kg) in male and female Wistar rats. Rats excreted 42% of the dose in the urine and 57% in the feces within 7 days. In biliary-cannulated rats, 14% of the dose was excreted in 4 days. Animals fed 1,000 ppm of DEHP in the diet for 7 days before administration of <sup>14</sup>C-labeled DEHP had 57% of the radioactive label in the urine and 38% in the feces in 4 days. Biliary-cannulated rats excreted 9% of the dose in 4 days. When fed continuously at concentrations of 1,000 ppm or 5,000 ppm, DEHP concentrations in the liver attained a steady state in 9-14 days. No further DEHP accumulation was observed when equilibrium was achieved. After rats were returned to a normal diet, the half-life of radioactivity declined in the liver (1-2 days) and fat (3-5 days).

Ikeda et al (1979) evaluated species differences in DEHP excretion patterns. DEHP (50 mg/kg) was administered in the diet to male Sprague-Dawley rats, beagle dogs, and miniature Hormel strain pigs for 21-28 days before a single dose of <sup>14</sup>C-labeled DEHP (9.74 mCi/mmol; 50 mg/kg).

Animals were killed at various times, and the distribution of radioactivity was analyzed in specific organs and tissues. The authors observed that approximately 84% of the labeled material was excreted in the urine and feces of rats during the first 24 hours. Excretion during this time was 67% of the labeled DEHP in dogs and 37% in pigs. For all three species, DEHP excretion was virtually complete within 4 days. Amounts of radioactivity detected in muscle and fat of both dogs and pigs did not decrease as rapidly as those in liver or lung tissue. The authors emphasized that the greater amounts of fat and muscle tissue in the dog and pig were probably responsible for the slower depletion of labeled material. In addition, these investigators noted four radioactive labeled substances in rat urine, three in dog urine, and five in pig urine. No more than a trace of unmetabolized DEHP could be detected in the urine of each species. In 1981, Albro et al reported evidence of species variations in rodents, African green monkeys, and humans. The investigators observed that primates excrete glucuronide conjugates of mono(2-ethylhexyl)phthalate (MEHP), and rats excrete alkyl diacids. Thus, the mechanisms for the elimination of DEHP in primates and rodents appear to differ.

Limited pharmacokinetic investigations of DEHP have been reported. Daniel and Bratt (1974) observed that DEHP was extensively metabolized following po administration to male and female Wistar rats. In rodents, DEHP is initially hydrolyzed to 2-ethylhexanol and MEHP (Albro et al, 1973; Daniel and Bratt, 1974). The remaining alkyl chain of the monoester may then be oxidized to form acids, alcohols, and ketones. Although hydrolysis of DEHP may occur in the liver or small intestine, Daniel and Bratt (1974) observed that DEHP was more rapidly hydrolyzed in vitro when incubated with pancreatic lipase than with rat liver homogenate.

### Carcinogenic Effects in Animals

Positive evidence of DEHP carcinogenicity resulted when the NTP tested the compound under the experimental conditions of the chronic bioassay protocol (NTP Technical Report, 1982). Testing results indicated that DEHP elicited treatment-related increases in the incidence of liver carcinomas in two rodent species. Groups of 50 male and 50 female Fischer 344 rats received either 12,000 ppm (the maximum tolerated dose) or 6,000 ppm of DEHP in their diets daily for 103 weeks. Mice, 50 males and 50 females of the B6C3F1 strain, were also fed diets containing DEHP at either 6,000 (maximum tolerated dose) or 3,000 ppm for 103 weeks. Results were compared with those of matched control animals, 50 untreated rats and 50 untreated mice, of both sexes. Statistical analysis of the data incorporated the one-tailed Fisher exact test to compare the tumor incidence observed in controls with that in dosed animals at each level.

Male rats exhibited an increased incidence of hepatocellular carcinomas or neoplastic nodules in a statistically significant positive relation ( $p = 0.01$  at the high dose when compared with untreated controls (Table II-2). In female rats, significant increase ( $p = 0.012$  in the low-dose

group and  $p < 0.001$  in the high-dose group) in the incidence of hepatocellular carcinomas or neoplastic nodules was reported. A significant increase ( $p = 0.003$ ) in hepatocellular carcinomas was found in female rats only at the high dose (a 16% final incidence of liver carcinomas, compared with 0% in controls). The incidence of hepatocellular carcinomas alone was significantly increased only in the female rats receiving the high dose. Neoplastic liver nodules, observed in both male and female rats, are thought by NTP to be precursors in a progression to hepatocellular carcinomas.

Histopathologic examination revealed that both male and female B6C3F1 mice exhibited significant increases in the incidence of hepatocellular carcinomas. Male mice had incidences of 29% (low dose) and 38% (high dose), compared with 18% in untreated matched controls; only the incidence of the high-dose group was significantly increased ( $p = 0.002$ ). Females had a 14% (low dose) and a 34% (high dose) incidence of hepatocellular carcinomas, compared with an absence of such carcinomas in matched controls (Table II-2). The incidence of liver carcinomas was significantly increased for females in both the low-dose ( $p = 0.006$ ) and high-dose ( $p < 0.001$ ) groups. Hepatocellular adenomas also resulted in mice of both sexes at incidences higher than matched controls in males (at both doses) and in females (at the low dose), although these differences were not reported to be statistically significant. However, hepatocellular carcinomas or adenomas of the liver in both sexes of mice were observed in a statistically positive relation when compared with controls. High-dose male mice exhibited a significant incidence ( $p = 0.002$ ) of hepatocellular carcinomas or adenomas; in the low-dose group, significant incidence ( $p = 0.013$ ) was observed. Females had a significant incidence ( $p = 0.001$ ) of hepatocellular carcinomas or adenomas at either dose.

A statistically significant positive dose-related trend of carcinogenicity was also reported for DEHP-treated rats and mice by the Cochran - Armitage test for linearity. Under this statistical test, the direction of a significant trend indicates a positive dose relationship to the carcinogenic response. Male rats exhibited hepatocellular carcinomas or neoplastic nodules with a linear trend statistically significant in the positive direction ( $p = 0.007$ ). The Cochran-Armitage test for female rats was also significant in the positive direction ( $p < 0.001$ ) for hepatocellular carcinomas and neoplastic nodules. In mice, hepatocellular carcinomas or adenomas of the liver showed a significant positive relation to dose ( $p = 0.002$  for linear trend in males and  $p < 0.001$  for females).

NTP concluded that DEHP was carcinogenic in the two species of rodents under the experimental conditions of the standard bioassay. A statistically significant increase in the incidence of hepatocellular carcinomas resulted in DEHP-treated male and female B6C3F1 mice and female Fischer 344 rats when compared with controls. This response was attributed to DEHP administration. Survival rates of the mice and rats exposed to DEHP did not differ from those of controls, according to the report. No statistically significant trends in mortality were associated with administration of DEHP.



TABLE II-2

INCIDENCE OF LIVER NEOPLASMS IN FISCHER 344 RATS  
AND B6C3F1 MICE AFTER A 2-YEAR  
DIET CONTAINING DEHP

Fischer 344 Rats	Untreated Matched Controls	Low Dose (6,000 ppm)	High Dose (12,000 ppm)
<u>Males</u>			
Liver			
Neoplastic nodules	2/50(4%)	5/49(10%)	7/50(14%)
Hepatocellular carcinomas	1/50(2%)	1/49(2%)	5/50(10%)
<u>Females</u>			
Liver			
Neoplastic nodules	0/50(0%)	4/49(8%)	5/50(10%)
Hepatocellular carcinomas	0/50(0%)	2/49(4%)	8/50(16%)*
<hr/>			
B6C3F1 Mice			
	Untreated Matched Controls	Low Dose (3,000 ppm)	High Dose (6,000 ppm)
<u>Males</u>			
Liver			
Hepatocellular adenomas	6/50(12%)	11/48(23%)	10/50(20%)
Hepatocellular carcinomas	9/50(18%)	14/48(29%)	19/50(38%)*
<u>Females</u>			
Liver			
Hepatocellular adenomas	1/50(2%)	5/50(10%)	1/50(2%)
Hepatocellular carcinomas	0/50(0%)	7/50(14%)*	17/50(34%)*

\*Significantly higher incidence of hepatocellular carcinomas in treated animals when compared with untreated matched controls (as determined by the one-tailed Fisher exact test).

Data excerpted from NTP Technical Report, 1982.

## Mutagenic Effects

No definitive evidence of mutagenicity has been reported for DEHP, although a variety of tests have been performed in bacteria and mammalian cell cultures. For example, in the Salmonella in vitro test for mutagenicity using a thousandfold range of concentrations up to 22,500 µg/plate (full-strength DEHP), no excesses (greater than 2 times that of control values) of revertants occurred either with or without liver microsomal (S-9) activation (Hanson, 1979). Similar results were reported with DEHP and its principal metabolite, mono-ethylhexyl phthalate (MEHP), at concentrations up to 1,000 µg/plate (Rubin et al, 1979). One report described excess revertants in Salmonella strain TA 100 with 5,000 µg DEHP plus S-9 (Tomita and Nakamura, 1978). However, no other concentrations or dose-response relationships were given by the authors. NIOSH recently conducted Salmonella mutagenicity testing of DEHP to assess the mutagenic response of test strains TA 98, TA 100, TA 1535, and TA 1537 (Ong, 1981). No mutagenic activity was evident in any of the strains, either with or without S-9 activation.

DEHP at 50 µg/disc yielded negative results in the B subtilis Rec-assay for DNA damage (Tomita and Nakamura, 1978). No increases in chromosomal aberrations were reported following in vitro exposures of up to 60 µg DEHP/ml in cultured human leukocytes or human fetal lung cells (Stenchever et al, 1976) or in Chinese hamster cells (Ishidate and Odashima, 1977).

## Teratogenic/Reproductive Effects

A single po dose of DEHP (1.0 ml/kg), administered on day 7 of gestation to a random mouse strain (ddY-Stc female x CBA male), elicited skeletal abnormalities in 18.4% of 38 live fetuses; untreated controls exhibited no adverse effects (Nakamura et al, 1979). Skeletal abnormalities included elongated and fused ribs, absence of tail bones, abnormal or incomplete skull bones, and incomplete or missing leg bones. In addition, the incidence of late fetal deaths was 58.5% as compared with 1.3% in untreated controls. When a higher dose of DEHP, 2.5 ml/kg, was given on day 7, an even greater incidence of skeletal malformations and early fetal deaths resulted. At doses of 5.0 or 10.0 ml/kg, an increase in the incidence of early fetal deaths was observed above that found in mice receiving 1.0 ml/kg. However, when DEHP was administered on earlier or later days of gestation, these effects were either reduced or absent. Fetuses from animals treated on day 7 with lower doses of DEHP (0.05 or 0.1 ml/kg) did not exhibit gross or skeletal malformations, and there was no evidence of an increase in the incidence of induced early fetal deaths. Because there was a linear relationship between fetal death and the dose of DEHP, the authors calculated the noneffective maximum dose in the mouse to be 0.065 ml/kg (64 mg/kg). Although a linear relationship was observed between dose and other fetotoxic effects, the authors concluded that exact values of the noneffective maximum dose for gross and skeletal abnormalities must await further study.

Maternal and fetal effects in rats exposed to DEHP over a wide range of doses have also been studied. Pregnant Wistar rats fed DEHP at doses ranging from 200 to 1,700 mg/kg/day during gestation had litters with reduced fetal body weights and increased resorptions only when maternal doses were 340 mg/kg or greater (Nikonorow et al, 1973; Onda et al, 1976). Furthermore, no adverse effect on fetal skeletal development could be related to doses ranging from 340 to 1,700 mg/kg (Nikonorow et al, 1973). DEHP (340 and 1,700 mg/kg/day) was also administered by gavage for 3 months prior to mating female Wistar rats, but not during gestation. While these doses produced a significant increase in mean maternal liver weights, no adverse embryo-fetotoxic effects were observed.

Embryo-fetotoxic effects observed in other rodent studies resulted only when DEHP was administered at exceedingly high doses. For example, groups of five pregnant female Sprague-Dawley rats, weighing 200-250 g, were given a single ip injection of either DEHP and/or di-n-octyl phthalate on day 5, 10, or 15 of gestation (Singh et al, 1972). Animals were injected with either 5,000 or 10,000 mg/kg, based on an acute LD<sub>50</sub> determination of over 50,000 mg/kg for both phthalate esters. Control animals received either normal saline (10,000 mg/kg) or cottonseed oil (10,000 or 5,000 mg/kg), or were not treated. Animals were monitored for resorptions, fetal deaths, gross malformations, and skeletal abnormalities. Twisted hind limbs appeared in 15/55 (27.3%) of the fetuses given the high dose of di-n-octyl phthalate and in 8/51 (15.7%) given the low dose. Hemangiomas of the limbs occurred in 9/41 (22%) fetuses following the high DEHP dose; one fetus also developed twisted hind limbs. No gross malformations were observed in the low-dose DEHP treatment group, and no skeletal abnormalities were seen with either phthalate ester.

Singh et al (1974) found a significantly ( $p < 0.01$ ) depleted number of embryonic implants and reductions in litter sizes following single ip injections of DEHP to male Harlan/ICR mice at 12,500, 18,800, or 25,000 mg/kg. Each male was mated sequentially with two virgin females each week for 12 weeks following DEHP exposure. Four of ten males that received the high dose died within 2 weeks of this single injection exposure, and a fifth male died within 10 weeks. Females mated with males given the high dose had fewer implants per pregnancy during the first 3 weeks than controls. No statistically significant dose-related differences were observed for implants per pregnancy, early fetal deaths, or litter sizes except during the first 3 weeks in the high-dose group. The authors interpreted their results to indicate reduced fertility and possible genotoxicity. However, both the liver and the testes of male rodents can be damaged at these toxic dose levels (Shaffer et al, 1945; Lake et al, 1976), making it difficult to assess potential genetic effects. Singh et al (1975) measured the distribution of radioactivity after ip injections of <sup>14</sup>C-labeled DEHP at 5 ml/kg in pregnant rats on day 5 or 10 of gestation. Even at these high doses, the maximum amounts of radioactivity detected were 0.016% in the amniotic fluid and 0.033% in fetal tissue. These data indicated to the authors that a low level of DEHP and/or its metabolites crossed the rat placenta on critical days during gestation.

## Human Health Effects

A morbidity study was conducted by Thiess et al (1978) on 101 workers at a German plant that manufactures DEHP. These workers were potentially exposed to DEHP over an average of 12 years (range: 0.3-35 years). Atmospheric concentrations of DEHP in the workplace, as determined by this study, ranged from 0.0006 to 0.01 ppm (approximately 0.01-0.16 mg/m<sup>3</sup>). The authors concluded that there were no significant differences in laboratory findings, illnesses, or injury rates in DEHP-exposed workers when compared with control workers from other areas of the same plant. In fact, the amounts of DEHP measured in the blood and urine of six DEHP-exposed workers (0.5-7.0 mg/l plasma; 0.2-7.5 mg/l urine) could not be distinguished from amounts in three medical department controls not directly exposed (0.5-10 mg/l plasma; 0.2-1.5 mg/l urine). This finding indicates the ubiquitous nature of DEHP in the total environment (Lawrence, 1978; Thomas et al, 1978). Thiess et al (1978) also reported no evidence for an increased rate of miscarriages or infant deformities among female workers or wives of male workers at the plant in Germany. Furthermore, no evidence of an increased rate of chromosomal aberrations was detected in leukocyte cultures taken from a group of 10 employees working with DEHP for 10-30 years (Thiess and Flieg, 1978).

## Characterization of DEHP Exposure

The exposure of workers to DEHP during respirator QNFT has been reported to be low (Kolesar, 1980). The challenge aerosol concentration of DEHP for most tests is 25 mg/m<sup>3</sup>, although some test chamber models can be adapted to challenge concentrations as high as 100 mg/m<sup>3</sup>. Maximum uptake of DEHP during fit testing can be calculated for a human test subject assuming a respiration rate of 20 l/min and total absorption of inhaled particles during a full 30-minute test period. An average facepiece leakage of 1% in a 25-mg/m<sup>3</sup> test atmosphere would result in uptake of 150 µg of DEHP. It should be emphasized that QNFT is usually performed only once or twice yearly, or in the initial selection of a respirator. Even in cases where the test might be performed more frequently, DEHP uptake would appear to be minimal under normal conditions. However, the DEHP exposure could vary if QNFT is improperly administered. The exposure to individuals administering the test could also be elevated, especially where portable equipment is used in the field. Exposures of field testers to DEHP may vary considerably because of differences in (1) the duration or frequency of the test and (2) the workplace environment and ventilation characteristics where the test is conducted.

NIOSH has evaluated health hazards in several plants using phthalate plasticizers in thermoplastic molding materials and in a screen printing operation (Health Hazard Evaluations, 1976; 1977; 1978). Based on analytical methods sensitive to 1 ppm, investigators were unable, with one exception, to detect DEHP in either personal or workplace general air samples. In the vicinity of a curing oven, 4 ppm of DEHP was measured in the screen printing plant (Health Hazard Evaluation, 1977). Health effects in workers were not investigated in these studies.

## Conclusions

After examining the data from the NTP study, NIOSH concludes that DEHP was carcinogenic to rodents under the experimental conditions of the standard bioassay protocol. This positive response indicates that DEHP should be considered as having a carcinogenic potential. No evidence of DEHP carcinogenicity in humans has been reported, but the scarcity of exposure data seriously limits conclusions that can be drawn.

Albro et al (1981) reported that the metabolism of DEHP in the rat may differ conspicuously from that observed in humans and African green monkeys. The authors found that primates excrete mainly glucuronides of mono(2-ethylhexyl)phthalate, while rats excrete unconjugated diacids. But until more definitive evidence resolves questions about (1) pharmacokinetic distinctions between rodents and humans and (2) the proximate agent that elicited the liver tumors in the NTP test animals, the relevance of these metabolic differences to DEHP carcinogenicity remains uncertain. It should also be emphasized that possible differences between rat and human metabolism of DEHP may or may not relate to the carcinogenic mechanism expressed under the conditions of rodent exposures in the NTP bioassay. Furthermore, the relevance of this species distinction between humans and rats for carcinogenesis awaits determinations of a probable mechanism of tumorigenic action for DEHP. Whether or not mice, which are susceptible to DEHP carcinogenicity, also metabolize DEHP under similar patterns reported for rats and humans is unresolved.

NIOSH, after reviewing all other available information, concludes that DEHP has exhibited negative results in several tests for mutagenicity including the Salmonella in vitro mutagenicity assay. Such negative results may suggest that DEHP is not a genotoxic carcinogen, although direct evidence of tumorigenic promoter activity for the compound is presently lacking. NIOSH also concludes that results of reproductive toxicity testing are inconclusive and that definitive evidence of teratogenicity is lacking. Additional teratogenicity testing is probably warranted from the positive results observed in several mammalian species, although any new experimental protocol should incorporate lower doses than those used in the widely reported studies.

The NTP report of DEHP carcinogenicity in rodents prompted NIOSH to examine the potential human health risks from this phthalate ester in QNFT. Although the available evidence indicates that DEHP exposure in QNFT would be low for the respirator wearer, the possibility for variations in conducting the test are noted by NIOSH. Exposures to the DEHP aerosol could vary if QNFT is improperly conducted in the field, especially where appropriate supervision in administering the procedure is lacking. Furthermore, when mobile portable equipment is used, there is also an increased risk to field practitioners administering QNFT, owing to difficulty in controlling the ventilation of the aerosol. For all these reasons, NIOSH concludes that an alternative QNFT aerosol must be found to reduce the potential health risk. Once identified, the alternative substance should be substituted for DEHP when conducting QNFT.

### III. ALTERNATE TEST AGENTS FOR RESPIRATOR QUANTITATIVE FIT TESTING (QNFT)

NIOSH has experimented with other substances to determine their suitability for generating a polydisperse aerosol test atmosphere in oil-mist aerosol QNFT systems designed for DEHP (Smith et al, 1980). Based on criteria such as density, vapor pressure, flash point, information about use in aerosol research, and available toxicologic data, four substances were selected for study (Table III-1). These agents included di-2-ethylhexyl sebacate (DEHS) and linoleic acid, which have been used in the past to generate aerosol particles of controlled size for lung deposition studies. Dimethicone, a silicone oil, and refined corn oil were also studied. The dimethicone used was Dow Corning 200 fluid (50-centistoke).

These four substances and DEHP were aerosolized in a Laskin-type nebulizer, common in QNFT systems. The concentration and size distribution of the aerosol particles were determined. In addition, the response of a forward light-scattering photometer, also used in QNFT systems in detection of these aerosols, was checked.

All the oils tested exhibited particle size characteristics similar to those of DEHP (Table III-2). In addition, they could all be detected at concentrations at or below 1/10,000 of the challenge aerosol concentration, which gives sufficient sensitivity for QNFT. Linoleic acid, however, reacted with brass fittings on the nebulizer. For this reason, and for the toxicologic implications of free fatty acids discussed later, the use of linoleic or similar acids is not advised. The NIOSH study noted that refined corn oil appeared to become thicker and cloudy after prolonged use. NIOSH recommends periodic replacement of unsaturated hydrocarbon oils when necessary to prevent problems due to oxidation of the oil. A Harvard University study (Hinds et al, 1981) also concluded that, from the standpoint of aerosol generation, a suitable polydisperse test atmosphere for QNFT may be generated with mineral oil and the polyethylene glycols as well as DEHP, DEHS, and refined corn oil. The Harvard report did not indicate testing of dimethicone.

Several different laboratories that conduct QNFT have investigated direct applications with either DEHS or refined corn oil aerosols in conducting fitting tests on respirator wearers. The Department of Energy, Richland, Washington (Musen, verbal communication to NIOSH, June 1981), and the OSHA Training Center, Des Plaines, Illinois (Saltsgaver, personal communication to NIOSH, February 1981), both indicated their success in the use of refined corn oil aerosols in QNFT equipment. NIOSH also generated refined corn oil aerosols in mannequin respirator tests and concluded that it performed as well as DEHP under test conditions (Myers, 1980). Askin (1980) reported a recommendation for using corn oil aerosols in QNFT. Fairchild and Talley reported in 1981 that the Los Alamos National Laboratory successfully used DEHS aerosols in QNFT.

TABLE III-1

## PHYSICAL PROPERTIES OF COMPOUNDS TESTED BY NIOSH

SUBSTANCE	PHYSICAL STATE (room temp.)	DENSITY (dyne/cm <sup>2</sup> )	MOLECULAR WEIGHT	REFRACTIVE INDEX	COLOR	FLASH POINT (°C)	BOILING POINT (°C)	VISCOSITY (centistoke)
Di-2-ethylhexyl phthlate (DEHP)	liquid	0.9861 (20°C)	390.6	1.4836	colorless	220.6	230.0	(25°C) 57.4
Di-ethylhexyl sebacate (DEHS)	liquid	0.913 (25°C)	426.0	1.447	colorless	215.0	248.0	(25°C) 27.4
Linoleic acid (9,12-octadecadienoic acid)	liquid	0.903 (10°C)	208.4	1.4699	colorless	188.9	230.0	(38°C) 28.0
Dimethicone (dimethyl polysiloxane)	liquid	0.960	—	(25°C) 1.401	colorless	285.0	250.0	(25°C) 50.0
Refined corn oil	liquid	0.922 (15°C)	—	1.4734	light yellow	—	—	—

Adapted from Smith et al, 1980

TABLE III-2

## AEROSOL CHARACTERISTICS OF COMPOUNDS TESTED BY NIOSH

SUBSTANCE	MASS MEDIAN AERODYNAMIC DIAMETER	GEOMETRIC STANDARD DEVIATION	MASS OUTPUT (mg/m <sup>3</sup> )	AEROSOL VOLUME CONCENTRATION ( $\mu\text{m}^3/\text{cc}$ )
DEHP	0.53	1.83	134.0	.169 x 10 <sup>5</sup>
DEHS	0.63	1.94	159.1	.169 x 10 <sup>5</sup>
Linoleic acid	.56	1.96	—	.357 x 10 <sup>5</sup>
Dimethicone	.57	1.84	121.0	.456 x 10 <sup>4</sup>
Refined corn oil	.53	1.85	118.1	.130 x 10 <sup>5</sup>

Adapted from Smith et al, 1980

Compounds were tested using a 2-jet generator at a pressure of 5.0 lb/in<sup>2</sup>.



Refined corn oil has an odor characteristic of vegetable oils in general. This odor is variously described as ranging from unobjectionable to "rancid," often depending on past experiences where commercial fryers are used. The degree to which the odor is objectionable frequently depends on the extent to which the oil is reused. Concern about undesirable odor prompted NIOSH to ask practitioners who were using corn oil in QNFT in the field. The users indicated that refined corn oil aerosols had not resulted in objectionable odor or prohibitive maintenance problems (written communications from: M. H. Marcus, Jr., Industrial Hygiene Consultant, May 1981; D. P. Askin, Energy Saving and Clean Air Technologies, May 1981; and L. Saltsgaver, OSHA Training Institute, February 1981; verbal communication from L. Musen, Department of Energy, June 1981). Collectively, these field practitioners have conducted over a thousand individual fittings using a corn oil aerosol in QNFT.

Being a vegetable oil, corn oil requires cleanup procedures that are different from those needed for DEHP. Slightly more maintenance seems to be needed for corn oil than for DEHP. However, the maintenance required for corn oil would be less than that required by another QNFT system that uses sodium chloride to generate an aerosol test atmosphere. If proper routine maintenance is performed when using fresh refined corn oil in QNFT, objectionable odors should be negligible. For example, the generator may need routine daily cleanup, and the test chamber or plastic shroud may require cleaning if an oil buildup becomes noticeable.

NIOSH investigated whether fungal growth could be a potential problem with corn oil use in QNFT. In one testing site, mold formation was observed on an exhaust filter after several weeks of corn oil use (T. Williams, verbal communication, November 1980). No fungal growth was observed elsewhere on the equipment, including the test chamber. Examination of the filter in question by the Mycology Division, Centers for Disease Control (CDC), revealed a minimal presence of four common molds: Alternaria species, Ulocladium species, Aureo basidium (pullulans), and Aspergillus fumigatus (L. Ajello, written communication, September 1981). The first three organisms are not considered to be pathogenic; however, Aspergillus fumigatus is a ubiquitous pathogen of low infectivity. CDC indicated that there was no visible sign of any fungal growth on the filter they received. Only a few colonies of molds could be cultured from the filter. The organisms identified from the cultures could have originated from the filter or sources other than from the use of corn oil in QNFT, especially since all the molds cultured are common. CDC also advised NIOSH that corn oil would not be a good growth medium for fungi because of its viscosity and immiscibility with water. Furthermore, although associated directly with corn, aflatoxin contamination directly from corn oil is not likely, since any possibility of it would be eliminated by an alkali step in the refining of corn oil (Bennett and Anderson, 1978). The Appendix discusses more details of processing procedures for corn oil.

#### IV. TOXICITY OF OTHER AGENTS SUITABLE FOR QNFT

##### Dimethicone (Dimethyl Polysiloxane)

A silicone oil consisting of dimethylsiloxane polymers and purified for pharmaceutical use, dimethicone has been used extensively as a vehicle for topical drug applications and as a skin protectant (Windholz et al, 1976). Also, because of its antifoaming properties, dimethicone may be added to cooking oils for deep-fat frying. In veterinary practice, it is used to prevent bloat in cattle. The polydimethylsiloxanes occur in a wide range of molecular weights and viscosities and are noted for their water repellency. This has led to a wide variety of cosmetic, medical, and industrial applications.

The toxicity of the polydimethylsiloxane compounds examined has been of a low order (Calandra et al, 1976). Kennedy et al (1976) studied the potential for mutagenic, reproductive, or teratogenic effects with selected polydimethylsiloxanes in rats and rabbits. Test results indicated that Dow Corning (DC) 700 vapor booster pump fluid, 7 centistokes (cs), was not teratogenic in rats at po doses as high as 1 g/kg and was not mutagenic at ip doses of 5 and 10 g/kg. DC 225 fluid (10 cs) was also not observed to be teratogenic in rabbits at a dermal application of 200 mg/kg. In other experiments by the same investigators, DC 360 medical grade fluid (350 cs) was administered sc to pregnant rats on days 6 to 11 of gestation at doses of either 200 or 1,000 mg/kg. The authors reported an increase of incompletely developed sternebrae in fetuses derived from dams at the high dose. This response was not concluded by the investigators to be significant. The only significant effect found was an apparent dose-related incidence of in utero mortality at both dose levels of the DC 360 fluid. However, no evidence of fetotoxicity was observed by the authors in a second study performed on rats at the same doses.

No detailed studies were found describing the direct inhalation toxicity testing of DC 200 fluid, the siloxane considered by NIOSH as a candidate QNFT substitute. In 1976, Calandra et al reported a low order of inhalation toxicity to an aerosol of mixed cyclic siloxanes. However, a subchronic 90-day inhalation toxicity test of a polyethylsiloxane aerosol at concentrations of 0.2, 2, and 10 mg/m<sup>3</sup> to rats produced both localized pulmonary irritation and other generalized toxic effects (Tikhonova and Bizin, 1976). Tracheal inflammation and lung lesions were also noted. Furthermore, pathologic cellular changes were found in cardiac muscle and in the liver for animals exposed at the highest concentration. No pathologic changes were reported in animals exposed at 0.2 mg/m<sup>3</sup>.

##### Di-2-Ethylhexyl Sebacate (DEHS)

A monodisperse DEHS aerosol is used extensively to evaluate human pulmonary deposition (Swift, 1967). Although no adverse human health effects have been indicated from this application, a characterization of

the inhalation effects for DEHS is lacking. If this agent is to be used in QNFT, the inhalation toxicity of the compound must be examined.

Limited data from previous studies have indicated a low toxicity for DEHS. Treon et al (1955), using small numbers of test animals, reported no fatalities in cats, guinea pigs, rabbits, or rats exposed to a mist of DEHS at 400 mg/m<sup>3</sup>, for 7 hours/day over a 10-day period. However, 2/4 rabbits, 3/4 rats, and 0/2 guinea pigs died within 7 hours after inhaling a DEHS mist at 940 mg/m<sup>3</sup>, generated at high temperature (371°C). A po LD<sub>50</sub> for DEHS in the rat and mouse was reported to be 12.8-25.6 g/kg (Sandmeyer and Kirwin, 1981). An ip LD<sub>50</sub> greater than 25.0 g/kg was also reported for the rat and mouse.

Like DEHP, DEHS is a 2-ethylhexyl ester, but of sebacic rather than phthalic acid. A metabolite of DEHS, 2-ethylhexanol, is currently suspected by the NTP of being the proximate carcinogen in its bioassay of DEHP. The NTP may conduct a chronic bioassay study of 2-ethylhexanol, because two esters with a 2-ethylhexanol moiety (DEHP and di-2-ethylhexyl adipate) were both found to be carcinogenic in previous bioassays (NTP Chemical Selection Working Group, 1980). The mechanism of DEHP's carcinogenicity remains unknown, and it is not clear whether DEHP was carcinogenic in the NTP study because it contains a 2-ethylhexyl moiety. Until more data are available on the metabolism of DEHP or DEHS and the potential carcinogenic mechanisms of 2-ethylhexanol, questions about this chemical moiety of both DEHP and DEHS will remain unresolved.

Moody and Reddy (1978) fed either di-2-ethylhexyl adipate, DEHP, or DEHS to male Fischer 344 rats at a dietary concentration of 2% for 3 weeks. Hepatic peroxisome proliferation, increases in liver size and activities of peroxisome-associated enzymes, and hypolipidemia were observed in animals fed each of these esters. The same results with 2-ethylhexanol in the diet indicated to the investigators that the alcohol may be the active portion of the molecule responsible for the observed peroxisome changes in all three esters.

### Corn Oil

An LD<sub>50</sub> for corn oil (refined, U.S.P. XVII) in rats was obtained when daily intragastric doses were administered over 5 days (Boyd et al, 1969). No single gastric dose of corn oil could be retained sufficiently to induce death in rats. The LD<sub>50</sub> was finally achieved after 5 days by administering a cumulative dose totaling 279<sub>+31</sub> ml/kg (256<sub>+28</sub> g/kg), a dosage approximately equivalent to one-quarter of the animal's body weight. The dosage and time required to attain the LD<sub>50</sub> indicate a very low order of acute toxicity for refined corn oil. The Appendix discusses the components of corn oil and describes its refining and processing procedures.

A chamber-scarification test for assessing skin irritancy of topically applied substances was used to evaluate irritancy properties of corn oil (Frosch, 1977). The author did not specify whether the corn oil was

refined. Test results were compared with other oily materials frequently used in cosmetics. Corn oil was rated as only a "slight" irritant when applied for 72 hours on scarified human skin. An erythema developed along scratch lines of the test patch. Under the conditions of this test, the corn oil was ranked as more irritating than lanolin, but less irritating than mineral oil.

Corn oil has been used extensively without incident as a vehicle to administer test chemicals by gavage to rodents in a variety of experimental laboratory procedures, including carcinogen bioassays conducted by the National Cancer Institute (NCI). For example, histopathologic examination of Fischer 344 rats administered refined corn oil vehicle only and untreated controls showed no essential difference for observed neoplasms in the NCI test of bis(2-chloro-1-methylethyl) ether (NCI Report No. 191, 1979). The refined corn oil vehicle was administered at 1 ml/kg body weight by gavage 5 days/week for 103 weeks. Similar observations resulted from other NCI studies (NCI Report Nos. 68, 73, and 110, 1978). NIOSH emphasizes that such data do not reflect results of standard test conditions for a direct bioassay of refined corn oil, which was not the prime material under test, and that predetermined maximum tolerated doses were not given. However, NCI's negative findings in literally thousands of applications to rodents suggest that there is no suspicion of carcinogenicity for refined corn oil.

When administered for prolonged periods as a major component of the diet, either 5% or 20% of the feed, corn oil was reported to enhance colon carcinogenesis induced by 1,2-dimethylhydrazine (DMH) in Fischer 344 rats (Reddy et al, 1976). The authors did not specify whether the corn oil was refined. The study's observations have been related to mechanisms of carcinogenesis for known carcinogens in test animals having a high fat intake. For example, Reddy et al (1977) showed that a high-fat diet of unstripped corn oil resulted in the excretion of elevated amounts of bile acids. They emphasized that it was the elevated levels of bile acids caused by a high fat diet, and not the corn oil itself, that acted as colon tumor promoters during DMH carcinogenesis. The same response was observed by these investigators when animals were on a diet high in animal fat.

The results of a dietary study of refined corn oil was negative in a test for carcinogenicity (Szepesenwol, 1978). Mice of the T.M. strain, each fed 200 mg of refined corn oil in a dish daily up to 540 days, exhibited essentially no difference in the incidence of forestomach tumors when compared with untreated control mice. However, treatment with either (1) free fatty acids (up to 1.5%) added to refined corn oil or (2) crude corn oil (containing free fatty acids) elicited a high incidence of forestomach tumors. The authors attributed this carcinogenic response to free fatty acids, either added to the refined oil or present in crude corn oil. They also concluded that refined corn oil was not carcinogenic under these experimental conditions because free fatty acids are eliminated in the refining process.

O'Gara et al (1969) studied the effects of commercial (cooking) corn oil as a 10% mixture in the diet in NIH black rats. When corn oil either (1)

heated at 200°C for 6-8 hours, or (2) used repeatedly for deep-fat frying, was given to rats for 17 months, a higher incidence of alimentary tract tumors resulted compared with those of untreated or fresh corn oil-treated rats. The authors concluded that heating or extensive reuse (in frying) of the corn oil, which can change the oil's chemical composition, led to the tumors. The investigators advised against excessive reuse of cooking fats in the preparation of food for human consumption.

Little information is available on mutagenic or teratogenic effects. Refined corn oil, administered orally at a dose of 10 mg/kg to CD-1 mice, was one of several negative control substances used in a test of the sensitivity and reproducibility of a dominant lethal assay system (Anderson et al, 1977). Responses to refined corn oil in terms of pregnancy frequency, implantations, and early deaths were similar to those of three other control substances, including water. The results differed markedly from those of substances eliciting mutagenic effects.

Shoshkes et al (1950) compared the inhalation toxicity of refined corn oil and petroleum-derived oil aerosols. Mice were exposed to 12.6 g/m<sup>3</sup> of corn oil aerosols with a mass median particle diameter of 2.6 µm. Lungs examined immediately after a 6-hour exposure showed that oil droplets were limited to the terminal bronchiolar areas, and they impinged on the walls of alveolar ducts opposite the air stream. There was an immediate and active phagocytosis by macrophages, and the lungs were essentially cleared within 48 hours. This macrophage response in the lungs occurred only after a single 2-hour exposure, or at longer exposures of 4-8 hours/day, 5 days/week. With longer exposures to the corn oil aerosol, the number of lung macrophages increased. In contrast, petroleum-derived oils under the same experimental conditions were not enzymatically degraded and were only slowly removed from the lungs through the lymphatic drainage system. Patches of acute inflammatory changes, consistent with a diagnosis of lipoid pneumonia, were observed in mice exposed to the petroleum-derived oil. Refined corn oil and other edible oils, on the other hand, were lipolyzed rapidly by lung macrophages. In addition, refined corn oil did not irritate the upper respiratory tract, and effects such as reflex apnea or bronchoconstriction were not observed.

Review of clinical cases of lipoid pneumonia, which frequently results from oil droplets in the lungs, showed an increase in phagocytic cells in the lungs (Keshishian et al, 1969). Vegetable oils were found to be metabolized and removed from the lung, whereas oils of mineral origin tended to remain in the lung tissue. Mineral oil does not undergo metabolic changes; thus, it remains and can cause the formation of a paraffinoma. Adenocarcinoma of the lung has also been reported clinically in a patient with chronic mineral oil pneumonia (Bryan and Boitnott, 1968). The tumor was thought to have resulted from the pulmonary fibrosis elicited directly by the exposure to the mineral oil. Cases of localized lipoid pneumonia due to exogenous oil in lung alveoli have been correlated clinically with the prolonged treatment of nasal sinusitis with mineral oil-based nose drops (Borrie and Gwynne, 1973). These studies of lipoid pneumonia have indicated that the disease can result from large exposures to mineral oil over extended periods of time.

## Other Substances

Mineral oil and the polyethylene glycols, in addition to refined corn oil and DEHS, have been proposed by other laboratories as having physical and chemical properties that may render them suitable for generating a QNFT aerosol test atmosphere (Hinds et al, 1981). The observation that mineral oil tends to remain in the lung for prolonged periods, and reports of clinical cases exhibiting adverse reactions to the oil, preclude further consideration of its use in QNFT. The glycols, on the other hand, could be potential interim test agents provided that consistent and reproducible data of their suitability for QNFT become available. The low order of toxicity of many of the lower molecular weight polyethylene glycols is well documented (Smyth et al, 1950; 1955), although the pharmacodynamics of these materials warrant study. The United States Army has informed NIOSH of its successful applications of PEG 400, a polyethylene glycol of low molecular weight, to QNFT (Gerber, written communication, January 1981). The Army is now testing the inhalation toxicity of these glycols in addition to reviewing reports of biologic effects.

Low molecular weight polyethylene glycol (PEG 200) given to Cynomolgus monkeys by gastric intubation at 2-4 ml/kg daily for 13 weeks, elicited intratubular deposition of oxalate crystals in the renal cortex (Prentice and Majeed, 1978). The investigators indicated that ethylene glycol, a probable metabolite of PEG 200, could have induced the renal changes. The authors concluded that polyethylene glycols are not devoid of toxic manifestations following po administration, and that they should be used with caution. However, this response appears to vary with those suggested by other reports (Smyth et al, 1950; 1955). In addition, the monkeys examined were treated at such a high dose that relevance to QNFT would be questionable. Results of the Army's toxicity testing of these agents may better resolve the biologic properties of the polyethylene glycols for QNFT.

## Conclusions

Although limited, more toxicity information is available on corn oil than on dimethicone or DEHS. Overall, the data reviewed here indicate that refined corn oil has a relatively low order of toxicity. Attempts to determine LD<sub>50</sub>'s for refined corn oil in animals has required inordinately large quantities of the oil to be given over 5 days. Toxic responses to corn oil occurred only when its chemical composition was altered, as in heating to high temperatures, or in reuse, as in the frying of foods. Corn oil's chemical composition and physical properties are summarized in the Appendix.

NIOSH recently tested refined corn oil and DEHS for mutagenic activity in the Salmonella mutagenicity assay using tester strains TA98, TA100, TA1535, and TA1537. No mutagenic activity was observed with or without S-9 activation in any of the strains studied. NIOSH also evaluated the mutagenic potential for 2-ethylhexanol, a possible metabolite of both DEHS and DEHP. The metabolite was observed to be toxic, but not mutagenic, up to 1.0 mg/plate for all tester strains (Ong, 1981).

Inhalation testing of refined corn oil in rodents indicated a low toxicity for the compound, although NIOSH recognizes that much is unknown about the use of a corn oil aerosol in QNFT. Data describing the inhalation toxicity of DEHS and dimethicone are even more limited than that of corn oil. To resolve these deficiencies, NIOSH is presently conducting both acute and subchronic inhalation toxicity tests in rodents at the Johns Hopkins University. The two leading QNFT candidates, refined corn oil and DEHS, are being examined using an experimental protocol specifically designed to expose the test animals to polydisperse aerosols as human test subjects would be in QNFT. This study is scheduled for completion in April, 1983. For the present, however, refined corn oil is the stronger candidate as an interim QNFT test agent.

## V. EVALUATION AND RECOMMENDATIONS

QNFT is performed to assure that a proper facial fit is established for the individual respirator wearer. Since only a limited number of respirator sizes and styles are marketed, QNFT is critical in the fitting process because of the infinite number of human facial size variations and characteristics. Hence, DEHP has become an integral component of sound occupational safety and health programs designed to reduce worker exposure to respiratory hazards. DEHP has been widely accepted in QNFT for years because of its suitable physical properties for nebulizing a polydisperse aerosol test atmosphere. This acceptance of the DEHP aerosol in QNFT was also encouraged by reports of low toxicity for the compound. However, after examining the data in the NTP study, NIOSH concludes that DEHP must be considered to have a carcinogenic potential. NIOSH believes that a prudent future course is necessary in conducting QNFT and recommends that DEHP be discontinued in the procedure. Because QNFT is indispensable in establishing proper respirator fit, NIOSH recommends that an alternate aerosol test agent replace DEHP. NIOSH tested several candidate agents to determine their adaptability to QNFT and reviewed the toxicity of each compound. Results of these studies have led NIOSH to conclude that there are several agents presently available for use in existing QNFT equipment, originally made for the DEHP aerosol. After reviewing the toxicity of the candidate agents, NIOSH recommends that refined corn oil is the best option as a DEHP substitute in QNFT.

NIOSH testing results show that several compounds have properties that make them suitable for use in QNFT. The results demonstrate the ease with which other compounds may be substituted for DEHP in the procedure. Refined corn oil, DEHS, and dimethicone all adapted well to existing QNFT equipment. Aerosols of these candidate agents exhibited particle characteristics essentially equivalent to those generated with DEHP. Furthermore, these substances appear to have a low order of toxicity, although information on dimethicone that is relevant to QNFT is sparse. Refined corn oil and DEHS appear to be the stronger candidates to replace DEHP in the procedure. A substantial amount of data on biologic effects reviewed here indicate that refined corn oil has a low overall toxicity. Furthermore, inhalation testing of refined corn oil suggested a low toxicity in the rodent lung (Shoshkes et al, 1950). Limited data on DEHS reviewed here also suggest a low toxicity for this candidate. DEHS has been used for years in pulmonary physiology to assess human lung deposition without reports of adverse health effects. However, the inhalation toxicity of this compound does not appear to have been characterized, leaving questions about the potential risks of using the compound in QNFT. NIOSH anticipates that its recently initiated inhalation toxicity testing of refined corn oil and DEHS will resolve these critical questions. Results of these NIOSH experiments, presently underway at the Johns Hopkins University, are expected in April, 1983. For the present, NIOSH concludes from the available information that refined corn oil is the best option for use as a DEHP substitute in QNFT.



A study at Harvard University also indicated successful generation of polydisperse aerosols suitable for QNFT with refined corn oil, DEHS, polyethylene glycol (PEG 400), and mineral oil (Hinds et al, 1981). The polyethylene glycols are widely known to exhibit a low order of toxicity, but the data are sparse on the metabolic fate of these polymers. On the other hand, mineral oil has been shown to accumulate in the lungs, a fact that discourages its consideration for use in QNFT.

Based on the type and extent of exposure in QNFT and the toxicologic data reviewed herein, refined corn oil, DEHS, the polyethylene glycols (PEG 400), and dimethicone (Dow Corning 200 fluid) may all be considered as candidates that should receive serious consideration in QNFT. Of the three substances that NIOSH found to be suitable for use in QNFT, refined corn oil has a very low order of toxicity and stands out from all other agents reviewed, especially because of the extensive negative data on carcinogenic potential. The remaining candidates warrant more thorough evaluations of their toxicities, especially after further experimental investigations are available. The U.S. Army's toxicity testing of the polyethylene glycols may provide greater insight about these potential QNFT agents. In conducting this review, NIOSH has learned that many QNFT field practitioners are already using corn oil aerosols as a test atmosphere. Field testers have indicated that, although some increased maintenance has been required with its use, refined corn oil is acceptable as an alternative test agent to DEHP in respirator fitting tests.

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## VII. APPENDIX

### PROPERTIES OF AND PROCESSING PROCEDURES FOR CORN OIL

Corn oil is a clear, light yellow, oily liquid with a faint characteristic odor and taste (Windholz et al, 1976). It is obtained from the embryo of Zea mays, Gramineae (L.) as a byproduct of milling during the manufacture of corn starch, corn syrup, glucose, dextrins, etc.

Corn oil has widespread use as a nutritive substance in the diet. During crop year 1975-1976,  $257 \times 10^9$  g (92% of the total crop yield) were used in edible products such as cooking oil, salad dressing, and margarine (Reiners, 1978; Sonntag, 1979). As a food, corn oil yields 8.9 cal/g (Mazola Data Sheet, 1979).

Refined corn oil is composed almost entirely (99%) of triglycerides, which are the primary forms of fats in the body. These triglycerides contain a mixture of saturated and unsaturated fatty acids that include linoleic acid, a polyunsaturated fatty acid comprising 34-62% of corn oil, one of the "essential fatty acids." Corn oil is known as a "semi-drying" oil (Windholz et al, 1976) because it contains traces of fatty acid fractions that readily oxidize and lead to a drying (hardening) of the oil.

Iodine Number A measures the degree of unsaturation among fatty acid components, and the saponification value indicates the mean molecular weight of the fatty acid constituents. Unsaponifiable matter consists of water-insoluble compounds. The unsaponifiable fraction of crude corn oil is partially removed during alkali refining (Sonntag, 1979), with less than 1.5% remaining in the refined product. Most of the remainder (0.6-1.2%) are sterols that are relatively inert and may occur in the free form or as wax-like esters. In the diet, these sterols may enter into the biosynthesis of Vitamin D or steroid hormones.

Other major components of the unsaponifiable fraction are the tocopherols. This group of closely related fat soluble compounds is collectively known as Vitamin E. Traces of triterpene alcohols, less than 0.1%, may also be present in the unsaponifiable fraction; nine components have been identified in corn oil (Sonntag, 1979). Small amounts of ubiquinone (0.02%), an electron carrier known as coenzyme Q found in chloroplasts, have also been identified in the unsaponifiable fraction (Mazola Data Sheet, 1979).

The refining process removes free fatty acids and most nonglycerides, such as phosphatides, carbohydrates, carbohydrate derivatives, protein fragments, and trace resinous and mucilaginous materials (Sonntag, 1979). These substances give a water-holding ability to the crude corn oil. After they have been removed, the refined oil is almost anhydrous (0.1% water).

The alkali refining step removes any aflatoxins or pesticide residues that may be present. Waxes, colored carotinoid compounds, and volatile materials



are removed during other refining steps (Sonntag, 1979; Reiners, 1978). The resulting product is a light, bland oil with a smoke point of about 238°C.

### Corn Oil Additives

Silicones or polydimethylsiloxanes, which are antifoaming agents and also considered as QNFT aerosols, may be added (0.1-1.0 ppm) to oils for deep-fat frying (Formo, 1979). Any such additive must be listed on the label according to FDA requirements (21 CFR 101.6).

While corn oil contains natural antioxidants, chiefly tocopherols, which confer a greater resistance to oxidative deterioration than found in pure triglycerides, manufacturers have also added other antioxidants, particularly for deep-fat frying. Two of the most common antioxidants are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). The total concentration of antioxidants, singly or in combination, may not exceed 200 ppm (0.02%) (21 CFR 182.3173).

### Oxidative Deterioration

Oxidative deterioration does occur, especially at elevated temperatures during deep frying (Sonntag, 1979). A total of 95 (30 acidic, 65 nonacidic) volatile decomposition products in small amounts have been isolated from corn oil heated at 185°C for 30 hours. Oxidation polymers impart disagreeable tastes and odors to the oil. In general, corn oil darkens and increases in viscosity as the composition of the oil changes with prolonged heating.

Unsaturated fatty acids of corn oil can also oxidize (autoxidation) during prolonged exposure to air (Sonntag, 1979). The initial uptake of oxygen is relatively slow and uniform; color and odor changes coincide with rapid oxygen uptake. Natural antioxidants in corn oil increase the resistance to oxidation and also the amount of oxygen required to cause deterioration in flavor and odor (rancidity). Unhydrogenated corn oil can absorb up to 150% oxygen by volume before becoming rancid. Linoleic acid is the primary polyunsaturated component in oxidative rancidity. The first step is a conjugation of monohydroperoxides at double bonds in linoleic components. This reaction proceeds by a free radical mechanism to further yield hydroperoxides and decomposition products. Such products include low- and medium-weight (C<sub>3</sub>-C<sub>11</sub>) saturated and unsaturated aldehydes, ketones, and acids with strong, unpleasant odors. Rancidity occurs when less than 0.1% of the fat is decomposed. Polymers are produced under more prolonged conditions of autoxidation. The rate of oxygen uptake, leading to oxidative rancidity, is accelerated by heat, ultraviolet light, and near-ultraviolet light (Sonntag, 1979). Metals, particularly copper, act as pro-oxidants.

Since corn oil aerosol in QNFT is generated at room temperature and is not reused, much less oxidation would occur than with deep-frying conditions. Nevertheless, to minimize the possibility of autoxidation in QNFT, it is advisable to remove any unused oil at the end of each test session.

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