



CPSC Staff Statement on University of Cincinnati Report “Toxicity Review for Epoxidized Soybean Oil (ESBO)”¹

June 2019

The U.S. Consumer Product Safety Commission (CPSC) contracted with the University of Cincinnati to conduct toxicology assessments for nine dialkyl o-phthalate (o-DAP) substitutes: phenyl esters of C10-C18 alkylsulfonic acid esters (ASE); glycerides, castor-oil-mono-, hydrogenated, acetates (COMGHA); dibutyl adipate (DBA) and di-isobutyl adipate (DiBA); di (2-ethylhexyl) sebacate (DEHS)/dioctyl sebacate (DOS); a mixture of 98% di-2-ethylhexyl terephthalate (DEHT) and 2% 2-ethylhexyl methyl terephthalate (2-EHMT); dibutyl sebacate (DBS); diisononyl adipate (DINA); epoxidized soybean oil (ESBO); and tributyl citrate (TBC). The reports will be used to inform staff’s assessment of products that may contain these compounds and is the first step in the risk assessment process.

CPSC staff assesses a product’s potential health effects to consumers under the Federal Hazardous Substances Act (FHSA). The FHSA is risk-based. To be considered a “hazardous substance” under the FHSA, a consumer product must satisfy a two-part definition. First, it must be “toxic” under the FHSA, or present one of the other hazards enumerated in the statute. Second, it must have the potential to cause “substantial personal injury or substantial illness during or as a proximate result of any customary or reasonably foreseeable handling or use.” Therefore, exposure and risk must be considered in addition to toxicity when assessing potential hazards of products under the FHSA.

The first step in the risk assessment process is hazard identification, which consists of a review of the available toxicity data for the chemical. If it is concluded that a substance may be “toxic,” then CPSC staff will pursue a quantitative assessment of exposure and risk to evaluate whether a specified product may be considered a “hazardous substance.”

The toxicity review for ESBO follows. Based on the research conducted by the University of Cincinnati, the animal data support the conclusion that ESBO does not fit the designation of acutely toxic under the FHSA following single oral or dermal exposures. No acute inhalation studies were located for ESBO.

¹ This statement was prepared by the CPSC staff, and the attached report was produced by the University of Cincinnati for CPSC staff. The statement and report have not been reviewed or approved by, and do not necessarily represent the views of, the Commission.

**TOXICITY REVIEW FOR
EPOXIDIZED SOYBEAN OIL (ESBO)**

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1 Introduction

This report summarizes available data on the identity, physicochemical properties, manufacture, supply, use, toxicity, and exposure associated with epoxidized soybean oil (ESBO). The available toxicological studies on ESBO (which at the time was a potential candidate for use in children's articles) were briefly identified in a previous contractor report to CPSC (Versar, 2010).

Literature searches for physico-chemical, toxicological, exposure, and risk information were performed in June 2018 using the CAS number and synonyms (see Appendix 1 for the full list of search terms), and using the following databases:

- EPA SRS
- PUBMED
- RTECS
- TSCATS (included in TOXLINE)
- TOXNET databases, including
 - TOXLINE
 - CCRIS
 - DART/ETIC
 - GENE-TOX
 - HSDB

Searches were conducted for studies indexed to PubMed and Toxline databases from all dates to the date of the search (June, 2018). Other databases and websites were also used to identify additional key information, particularly authoritative reviews. Authoritative reviews for general toxicity and physicochemical information were identified in the following databases using the CAS number for DEHS/DOS and synonyms. Downloaded documents were saved as pdfs.

Websites reviewed included:

- ANSES Information on Chemicals (<https://www.anses.fr/en>)
- ChemIDPlus (<https://chem.nlm.nih.gov/chemidplus/>)
- ECHA Information on Chemicals (<https://echa.europa.eu/information-on-chemicals>)
- EFSA (<https://www.efsa.europa.eu/>)
- EPA chemistry dashboard (<https://comptox.epa.gov/dashboard>)
- EPA Chemview (<https://chemview.epa.gov/chemview>)
- EPA (<https://www.epa.gov/>)
- EPA IRIS (<https://www.epa.gov/iris>)

- FDA (<https://www.fda.gov/>)
- Health Canada (<https://www.canada.ca/en/health-canada.html>)
- IARC (<https://www.iarc.fr/>)
- INCHEM (<http://www.inchem.org/>)
- JEFCA (http://www.who.int/foodsafety/areas_work/chemical-risks/jecfa/en/)
- NICNAS (<https://www.nicnas.gov.au/>)
- NTP (<https://ntp.niehs.nih.gov/>)
- OECD (<http://www.oecd.org/>)
- WHO (<http://www.who.int/en/>)

Some limited supplemental searching using Google was conducted in February, 2019. In addition to identifying some additional exposure information, this supplemental searching identified a 2018 review article written by FDA scientists (Bandelet et al., 2018) that reviewed several toxicity studies that were not available from other sources.

2 Physico-Chemical Characteristics

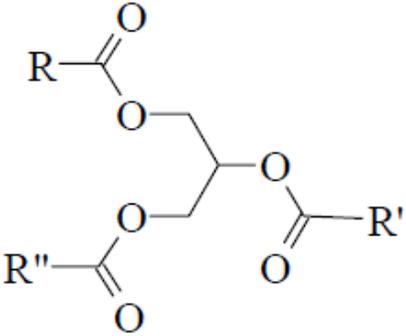
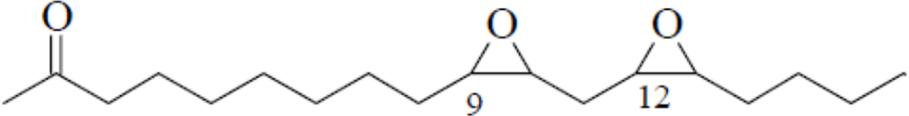
Epoxidized soybean oil (ESBO) is a mixture formed by the epoxidation of soybean oil, and so its fatty acid composition reflects the composition of soybean oil triglycerides. The major unsaturated fatty acid components of soybean oil triglycerides (which are thus susceptible to epoxidation) are 49-57% linoleic acid (containing two points of unsaturation), 26-36% oleic acid (mono-unsaturated), and 2-11% linolenic acid (three points of unsaturation) (BIBRA, 1997).¹ The remainder of the soybean oil consists of saturated fatty acids that are not susceptible to epoxidation because they lack double bonds, such as palmitic and stearic acids (Bandelet et al., 2018). The degree of epoxidation in ESBO is approximately 50% diepoxidized, 26% monoepoxidized, and 11% triepoxidized.

ESBO and other epoxidized fat compounds are typically graded based on the degree of epoxidation and the number of unsaturated carbon bonds. The extent of epoxidation is most often denoted by an oxirane oxygen content value, which is the average percentage of the compound composed of epoxide oxygen by weight. The prevalence of non-epoxide unsaturated carbon bonds is represented by an iodine number, the amount of iodine in g that can react fully with 100 g of the non-saturated fat. Some older references may use an “epoxide (or epoxidation) number”. This typically represents the extent/number of epoxide groups but does not have a uniform definition (Hang et al., 1999).

Some physical and chemical properties of ESBO are summarized below in Table 1.

¹ References differ in the reported fatty acid composition of ESBO. For example, EFSA (2004) reported 11% palmitic acid, 4% stearic acid, 23% oleic acid, 55% linoleic, and 8% linolenic acid.

Table 1: Physicochemical Properties and Identification Information for Epoxidized Soybean Oil (ESBO)

Chemical Name	Epoxidized soybean oil
Synonyms	2,3-bis[8-[3-[(3-pentylloxiran-2-yl)methyl]oxiran-2-yl]octanoyloxy]propyl 8-[3-[(3-pentylloxiran-2-yl)methyl]oxiran-2-yl]octanoate
CAS Number	8013-07-8
Structure	 <p>Where R, R' and R'' = an epoxidized hydrocarbon chain (sample represented with linoleic acid, including the ketone group before the R, R' or R'')</p>  <p>(EFSA, 2004)</p>
Chemical Formula	C ₅₇ H ₉₈ O ₁₂ (based on linoleic acid for all three R groups) (PubChem, 2019) Unspecified, due to mixture of variable R groups (OECD, 2006b)
Molecular Weight	975.399 g/mol (based on linoleic acid for all three R groups) (PubChem, 2019) 940 – 950 g/mol (OECD, 2006b)
Physical State	Oily liquid (PubChem, 2019)
Color	Pale yellow (PubChem, 2019)
Melting Point	-2.2°C (measured) (OECD, 2006b)
Boiling Point	Very high (PubChem, 2019) Decomposes at 176-204°C (OECD, 2006b)
Vapor Pressure	<0.001 Pa at 25°C (estimated) (OECD, 2006b) ¹

Water Solubility	0.00099 g/L at 20°C (estimated) (OECD, 2006b)
Log Kow	6.2 (estimated) (OECD, 2006b)
Flashpoint	585°F (PubChem, 2019) 315°C (OECD, 2006b)
Density	0.9875-0.9930 g/cm ³ at 25°C (measured) (OECD, 2006b)
BCF	375 (OECD, 2006b)
Source	As stated

K_{ow} is the octanol-water partition coefficient. BCF is the bioconcentration factor. See Appendix 2 for more detail.

¹ OECD (2006b) reports that “The vapor pressure of the EOD materials is below the ability to measure, which is expected based on the high molecular weights of these materials. The reported vapor pressure values are presumed to reflect the influence from minor impurities having relatively low boiling points.”

3 Manufacture, Supply, and Use

Manufacture and Supply

ESBO is a high production volume chemical with U.S. manufacture and imports reported between 100 and 250 million pounds (50,000 to 125,000 tons) per year for 2015 (U.S. EPA, 2019). The Chemical Economics Handbook reported U.S. production of ESBO to be 42,000 metric tons in 2014, which was about 6% of total plasticizer production in the U.S. (Bandelet et al., 2018). ESBO is manufactured and/or imported in the European Economic Area at a rate of 10,000 – 100,000 tons per year (ECHA, 2019).

Use

ESBO is used as a plasticizer, particularly for polyvinyl chloride (PVC). It also acts as a scavenger for hydrochloric acid (HCl) (which is formed by the thermal degradation of PVC, such as occurs during sterilization), thus stabilizing the plastic (Barone et al., 2015). ESBO is used to improve heat stability during the production and processing of PVC/PVDC (polyvinylidene chloride) articles (Bandelet et al., 2018).

A major use of ESBO is as a plasticizer in PVC gaskets for metal lids of glass jars, but it is also used in paints and lacquers, adhesives and sealants, polymers, lubricants and greases, printing inks, packaging, and finger paints, (Lowell Center, 2011; ECHA, 2019). ECHA notes that ESBO can be found in additional types of complex products, such as those made of plastic, metal, textiles, rubber, stone, plaster, cement, glass, or ceramic (ECHA, 2018). In Europe ESBO is reportedly used in consumer products such as tablecloths, curtains, and shower curtains (ECHA, 2012). As noted in Section 6.0, several studies reported levels of ESBO in toys. Epoxidized oils such as soybean and linseed oils are used in rubbers, epoxy resins, paints, and coatings, where

they act as lubricants and HCl scavenger (DEZA, 2013). ESBO is also approved for use as an inert ingredient in pesticides, where it serves as an acid scavenger to protect equipment (U.S. EPA, 2004, as cited by OECD, 2006).

ESBO is one of most commonly used additives for PVC, especially for food-contact applications (EFSA, 2004). Concentrations can reach 40% in sealing closure gaskets in lids for glass jars and 10% in PVC stretch food wrap films (EFSA, 2004). ESBO is used at concentrations of 8-13 % in food wraps, greater than 8% in adhesive-backed film for surgical and industrial tape, and 6-8% in medical applications (Bandelet et al., 2018). It is also mixed with PVC-based lacquers that are used to coat metal food cans (EFSA, 2004). ESBO is permitted by FDA for use in foods as a formulation aid, lubricant or release agent, and stabilizer or thickener and as an indirect additive to foods via use in food contact surfaces in packaging (FDA, 2019).

Versar (2010) noted that epoxidized oils such as ESBO are attractive candidates for replacing phthalates in toys or children's articles, based upon their approved use for food contact materials, their higher biodegradability than other plasticizers, and because they do not need metal stabilizers.

4 Toxicokinetics

No detailed toxicokinetic studies were located for ESBO. Although information specific to ESBO is not available, it is assumed that the absorption and metabolism of ESBO is similar to that of other triglycerides. In the gastrointestinal (GI) tract, pancreatic lipase hydrolyzes triglycerides into mono- and diglycerides and free fatty acids, which are then absorbed into the body. Metabolism via esterases (carboxylesterase and similar enzymes) eventually yields glycerol and free fatty acids (OECD, 2006a). Oral absorption of ESBO has been studied in a small number of volunteers (see below). Absorption of ESBO via inhalation or dermal routes has not been characterized in any available literature, but with its high molecular weight, insolubility in water, and high log Kow, dermal absorption is not likely to be significant.

Wilson et al. (2002) measured the kinetics of synthetic epoxidized fatty acids in healthy female volunteers, primarily to judge the extent of absorption. In this study, 6 and 7 women per group, respectively, consumed triglycerides containing uniformly [¹³C]-labeled 20 mg monoepoxy or 25 mg diepoxy stearic acid. The triglycerides were provided in a 100 mL vehicle comprising walnut oil, low-fat milk, and milkshake powder (approx. 29 g fat total). The women were in a fasting state (morning) at administration and were given a low-fat lunch during the day (subjects were hospital workers at work). Plasma levels of the radiolabeled fats were measured at 0, 2, 4, 6, 8, and 24 hours. It was estimated that 17±4% of the monoepoxy fatty acids were absorbed and peak plasma levels were seen at 6 hours. Absorption of the diepoxy fatty acids was estimated at 8±1%, and peak plasma levels were seen at 2-4 hours. The plasma concentrations of the [¹³C]-labeled lipids varied considerably between individuals, and area under the concentration-time curve (AUC) and half-life values were not reported. The authors reported that monoepoxidized and diepoxidized fatty acids make up 5% and 16% of triglyceride-bound fatty acids, respectively, in

fully epoxidized soybean oil, and so this study provides some information related to absorption of two components of ESBO. (BIBRA, 1997, cites approximately 26% and 50% of fatty acids in ESBO being monoepoxy and diepoxy species, respectively.)

The excretion of ESBO was investigated by Eagle and Harlan (1960, as cited by Bandele et al., 2018). Seven rats (strain and sex not provided) were given an unspecified amount of [¹⁴C]-labeled epoxidized fatty acid methyl esters via gavage. The esters comprised a mix of saturated and unsaturated C16 and C18 fatty acids that were specified to resemble the natural composition of soybean oil. Animals were kept in metabolic cages for 80 hours, and then sacrificed. Approximately 93% of the administered radiolabel was recovered, with 68% recovered in expired CO₂, 13.5% in feces, 3.5% in urine, and 8% in the remaining tissues/carcass. The radiolabel in the carcass was broadly distributed and was not concentrated in any particular tissues. These data support the assumption that ESBO is metabolized similarly to other fats, eventually being completely catabolized and predominantly exhaled as CO₂.

5 Hazard Information

5.1 Acute Single Dose Toxicity

5.1.1 Acute Oral Toxicity

Acute toxicity of ESBO by the oral route is low. Two studies in rats (10/group, strain/sex not provided) reported slight breathing difficulty, lethargy, and diarrhea following gavage administration of 5000 mg/kg or 5 mL/kg ESBO (oxirane oxygen 6.4%, iodine value 4.3). No deaths were seen. Tissues (not further specified) showed no gross abnormalities, but hunched posture and ruffled fur was observed for 1-4 days (Ciba-Geigy, 1981d, HRC, 1973, as cited by BIBRA, 1997). LD₅₀ values for rats have been reported as greater than 5000 - 40,000 mg/kg but the grade of the ESBO was not specified in these reports (BIBRA, 1997).

5.1.2 Acute Dermal Toxicity

ESBO has low toxicity by the dermal route. LD₅₀ values were reported as greater than 20 mL/kg in 24-hour contact studies in rabbit (Weil et al., 1963, as cited by BIBRA, 1997). The grade of the tested ESBO was not specified.

5.1.3 Acute Inhalation Toxicity

No acute inhalation studies were located for ESBO.

5.1.4 Irritation/Sensitization

ESBO has the potential to cause mild-to-minimal irritation to the skin. Commercial grade ESBO (oxirane oxygen 6.4%, iodine value 4.3) caused mild reddening and minimal swelling of intact skin and was moderately irritating to abraded skin following covered contact for 24 hours in rabbits (Ciba-Geigy, 1981a, as cited by BIBRA, 1997). Another study using an unspecified grade

of ESBO reported slight skin irritation following 24 hour uncovered contact in rabbits (Weil et al., 1963, as cited by BIBRA, 1997).

ESBO may be mildly irritating to the eyes. Reddening of the conjunctiva was reported following instillation of 0.1 mL ESBO (oxirane oxygen 6.4%, iodine value 4.3) in rabbit eyes (Ciba-Geigy, 1981b, as cited by BIBRA, 1997). In contrast, no eye irritation was reported following instillation of 0.5 mL ESBO in rabbit eyes (Weil et al., 1963, as cited by BIBRA, 1997). The different results of the two studies may reflect the testing of different grades of ESBO; the grade was not specified in the latter study.

Sensitization to ESBO has been reported anecdotally but lab-based testing has failed to demonstrate this. Pauli et al. (1980) presented three occupational case reports of “meat wrapper’s asthma” associated with PVC films containing ESBO and other compounds. One case received a follow-up where asthmatic signs could be elicited with PVC films or paper labels. Experimental inhalation exposure to either phthalic anhydride (a known sensitizer and constituent in paper labels) or ESBO, but not other constituent compounds, elicited asthmatic signs. Airway obstruction was seen 5 minutes after ESBO exposure and persisted for 12 hours. The grade specifications of the ESBO were not reported.

Two studies have attempted to demonstrate sensitization via intracutaneous injections of ESBO in guinea pigs. Ciba-Geigy (1981c, as cited by BIBRA, 1997) injected the guinea pigs (20/group) with 0.1 mL of 0.1% ESBO (oxirane oxygen 6.4%, iodine value 4.3) on alternating days for 10 injections. The guinea pigs were challenged 3 weeks later by injection with the same dose, and were challenged a second time 2 weeks later with dermal exposure to a 30% solution. Both challenge tests were negative. Another study in 20 guinea pigs used eight injections of diluted ESBO (concentration and grade not provided) on alternating days, followed by challenge injection 3 weeks later (Weil et al., 1963, as cited by BIBRA, 1997). This study was also negative.

A case study reported a worker developing eczema due to exposure to materials used in screwdriver handles containing cellulose acetate, ESBO, and unhardened low-molecular-weight epoxy resin. The unhardened resin was presumed to be the primary sensitizing agent and it was not clear if ESBO played a role (Fischer et al., 1987).

5.2 Repeated Dose Toxicity

Several reports on the oral repeat-dose toxicity of ESBO are available, but several are unpublished and documentation is often limited. Overall, the repeat-dose toxicity of ESBO appears to be low by the oral route, but certain grades appear more toxic than others. Where toxicity is documented, target organs are liver, kidneys, and reproductive tissues (uterus and testes). Decreased growth rates are documented in some cases.

A limitation of the toxicology database is that almost all of the studies were available only from secondary sources, and several were conducted using methods that were incomplete compared to modern testing standards. However, a recent review by FDA scientists (Bandelet al., 2018) focused on the higher-quality studies, including several unpublished studies that the authors had directly reviewed and reported on in some detail.

A 12-day feeding study (Mounie et al. 1988, as cited by BIBRA, 1997) in rats (6/group, sex and strain not provided) given dietary ESBO (oxirane number 6-6.8%, iodine value not given) at a dose of approximately 2500 mg/kg-day showed decreased growth rates, but the degree of change was not reported. Liver weights and serum transaminase levels were not affected. The summary by BIBRA (1997) reported that epoxide hydratase (which metabolizes epoxide groups to diols) was upregulated by ESBO. The full range of endpoints examined was not clear.

BIBRA (1997) summarized a 10-week feeding study (Kieckebusch et al., 1963) in rats (sex, strain, and group size not given) reporting dietary exposure to a range of ESBO variants that did not “match the specification of those in commerce today.” Five variants with different epoxide numbers ranging from 14.6 to 111.5 were tested and iodine values ranged from 0.9 to 113.2. A level of 20% dietary ESBO (approx. 10,000 mg/kg-day²) was tested against a 20% soybean oil control and the testes, liver, kidneys, heart, and intestines were examined microscopically. Growth retardation, testicular degeneration, and deaths were observed in rats given ESBO of epoxide numbers 105 and 111.5. Fatty degeneration of the liver and kidneys occurred with lower epoxide numbers and also in the soybean oil controls. All ESBO variants caused decreased growth rates. Another rat experiment reported in Kieckebusch et al. (1963, as cited by BIBRA, 1997; strain, sex, and group sizes not given) treated rats with dietary ESBO with epoxide number 220 for 8 weeks. This study observed growth retardation at a dose of approximately 500 mg/kg-day (1% in the diet) in a 20% soybean oil diet. Increasing the dose to approximately 2500 mg/kg-day (5% in diet) caused deaths in 6/54 rats. No other details were available.

In perhaps the best-documented subchronic study of ESBO, Holtzman albino rats (10/sex/dose) were fed diets containing 0, 0.1, 0.5, 1.0, 5.0, or 10% ESBO (epoxide content 6.8%, iodine number 1.1) for 90 days (Eagle, 1960b, as cited by Bandele et al., 2018). Bandele et al. (2018) reported that the corresponding dose levels were 0, 25, 125, 250, 1250, and 2500 mg/kg-day for both sexes. One high-dose rat died on day 51, due to abdominal hemorrhage. Adverse effects were limited to the high dose, and were reported to include growth suppression, and increased liver and kidney weight. Increased liver weight was also reported at 1250 mg/kg-day, but was considered equivocal, and there were no reported treatment-related effects at lower doses. There was no evidence of histopathology at any dose, but no information was provided on which tissues were evaluated. No data on urine or hematology parameters were reported. Bandele et al. (2018) reported that FDA considered the NOAEL to be 1250 mg/kg-day, based on growth suppression and increased liver and kidney weight at 2500 mg/kg-day.

² Dose conversion provided by BIBRA (1997). Bandele et al. (2018) reported the corresponding dose as 5000 mg/kg-day, which is more consistent with the conversion reported for the Mellon Institute of Industrial Research (1960) study.

In another unpublished subchronic study reviewed by FDA scientists, beagle dogs (2/sex/dose) were fed diets containing 0, 1.0, 2.5, or 5.0% ESBO (oxirane content 6.8%, iodine number 1.1) for 14 weeks (Eagle, 1960a, as cited by Bandele et al., 2018). The corresponding doses were reported as 0, 250, 625, and 1250 mg/kg-day. “Decreased feed efficiency” and decreased growth were reported at the high dose (degree of change not reported), but there were no gross or microscopic findings (tissues evaluated not reported). Bandele et al. (2018) reported the study NOAEL as 625 mg/kg-day.

Bandele et al. (2018) also reported on subchronic rat studies for which only summary data were available. In the first study, albino rats (strain not reported; 10/sex/dose) were fed 0, 0.04, 0.2, 1.0 or 5.0% ESBO in the diet (epoxide content 6.3%, iodine number of 6-14; described as Plasticizer EPO and Paraplex G-62) for 90 days (Mellon Institute of Industrial Research, 1960, as cited by Bandele et al., 2018, OECD, 2006b). The corresponding doses were reported by Bandele et al. (2018) as 0, 10, 50, 250, and 1250 mg/kg-day. It appears that Bandele et al. (2018) and OECD (2006) were describing the same study, but there are some slight differences in the reported results, with Bandele et al. (2018) reporting based on greater generalizations. OECD (2006) reported that males had decreased body weight gain (both compounds) and food consumption (Plasticizer EPO only) at the high dose, but only during the first weeks of dosing. In the females, decreased food consumption was reported at the two top doses with both test materials, but there was no effect on body weight gain. Liver weight was increased in both sexes at the high dose, and in only one sex at the second highest dose. OECD, 2006 reported the males as being affected at 1%, while Bandele et al., 2018 described the females as being affected at 1%. Gross findings in kidneys (not further described) were reported in males at the two top doses, and in the liver of both sexes at the high dose (Paraplex G-62) or the two top doses (Plasticizer EPO). Based on the liver effects, the NOAEL was reported by the authors at 50 mg/kg-day, with a LOAEL of 250 mg/kg-day.

An unpublished 2-year feeding study (BIBRA, 1986, as cited by BIBRA, 1997, Bandele et al., 2018, OECD, 2006b) in Wistar rats (48/sex/dose) administered ESBO (oxirane oxygen 6.3-6.4%, iodine number 7-8) in the diet at 0, 0.025, 0.25, or 2.5%. The corresponding doses were reported as 0, 10, 100, and 1000 mg/kg-day for males, and 0, 14, 140, and 1400 mg/kg-day for females. The study measured survival, blood parameters, urinalysis, and microscopic examination of a comprehensive range of organs. There were no deaths and no treatment-related tumors. The high-dose males had increased body weights, while the high-dose females had slightly decreased body weight and water consumption at the high dose. Food consumption was decreased in both males and females, with a larger decrease in females. Effects at the high dose included “slight changes” (histopathology) in the uterus of females at the high dose (described by Bandele et al., 2018 as increased uterine weight), and increased liver and kidney weights in males (no quantitative information reported). The study NOAEL was defined as 100 mg/kg-day by the United Kingdom Committee on Toxicity, based on changes in organ weight. Applying a safety factor of 100 to this NOAEL, the Committee established a tolerable daily intake (TDI) of 1 mg/kg-day (U.S. Department of Health, 1995, as cited by Bandele et al., 2018). That TDI was also adopted by the European Union Scientific Committee on Food and confirmed by the European Food Safety Authority (EFSA, 2004).

Another study (BIBRA, undated, as cited by BIBRA, 1997), using apparently the same material, reported liver and kidney enlargement and fatty liver accumulation in rats following dietary exposure at 2.5% (approx. 1300 mg/kg-day) for only 15 weeks. No other details were given in BIBRA (1997), but a brief summary of this study by OECD (2006a) reported a NOAEL of 1.5% (approx. 780 mg/kg-day based on the above conversion) with critical effects being liver and kidney enlargement and transiently decreased growth. OECD (2006a) also reported that the doses in this study ranged up to 5.0% diet.

Another 2-year feeding study (Larson et al., 1960) in albino rats (15/sex/dose, strain not specified) tested two ESBO products (Paraplex G-60 and Paraplex G-62). The specifications were not provided by product, but Bandele et al. (2018) stated that one product had an epoxide content of 6.3% and iodine number of 6-14, and the other had an epoxide content of 6.8% and iodine number of 1.1. The compounds were added to the diet at 0, 0.1, 0.5, 1.0, 2.5, or 5.0% (approximately 0, 50, 250, 500, 1250, and 2500 mg/kg-day based on conversions given in BIBRA, 1997 summary³). This study used different endpoints at intermediate and 2-year time points. In the Paraplex G-60 group (6.3% epoxide), 5 rats/sex/dose were assessed at 1 year for: hematology, organ weights, and histopathology (heart, lung, liver, kidney, spleen, GI tract, thyroid, adrenal, pancreas, gonads, muscle, bone marrow). Early depression of growth was observed at 2500 mg/kg-day in both sexes, which resolved by 8 weeks with continued feeding. The only other effect of Paraplex G-60 was elevated liver-body weight ratios in male rats given 2500 mg/kg-day for 1 year. In the Paraplex G-60 group, 3-8 rats/sex/dose survived until 2 years (survival was not affected by treatment). In 2-year groups, only hematology and histopathology were assessed and no significant effects were noted. In the Paraplex G-62 groups, the 5 rats/sex/dose in the interim sacrifice group were assessed at 6 months in the same way as above. In the 6-month groups, Paraplex G-62 caused decreased growth rates in both sexes (resolving by 8 weeks for females but not males), elevated liver-body weight ratios in both sexes and also caused elevated kidney-body weight ratios in females. Regression analysis by the authors predicted the following threshold doses (in diet percentage) for effects, based on the 95% confidence intervals: 1.0% in females and 1.6% in males for decreased growth, 0.5% in females and 2.3% in males for elevated liver weights, and 1.6% for increased kidney weight in females. However, the degree of change for these “effective concentrations” was not reported. Histopathology showed no changes in organs at 6 months. Rats surviving to 2 years (3-8/sex/dose) were assessed as above and showed no effects attributable to treatment.

Larson et al. (1960) also exposed mongrel dogs (3/group) to either ESBO product in the diet at 0.1, 1.0, and 5.0% (25, 250, 1250 mg/kg-day based on conversion by BIBRA, 1997) for 1 year; no control group was included. The same endpoints were evaluated as in the rat study. No effects were seen except for weight loss in the 1250 mg/kg-day group for either product. The weight loss appeared to be due to aversion to the food. One dog had a slightly fatty liver in the 1250 mg/kg-day Paraplex G-62 group.

In a reproductive study (CIT, 1993a, as cited by BIBRA, 1997, Bandele et al., 2018) that also recorded a range of parameters in Sprague Dawley rats (28/sex/dose, strain not given), ESBO

³ Bandele et al. (2018) reported the doses as 0, 25, 125, 250, 625, and 1250 mg/kg-day.

(oxirane oxygen 6.4%, iodine value 4.3) was administered by gavage at doses of 0, 100, 300, or 1000 mg/kg-day. Treatment was daily from 71 days prior to mating (males) or 15 days prior to mating (females) until pups were 21 days old. No effects on growth, blood chemistry, and liver, ileum, kidneys, and reproductive organ histopathology were seen, and the high dose of 1000 mg/kg-day was a systemic NOAEL.

Another citation reported “no adverse findings” following twice-weekly administrations of 1.4 g/kg to rats for 16 months and dogs for 12 months (Krauze & Homrowski, 1961, as cited by BIBRA, 1997). No further details were given.

5.3 Chronic Toxicity/Carcinogenicity

Chronic toxicity data for ESBO is available from two 2-year oral studies in rats, and one dermal study in mice (Weil et al., 1963, as cited by BIBRA, 1997). There is no evidence of carcinogenicity based on these reports, although only one study, the rat study by BIBRA (1986, as cited by BIBRA, 1997, Bandele et al., 2018) was sufficiently well-conducted with an adequate number of animals to detect a response.

In the unpublished 2-year study originally reported in BIBRA (1986, as cited by BIBRA, 1997, Bandele et al., 2018), no evidence of carcinogenicity was seen in rats (48/sex/dose) given up to 2.5% (up to approx. 1000 mg/kg-day for males and 1400 mg/kg-day for females) ESBO (oxirane oxygen 6.3-6.4%, iodine number 7-8) via diet. A “comprehensive” range of tissues was examined.

In the 2-year study by Larson et al. (1960), major tissues were examined in 3-8 animals from each of the control, 2.5%, and 5% dietary dose groups (approx. 0, 1250, and 2500 mg/kg-day). No evidence of carcinogenicity was seen in rats given ESBO in the form of Paraplex G-60 or Paraplex G-62 (not further specified) up to approx. 2500 mg/kg/day by diet.

A brief abstract (Weisburger et al., 1965, as cited by BIBRA, 1997) reported a “slight enhancing effect” on the effects of an unnamed co-administered primary liver carcinogen in rats (sex/strain not given) administered 400 mg/kg-day unspecified ESBO via gavage 5 days/week for 1 year.

Skin tumors were not observed when an unspecified amount of undiluted ESBO was applied to the skin of 40 mice (sex/strain not given) three times weekly for the lifetime of the animals (Weil et al., 1963, as cited by BIBRA, 1997). All of the mice died before the end of 2 years. BIBRA (1997) noted that this study had limited potential for detecting carcinogenesis, due to the small sample size, the intermittent treatment, and limited extent of tissue examination.

5.4 Reproductive Toxicity

Although repeated dose studies have occasionally reported some effects on male and female reproductive organs (see Section 5.3), no effect on reproductive function was observed in a standard reproductive toxicity study in rats. ESBO has also tested negative in mechanistic assays for human endocrine activity related to reproduction.

In a Good Laboratory Practices (GLP)-compliant reproductive study (CIT, 1993a, as cited by BIBRA, 1997, Bandele et al., 2018), Sprague-Dawley rats (28/sex/dose) were administered up to 1000 mg/kg-day ESBO (oxirane oxygen 6.4%, iodine value 4.3) via gavage from day 71 (males) and day 15 (females) prior to mating until postnatal day (PND) 21. There were no effects on fertility, number of live births, offspring birth weight, sex ratio, pup growth, physical or reflex development of the offspring, or macroscopic appearance of a wide range of tissues in the pups. There was also no effect on the adults, based on growth, blood composition, or microscopic appearance of the liver, ileum, kidneys, and reproductive organs. The high dose of 1000 mg/kg-day was a reproductive and systemic NOAEL.

In a related range-finding study, (CIT, 1993c, as cited by BIBRA, 1997, Bandele et al., 2018) Sprague-Dawley rats (12/sex/dose) were gavaged with the same form of ESBO at 0, 150, 450, or 1000 mg/kg-day for 15 days prior to mating until PND 7. No effects were seen in a similar range of endpoints as above, with examination for malformations limited to external, heart, liver, kidneys, stomach and intestine.

Endocrine toxicity of ESBO has been investigated in a few mechanistic studies. This testing was motivated by an interest in the endocrine activity of PVC ingredients, given the prevalence of endocrine effects associated with PVC mixtures. An *in vivo* study by Ohta et al. (2003)⁴ measured uterine wet weight, hyperplasia of endometrium, and cornification of vaginal mucosa in ovariectomized female Crl:CD (SD) rats (10/dose) following 3-day exposures to ESBO (grade not specified) at gavage doses of 0.5 or 500 mg/kg-day (gavage) or subcutaneous doses of 0.5 or 100 mg/kg-day. No changes were observed. In the same study, ESBO was negative in competitive binding assays for the human estrogen receptors ER α and ER β , as well as rat androgen receptor, at concentrations up to approximately 100 nM. Another study (Ohashi et al., 2005) tested commercial ESBO (grade not specified) and found no affinity for human ER α at concentrations up to 100 mM.

Slight histopathology changes in the uterus, accompanied by slight changes in uterus weight (further details not available) were reported in Wistar rats fed 2.5% ESBO (6.3-6.4% epoxide, iodine number 7-8) in the diet (about 1400 mg/kg-day) for 2 years (BIBRA, 1986, as cited by BIBRA, 1997, Bandele et al., 2018). In the repeat-dose study by Kieckebusch et al. (1963, as cited by BIBRA, 1997) testicular degeneration was observed in male rats (number/strain not given) fed dietary ESBO at a concentration corresponding to about 10,000 mg/kg-day for 7 weeks. The grades of ESBO in this case (epoxide numbers ranging from 105 to 111.5) was not comparable to modern commercial ESBO grades.

⁴ This document was predominately in Japanese and some details reported here based on partial translations using Google Translate (v.5.26.59113).

5.5 Prenatal, Perinatal, and Post-natal Toxicity

In the only developmental toxicity study available for ESBO, pregnant Sprague-Dawley rats (25/dose) were administered gavage doses of 0, 100, 300, or 1000 mg/kg-day ESBO (epoxide content 6.4%, iodine number 4.3) on gestation days (GD) 6-15 (CIT, 1993b, as cited by BIBRA, 1997, Bandele et al., 2018). There were no clinical signs of maternal toxicity. There was also no effect on the number of corpora lutea, implantation sites, number of live fetuses, or post-implantation loss. In offspring, a non-significant increase in skeletal anomalies was seen; no other effects on fetal weight, sex ratio, or external, skeletal, or soft-tissue malformations were observed. The study authors considered the high dose of 1000 mg/kg-day to be a maternal and developmental NOAEL.

In an *ex vivo* embryotoxicity experiment comparing ESBO with various phthalate esters (Rhee et al., 2002), no effects on the growth or morphological differentiation of D9.5 whole rat (Wistar) embryos were seen when cultured with up to 750 µg/mL ESBO for 48 hours. Impairments in these endpoints were seen with exposure to diethyl hexyl phthalate, butylbenzyl phthalate, and dibutyl phthalate at considerably lower concentrations in this format.

5.6 Genotoxicity

ESBO has been tested in a number of bacterial mutation assays, and was consistently negative. ESBO was not mutagenic in a conventional Ames assay using Salmonella strains TA98 and TA100 with or with S9 mix from rat liver (Monsanto, 1986). ESBO (oxirane oxygen 6.4%, iodine number 4.3) was not mutagenic in Ames tests, with or without S9 (Ciba-Geigy, 1981e, Hazleton, 1992, both as cited by BIBRA, 1997). ESBO was also negative in Ames assays using Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538 (Heath and Reilly, 1982). In an *in vivo/in vitro* modification of the Ames assay, male Long-Evans rats were gavaged with 1000 mg/kg ESBO, and homogenates of the stomach and small intestine were tested in the Ames assay with the bacteria exposed for 1 or 6 hours. In this assay, TA98 and TA100 Salmonella strains were tested; the results were not different from control homogenates from rats administered distilled water (Monsanto, 1987a). ESBO was also tested for mutagenicity using the HGPRT mutation assay in CHO cell cultures. Mutagenicity was not evident at exposures up to 2 mg/mL either with or without metabolic activation by S9 mix (Monsanto, 1987b).

ESBO (oxirane oxygen 6.4%, iodine number 4.3) was tested for chromosomal damage in human and mouse lymphoma cells. No chromosomal aberrations or aneuploidy were observed with or without activation by S9 mix from rat liver in either cell type (Hazleton, 1992, as cited by BIBRA, 1997).

5.7 Mechanistic Studies

ESBO tested negative in mechanistic assays for human endocrine activity related to reproduction. It did not exhibit estrogen-like effects on ovariectomized rats (Ohta et al., 2003), and did not bind to estrogen receptors *in vitro* (Ohta et al., 2003; Ohashi et al., 2005).

No other mechanistic studies of ESBO were located.

5.8 Mode of Action

Detailed mode-of-action information specific to ESBO is not available. Epoxidation of unsaturated fatty acids occurs in normal hepatic metabolism and it is likely that some effects seen in animals at high doses (such as fatty accumulation in liver) are not different from the general effects of increased fat intake. Some grades of ESBO appear more toxic than others, especially those with a high epoxide number (Kieckebusch et al., 1963, as cited by BIBRA, 1997), but an underlying mode of action is not known at this time.

5.9 Lowest Hazard Endpoints by Organ System and Exposure Duration

Although the database for ESBO is relatively rich, identification of effect levels is complicated by the variety of degrees of epoxidation of the test material. Evaluation of ESBO toxicity is further complicated because most of the toxicity studies are unpublished and available only via secondary sources, usually without quantitative data.

Despite these challenges, some clear generalizations about ESBO toxicity are possible. It appears that the higher epoxide numbers are associated with higher toxicity (Kieckebusch et al., 1963, as cited by BIBRA, 1997). Based on the higher quality studies, the most consistent effects are growth suppression, and increased liver and kidney weights. All three effects appear to occur at about the same doses, and show the expected pattern of higher effect levels at shorter durations. Thus, 2500 mg/kg-day was a LOAEL and 1250 mg/kg-day was a NOAEL for these effects in rats treated for 90 days (Eagle, 1960b, as cited by Bandele et al., 2018). There was no clear pattern for body weight changes in rats exposed for 2 years, but increased liver and kidney weights were seen in males at 1000 mg/kg-day with the corresponding NOAEL of 100 mg/kg-day (BIBRA, 1986, as cited by BIBRA, 1997, Bandele et al., 2018). No histopathology was seen in the liver or kidney across the studies, aside from sporadic reports of fatty liver at 1300 mg/kg-day in rats exposed for 15 weeks (BIBRA, undated, as cited by BIBRA, 1997 and OECD, 2006) and in a dog treated with 1250 mg/kg-day for 1 year (Larson et al., 1960). Fatty liver accumulation may be related to the oily nature of the test material.

ESBO did not cause any reproductive toxicity study in rats at doses up to 1000 mg/kg-day in a study with appropriate pre-mating exposure of males and females (CIT, 1993a, as cited by BIBRA, 1997, Bandele et al., 2018). ESBO also did not exhibit estrogen-like effects on ovariectomized rats (Ohta et al., 2003), and did not bind to estrogen receptors *in vitro* (Ohta et al., 2003; Ohashi et al., 2005). “Slight” histopathology changes in the uterus and slight changes in uterine weight were reported in rats treated with 1400 mg/kg-day in the diet for 2 years (BIBRA, 1986, as cited by BIBRA, 1997 and Bandele et al., 2018). In the absence of more details it is not possible to determine the significance of these changes, but any apparent difference from the reproduction study could be related to the higher dose tested and the longer duration of the 2-year study. Testicular degeneration was reported in rats treated in the diet with

approximately 10,000 mg/kg-day for 10 weeks (Kieckebusch et al., 1963, as cited by BIBRA, 1997), but this may not have been a testes-specific effect, since increased lethality was also reported at this dose. In addition, testes effects were seen only when using ESBO grades with epoxide numbers greater than 100.

ESBO did not cause developmental toxicity in the offspring of pregnant rats gavaged with doses up to 1000 mg/kg-day (CIT, 1993b, as cited by BIBRA, 1997, Bandele et al., 2018), and there was no evidence of developmental toxicity in the one-generation reproductive toxicity study (CIT, 1993a, as cited by BIBRA, 1997, Bandele et al., 2018).

ESBO was negative for gene mutation in several bacterial mutation assays (Monsanto, 1986, 1987a; Heath and Reilly, 1982; Ciba-Geigy, 1981, Hazleton, 1992, both as cited by BIBRA, 1997). ESBO was also negative for gene mutation in mammalian cells (Monsanto, 1987b), and for inducing chromosome damage in human and mouse lymphoma cells (Hazleton, 1992, as cited by BIBRA, 1997).

ESBO was not carcinogenic in an adequately-conducted 2-year feeding study in rats that examined a comprehensive range of tissues (BIBRA, 1986, as cited by BIBRA, 1997, Bandele et al., 2018). No mouse carcinogenicity study is available, but ESBO was also negative in another 2-year bioassay with an inadequate number of rats (Larson et al., 1960), and in a 1-year dog study with a small sample size (Larson et al., 1960).

5.10 Uncertainties and Data Gaps

Several uncertainties of varying importance were identified in this assessment.

Database:

The overall database on ESBO is fairly complete, including many of the key studies. Subchronic and chronic systemic toxicity studies are available in rats and dogs (Eagle, 1960a, 1960b, as cited by Bandele et al., 2018; BIBRA, 1986, as cited by BIBRA, 1997, Bandele et al., 2018; Larson et al., 1960), although some of the chronic studies were conducted with an inadequate number of animals. Reproductive and developmental toxicity studies (CIT, 1993a, 1993b, as cited by BIBRA, 1997, Bandele et al., 2018). No studies beyond acute duration are available for the inhalation or dermal routes, aside from one poorly documented 2-year dermal bioassay in mice with several significant design limitations. In addition, the 2-year dermal mouse study was the only study on ESBO conducted in mice (Weil et al., 1963, as cited by BIBRA, 1997).

The key limitation to the database is that, with the exception of a study from the 1960's and some mechanistic studies, all of the repeated dose, reproductive, and developmental toxicity studies were available only from secondary sources, and none of the secondary sources provided detailed quantitative results. This substantially limited the potential for independent evaluation of the results.

Table 2. Summary of NOAELs/LOAELs Identified for ESBO by Organ System

Species (Sex), Reference	Exposure Regimen ⁵	Effect Category	Toxicological Endpoint (mg/kg-day) ⁶	Toxicological Basis	Comments
Unknown strain, rat (sex not given) 6/dose Mounie et al., 1988, as cited by BIBRA, 1997	12 days Diet 0, 5% Approximately 0, 2500 mg/kg-day	Body weight	NOAEL = N/A ⁷ LOEL = 2500	Decreased growth rates	Study limited by reporting and small sample size; degree of decrease of growth rate not reported Liver weights and serum transaminase were not affected, epoxide hydratase was elevated Full range of endpoints not reported.
Unknown strain, rat (sex not given) Group size not given Kieckebusch et al., 1963, as cited by BIBRA, 1997	8 weeks Diet 0, 1, 5% Approximately 0, 500, 2500 mg/kg-day	Body Weight Systemic	NOAEL = N/A LOAEL = 500 NOAEL = 500 LOAEL = 2500	Decreased growth rates Deaths in 6/54 animals	Study used ESBO with very high epoxide number (220) Diet adjusted to 20% soybean oil in all groups No other details given

⁵ Dose conversion from dietary percentages are partially based on proportional scaling of mg/kg conversion referred to in the comments column

⁶ All effect levels as identified by the authors of this assessment. Effect levels identified by previous assessments, when different or of note, are in the comments section.

⁷ N/A = Not applicable

Species (Sex), Reference	Exposure Regimen ⁵	Effect Category	Toxicological Endpoint (mg/kg-day) ⁶	Toxicological Basis	Comments
Unknown strain, rat (sex not given) Group size not given Kieckebusch et al., 1963, as cited by BIBRA, 1997	10 weeks Diet 0, 20% Approximately 0, 10,000 mg/kg-day	Body weight	NOAEL = N/A LOAEL = 10,000	Retarded growth rates	Five ESBO variants tested with low to high epoxide numbers (higher numbers caused more toxicity) Dose conversion based on summary in BIBRA, 1997 ⁸ Fatty degeneration of liver and kidney occurred in all groups including 20% soybean oil controls Limited reporting of study details
		Systemic	NOAEL = N/A LOAEL = 10,000	Death	
		Reproductive	NOAEL = N/A LOAEL = 10,000	Testicular degeneration	
Holtzman rat (M & F) 10/sex/dose Eagle, 1960b, as cited by Bandele et al., 2018	90 days Diet 0, 0.1, 0.5, 1.0, 5.0, or 10% Approximately 0, 25, 125, 250, 1250, 2500 mg/kg-day	Systemic	NOAEL = 1250 LOAEL = 2500	Growth suppression	No quantitative data reported. No evidence of histopathology at any dose, but no information on which tissues were evaluated. No data on urine or hematology parameters were reported. FDA considered the change in liver weight adverse, but it is unclear whether modern interpretation would agree, in the absence of evidence of damage to the liver. FDA considered the NOAEL
		Liver	NOAEL = 1250 LOAEL = 2500	Increased liver weight	
		Kidney	NOAEL = 1250 LOAEL = 2500	Increased kidney weight	

⁸Baderle et al. (2018) reported the corresponding dose as 5000 mg/kg-day, which is more consistent with the conversion reported for the Mellon Institute of Industrial Research (1960) study.

Species (Sex), Reference	Exposure Regimen ⁵	Effect Category	Toxicological Endpoint (mg/kg-day) ⁶	Toxicological Basis	Comments
					to be 1250 mg/kg-day, based on growth suppression and increased liver and kidney weight at 2500 mg/kg-day (Bandelet et al., 2018).
Beagle dog (M & F) 2/sex/dose Eagle 1960a, as cited by Bandelet et al., 2018)	14 weeks Diet 0, 1.0, 2.5, or 5.0% 0, 250, 625, and 1250 mg/kg-day	Systemic	NOAEL = 625 LOAEL = 1250	Decreased feed efficiency and decreased growth	Dose conversions reported by Bandelet et al. (2018) No gross or microscopic findings (tissues evaluated not reported)
Unknown strain, rat (sex/number not given) BIBRA, undated, as cited by BIBRA, 1997, and OECD, 2006	15 weeks Diet 0, 1.5, 2.5, 5.0% Approximately 0, 780, 1300, 2600 mg/kg-day	Body weight Liver Kidney	NOAEL = 780 LOAEL = 1300 NOAEL = 780 LOAEL = 1300 NOAEL = 780 LOAEL = 1300	Transiently decreased growth Enlarged liver and fat accumulation Enlarged kidney	Full range of endpoints not available ESBO material appeared identical to 2-year feeding study reported in BIBRA, 1986 Dose conversions based on conversion given in BIBRA, 1997 summary No other details available
Albino rat (M & F) (10/sex/dose)	90 days Diet	Liver	NOAEL = 50 LOAEL = 250	Increased liver weight, “test article-related effects” from gross examination	Range of parameters examined was not available

Species (Sex), Reference	Exposure Regimen ⁵	Effect Category	Toxicological Endpoint (mg/kg-day) ⁶	Toxicological Basis	Comments
Mellon Institute of Industrial Research, 1960, as cited by OECD, 2006b, Bandele et al., 2018	0, 0.04, 0.2, 1.0, 5.0% Approximately 0, 10, 50, 250, 1250 mg/kg-day	Kidney	NOAEL = 50 LOAEL = 250	“test article-related effects” from gross examination	Although there appeared to be some small sex-related differences in response, these could not be definitively identified, due to inconsistencies in the reporting by secondary sources. Dose conversion reported by Bandele et al. (2018)
Wistar rat (M & F) 48/sex/dose BIBRA, 1986, as cited by BIBRA, 1997, Bandele et al., 2018	2 years Diet 0, 0,025, 0.25, 2.5% Approximately: M: 0, 10, 100, 1000 mg/kg-day F: 0, 14, 140, 1400 mg/kg-day	Systemic	NOAEL = 1000 (M, F) LOAEL = N/A	Slight changes in body weight	Dose conversions based on Bandele et al., 2018
		Kidney	NOAEL = 100 (M) LOAEL = 1000 (M) NOAEL = 1400 (F) LOAEL = N/A (F)	Increased kidney weight	Endpoints included blood parameters and examination of a “comprehensive range of tissues” No quantitative information provided
		Liver	NOAEL = 100 (M) LOAEL = 1000 (M) NOAEL = 1400 (F) LOAEL = N/A (F)	Increased liver weight	Basis for ADI

Species (Sex), Reference	Exposure Regimen ⁵	Effect Category	Toxicological Endpoint (mg/kg-day) ⁶	Toxicological Basis	Comments
		Reproductive	NOAEL = 140 (F) LOAEL = 1400 (F)	“slight changes” in uterus upon microscopic examination; slight changes in uterine weight	
Unknown albino strain, rat (M F) 15/sex/ dose Larson et al., 1960	2 years Diet 0, 0.1, 0.5, 1.0, 2.5, 5.0% Approximately 0, 50, 250, 500, 1250, 2500 mg/kg-day ⁹	Body weight	NOAEL = 1250 (M,F) LOAEL = 2500 (M,F)	Transiently decreased growth rate (recovers by ~8 weeks)	ESBO was “Paraplex G-60” Study limited by small sample size; interim sacrifice of 5/sex/dose Dose conversions based on BIBRA, 1997
		Liver	NOAEL = 500 (M) LOAEL = 1250 (M) NOAEL = 2500 (F) LOAEL = N/A (F)	Elevated liver-body weight ratio at 1 year	Hematology, organ weights, and histopathology assessed at 1 year (5/group). Hematology and histopathology assessed at 2 years (3-8/group)
Unknown albino strain, rat (M/F) 15/sex/ dose Larson et al., 1960	2 years Diet 0, 0.1, 0.5, 1.0, 2.5, 5.0% Approximately 0, 50, 250, 500,	Body weight	NOAEL = N/A (M, F) LOAEL = N/A (M, F)	Transiently decreased growth rate. Recovered by 8 weeks in females, did not recover fully in males	ESBO was “Paraplex G-62” Study limited by small sample size; interim sacrifice of 5/sex/dose
		Liver	NOAEL = N/A (M, F) LOAEL = N/A (M, F)	Elevated liver-body weight ratio at 6 months	Dose conversions based on summary in BIBRA, 1997

⁹ Bandele et al. (2018) reported the doses as 0, 25, 125, 250, 625, and 1250 mg/kg-day.

Species (Sex), Reference	Exposure Regimen ⁵	Effect Category	Toxicological Endpoint (mg/kg-day) ⁶	Toxicological Basis	Comments
	1250, 2500 mg/kg-day ⁴	Kidney	NOAEL = N/A (F) LOAEL = N/A (F) NOAEL = 2500 (M) LOAEL = N/A (M)	Elevated kidney-body weight ratio at 6 months	NOAELs and LOAELs were not provided by the authors, and no pairwise statistical analyses were performed. Instead, the authors conducted a regression analysis of data to estimate thresholds, but the degree of change considered to be a “threshold” was not reported. Hematology, organ weights, and histopathology assessed at 6 months (5/group). Hematology and histopathology assessed at 2 years (3-8/group)
Mongrel dog (sex not given) 3/dose/compound Larson et al., 1960	1 year Diet 0.1, 1.0, 5.0% Approximately 25, 250, 1250 mg/kg-day	Body weight	NOAEL = 250 LOAEL = 1250	Decreased body weight and food intake	Dogs received Paraplex G-60 or Paraplex G-62 (effects were similar) Study limited by small sample size and absence of a control group Dose conversion based on summary in BIBRA, 1997 One dog in high dose group had slightly fatty liver

Species (Sex), Reference	Exposure Regimen ⁵	Effect Category	Toxicological Endpoint (mg/kg-day) ⁶	Toxicological Basis	Comments
Sprague-Dawley rat (M & F) 28/sex/dose CIT, 1993a, as cited by BIBRA, 1997, Bandele et al., 2018	71 (M) or 15 (F) days before mating to PND 21 Gavage 0, 100, 300, 1000 mg/kg-day	Reproductive	NOAEL = 1000 (M, F)	No effects	Growth, hematology, and histopathology (liver, ileum, kidneys, reproductive organs), fertility, and number of live births assessed in adults
		Developmental	NOAEL = 1000 LOAEL = N/A	No effects	Birth weights, growth, physical development, and gross tissue appearance assessed in offspring
		Systemic	NOAEL = 1000 (M,F) LOAEL = N/A	No effects	
Sprague Dawley rat (F) 25/dose CIT, 1993b, as cited by BIBRA, 1997, Bandele et al., 2018	GD 6-15 Gavage 0, 100, 300, 1000 mg/kg-day	Maternal	NOAEL = 1000 LOAEL = N/A	No effects	Assessment included numbers of corpora lutea, implantation sites, and live fetuses in adults
		Developmental	NOAEL = 1000 LOAEL = N/A	No effects	Sex ratio, birth weight, and external, skeletal, and soft-tissue malformations assessed in offspring Non-significant increase in skeletal anomalies seen in offspring
Sprague-Dawley rat (M & F) 12/sex/dose	15 days before mating until PND 7 Gavage	Reproductive	NOAEL = 1000 LOAEL = N/A	No effects	Full dose range not reported Assessment included numbers of corpora lutea, implantation sites, and live fetuses in adults

Species (Sex), Reference	Exposure Regimen ⁵	Effect Category	Toxicological Endpoint (mg/kg-day) ⁶	Toxicological Basis	Comments
CIT, 1993c, as cited by BIBRA, 1997, Bandele et al., 2018	0, 150, 450, 1000 mg/kg-day Range-finding study	Developmental	NOAEL = 1000 LOAEL = N/A	No effects	Sex ratio, birth weight, and external, heart, liver, kidneys, stomach, and intestine malformations assessed in offspring
CrI:CD (SD) rats (F) 10/dose Ohta et al., 2003	3 days Gavage 0, 0.5, 500 mg/kg-day	Reproductive	NOAEL = 500	No effects	Rats ovariectomized prior to exposure and were assessed for uterine wet weight change, uterine endometrium hyperplasia, and vaginal mucosa cornification (Primarily in Japanese, portions translated with aid of Googletranslate.)

Hazard:

For all of the observed effects, there is at least some uncertainty regarding the adversity of the observed change, due to the absence of quantitative results.

Body weight: There is uncertainty whether “slight” decreases in body weight were of sufficient magnitude to be considered adverse, as well as uncertainty in interpreting the body weight changes in the chronic rat study (BIBRA, 1986, as cited by BIBRA, 1997, Bandele et al., 2018), for which there were some differences in reporting across secondary sources.

Liver weight: There is uncertainty as to whether the liver weight changes occurring in the absence of other supporting changes would be considered adverse in a modern risk assessment context. Recent guidance by U.S. EPA (2002) provides that hepatocellular hypertrophy and/or liver size/weight changes should not be considered adverse unless there is a known mode of action for toxicity and/or the other study data (e.g., clinical chemistry and histopathology) indicate adverse changes.

Kidney weight: There is some uncertainty in interpreting the adversity of kidney weight changes in the absence of quantitative data.

Uterus: There is uncertainty in interpreting the significance of the “slight” microscopic changes in the uterus and slight changes in uterine weight, in the absence of further details. Adding to the uncertainty, the uterine changes were observed only in the chronic rat study at 1400 mg/kg-day (BIBRA, 1986, as cited by BIBRA, 1997, Bandele et al., 2018), but not in any other study, including the subchronic rat study at 2500 mg/kg-day (Eagle, 1960b, as cited by Bandele et al., 2018).

6 Exposure

The use of ESBO in consumer products has been described in Section 3 of this report. The general population is exposed to ESBO via ingestion of foods packaged in containers that utilize ESBO in lid gaskets or can coatings, and through PVC cling films used for wrapping and packaging foods. Infants and children may be exposed via mouthing of products (e.g., children’s toys) containing ESBO. Dermal contact with toys or consumer products may also be a route of exposure. However, ESBO has a high molecular weight, is insoluble in water, and has a high log K_{ow} , which would indicate that dermal absorption is not likely to be significant.

ESBO will react with HCl from PVC degradation and form chlorohydrins, with up to 5% of the fatty acids in jar gaskets made with ESBO converted into these derivatives (EFSA, 2004). These chlorohydrins may then be present in foods, and their potential health effects would need to be evaluated separately; consideration of the health effects of chlorohydrins is beyond the scope of this report.

Workers may be exposed to ESBO dermally from handling of materials, although the likelihood of such exposure is low, because ESBO is stored and transported in closed containers (OECD

2006). Dermal exposure may occur to pesticide applicators (OECD, 2006). ESBO has a very low vapor pressure and so airborne exposure to ESBO vapor is unlikely (OECD, 2006).

Several studies have measured levels of ESBO in plastic toys and childcare articles. A 2007 survey of toys and childcare products in Germany, Austria, and Switzerland (252 samples from 172 items) found ESBO in 1% of the tested materials, with a mean concentration of 13% ESBO (Maag et al., 2010). In 2015, McCombie et al. (2017) tested 118 samples from 88 toys from the Swiss market for compliance with the EU's 0.1% restriction for phthalate content. ESBO was found in over 80% of the samples in amounts over 0.1% (range 0.6 % - 13% by weight; average 4.6%). However, no studies were located that quantified estimates of exposure from exposure to consumer products, including toys.

Estimates of human exposure to ESBO are available only for exposures resulting from use in food packaging. The following discussion briefly summarizes concentrations of ESBO in foods and estimates of daily dietary intake. Considerable attention has been paid to ESBO migration from food packaging, particularly in Europe where food products have been found to often exceed the migration limit of 60 mg/kg.

Levels of ESBO in various foods and food simulants have been examined and discussed by numerous authors (e.g., Bueno-Ferrer, 2010; Ezerski et al., 2007; Pedersen et al., 2008, as cited by Bui et al., 2016; Fankhauser-Noti et al., 2005, 2006a; Hanusova et al., 2013; Kawamura et al., 2006; Fankhauser-Noti et al., 2006a, as cited by Bandele et al., 2018; McCombie et al., 2012). Common findings are that ESBO migrates from lid gaskets, particularly into fatty or higher "fluidity" foods, most of the studies identified some samples that exceeded the migration limit of 60 mg/kg food. For example, Fankhauser-Noti and colleagues (Fankhauser-Noti et al., 2006b, as cited by Bandele et al., 2018) analyzed 158 oil-based foods that were packaged in glass jars with metal lids using PVC gaskets, and measured migration into foods with fat/oil content ~3% or greater. Concentrations in the food exceeded the migration limit of 60 mg/kg for 89% of the 104 products that had ESBO in the lid gaskets, with an average concentration of 216 mg/kg. A more recent investigation of 411 food products in Europe (testing conducted in 2011) identified ESBO in 73% of the PVC gasket lids (average concentration of ESBO in gaskets was 17%) (McCombie et al., 2012), but this study did not evaluate migration.

Other studies focused on baby foods. FASFC (2014) reported that ESBO was found in approximately 50% of Belgian baby food samples collected from 2008 until 2012 (range of concentrations from 1 to 55 mg/kg, with 4.6% exceeding the 30 mg/kg limit [the EU specific migration limit for baby food]) and in almost half of the other food category samples (3.5% above 30 mg/kg). Fantonian and Simoneau (2003) found ESBO in 38% of 248 baby food samples from Europe. Levels ranged from 1.5 to 135.2 mg/kg, with 4% exceeding the 60 mg/kg overall migration limit in Europe, and 15% over the 30 mg/kg limit. A Swedish study (Hammerling et al., 1998) found residues of ESBO in almost all the baby foods they sampled

(mean 11.9 mg/kg and median 7.8 mg/kg for foods with detectable levels). The authors also measured food samples before packaging and did not detect ESBO, leading them to conclude that the source of ESBO was the packaging.

Numerous authors have investigated levels of migration of ESBO from the PVC gaskets of commercial lids used to seal jars. ESBO was the most frequently-measured plasticizer (64%) in lid gaskets studied by Fankhauser-Noti and colleagues in Swiss market foods (Fankhauser-Noti et al., 2006b, as reported by Bandele et al., 2018). They found concentrations in the gaskets ranging from 35% - 45%. Carlos et al. (2018) measured ESBO content in 56 food contact materials (domestic and international sources purchased in Maryland) and found ESBO was the primary plasticizer in one-third (2 of 6) of the bottled beverage cap gaskets (concentrations of 20.4% and 15%, respectively) and almost half (12 of 25) of the jarred products lid gaskets (concentration range 18-36%).

Others have demonstrated migration of ESBO from plasticized film wraps into foods. Choi et al. (2018) demonstrated that ESBO is capable of migrating into amphiphilic/oily foods from PVC and PVDC cling film. Castle et al. (1990) measured the level of ESBO migration into retail packaged foods and into foods wrapped in PVC film in the home. They found that levels varied based upon type of food and length of contact time with the packaging or film. Direct contact with fatty foods resulted in the highest concentrations. They found lower levels of ESBO in infant foods packaged in glass jars with PVC gaskets and in cans with ESBO in the can lining. In a study of ESBO migration from commercial PVC-wrapped foods, levels of ESBO increased with fat content of the food, with increasing time in contact with the food, and with increasing temperature (Lee et al., 2003, as cited by Bandele et al., 2018).

Migration rates into foods from jar lid gaskets and film wraps, are difficult to estimate. These rates vary with numerous factors, including (1) ESBO concentration in the contact medium, (2) the surface area in contact with the food, (3) the fat and oil content of the food, (4) the length of time the food is in contact with the ESBO-containing materials, and (5) the temperature during contact (Bandele et al., 2018; Hansova et al., 2013; Lee et al., 2003, as cited by Bandele et al., 2018; Castle et al., 1990). Time of contact involves the time from canning to consumption, but also must consider the handling of the product (e.g., a jar stored upside down will have greater migration due to full contact with the sealing gasket). Temperature can vary greatly for processing and storage conditions.

EFSA (2004) reported that the highest ESBO residues have been found in baby foods from glass jars. They noted that adult exposure is likely to be lower than for infants, due to smaller contribution from jar foods to adult diets, and less ESBO in lid gaskets of adult foods. They reported that adult intake of ESBO comes primarily from PVC film wraps. In a screening level assessment, EFSA estimated the daily adult dietary intake from migration of ESBO from foods

packaged in glass jars with PVC lid gaskets using conservative assumptions regarding packaging, migration levels, and food consumption. A daily intake of 0.25 mg/kg-day was estimated based upon average concentration levels of ESBO in the food, while an intake of 0.64 mg/kg-day was estimated based upon the 90th percentile of the concentration of ESBO in the food (EFSA, 2006). In addition, they estimated that adult exposure to ESBO from foods packaged in cling films would not be greater than 0.2 mg/kg-day. They concluded that these conservative estimates of intake are below the TDI of 1 mg/kg-day set by the EU SCF (SCF, 1999, as cited by EFSA, 2004, 2006), and therefore they did not refine the exposure estimates.

Infant ESBO intake was estimated by EFSA (2004) using various scenarios with estimated intake ranging from 0.34 mg/kg-day to 4.65 mg/kg-day. The estimates were based on reasonable worst-case contamination concentrations of 12.0 to 50.0 mg ESBO/kg food, and food intake estimates ranging from average to 97.5th percentile (28 g food/kg-day to 93 g food/kg-day, respectively).

Several European countries have estimated intakes based upon data for their individual country/population. Intakes estimated for Belgian adults and infants were lower than those estimated by EFSA (FASFC, 2014). Similarly, the Norwegian Scientific Committee for Food Safety estimated lower infant intakes (mean intake of 0.2 mg/kg-day, worst case intake of 1.6 mg/kg-day) based upon samples of baby foods from the Norwegian market (VKM, 2005). In Ireland, exposure to ESBO was estimated for 594 children aged 5-12 years using data from the Irish National Children's Food Survey (NCFS) collected in 2003-04 by Duffy and Gibney (2007). Information on the contact layer of the food packaging was also collected and used to estimate exposure to ESBO, using a method similar to that used by EFSA in their adult exposure assessment. The authors of the Irish study assumed that all packaging types that could contain ESBO did contain ESBO, and they used the 90th percentile migration values from the literature, resulting in a worst-case type of estimate. Mean intake of ESBO for these children was estimated to be 0.023 mg/kg-day, with a maximum intake rate of 0.385 mg/kg-day. Tomato sauces packaged in jars with PVC lined metal lids contributed almost 50% to intake. The authors noted that these exposures were well below the TDI of 1 mg/kg-day set by the EU Scientific Committee for Food in 1999 (SCF, 1999, as cited by Duffy and Gibney, 2007).

More recently, Bandele and colleagues from the U.S. Food and Drug Administration (FDA) estimated exposure of ESBO from PVC lid gaskets and PVC film wraps (Bandele et al., 2018). They used the average migration of 216 mg/kg from the Fankhauser-Noti et al. (2006b, as cited by Bandele et al., 2018) to calculate a weighted average dietary concentration of 1 mg ESBO/kg food for contamination from lid gaskets and 1.6 mg ESBO/kg food from contamination from food-contact films. Combining the contribution from PVC gaskets and films, they estimated a cumulative dietary concentration (CDC) of 2.6 mg/kg food and a cumulative estimated daily intake (CEDI) for the general population of 0.13 mg/kg-day (Bandele et al., 2018). The authors

noted that this CEDI is well below the most conservative animal NOAEL of 100 mg/kg-day (BIBRA, 1986, as cited by Bandele et al., 2018) and the corresponding TDI of 1 mg/kg-day.

7 Discussion

7.1 Toxicity Under FHSA

Animal data support the conclusion that **ESBO does not fit the designation of acutely toxic under the Federal Hazardous Substances Act (FHSA) (16 CFR§1500.3(c)(2)(i)(A))** following single oral or dermal exposures. Multiple rat studies have reported rat oral LD₅₀ values as greater than 5000 mg/kg (Ciba-Geigy, 1981d, HRC, 1973, as cited by BIBRA, 1997). The dermal LD₅₀ in rabbits is greater than 20 mL/kg (Weil et al., 1963, as cited by BIBRA, 1997).

ESBO caused mild-to-minimal skin irritation (Ciba-Geigy, 1981a, Weil et al., 1963, both as cited by BIBRA, 1997). ESBO may be mildly irritating to the eyes. Reddening of the conjunctiva was observed in one rabbit study (Ciba-Geigy, 1981b, as cited by BIBRA, 1997), but there was no eye irritation in another study testing a larger dose of ESBO (Weil et al., 1963, as cited by BIBRA, 1997). The different results of the two studies may reflect the testing of different grades of ESBO. Sensitization to ESBO has been reported anecdotally in workers (Pauli et al., 1980), but reports are limited and controlled studies in guinea pigs did not find any evidence of sensitization (Ciba-Geigy 1981c, Weil et al., 1963, both as cited by BIBRA, 1997).

The systemic toxicity of ESBO is low, with reproducible effects limited to changes in body weight, liver and kidney weight, and fatty liver (Eagle, 1960a, 1960b, as cited by Bandele et al., 2018; BIBRA, 1986, as cited by BIBRA, 1997, Bandele et al., 2018; Larson et al., 1960).

ESBO did not cause reproductive or developmental toxicity in rats (CIT, 1993a, 1993b, both as cited by BIBRA, 1997, Bandele et al., 2018).

ESBO was negative for gene mutation in several bacterial mutation assays (Monsanto, 1986, 1987a, 1987b; Heath and Reilly, 1982; Ciba-Geigy, 1981, Hazleton, 1992, both as cited by BIBRA, 1997), and negative for inducing chromosome damage in human and mouse lymphoma cells (Hazleton, 1992, as cited by BIBRA, 1997).

ESBO was not carcinogenic in an adequately-conducted 2-year feeding study in rats that examined a comprehensive range of tissues (BIBRA, 1986, as cited by BIBRA, 1997, Bandele et al., 2018). No mouse carcinogenicity study is available.

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APPENDIX 1

Search Terms Used

Toxline	"Epoxidized soy bean oil" OR "Oils, soybean, epoxidized" OR "Soybean oil, epoxidized" OR "ESBO" OR "Paraplex G-62" OR "Paraplex G-60" OR "Fatty acid, soybean oil, epoxidized" OR "Epoxidized soy bean oil fatty acid" OR "Flexol EPO"
Toxline	(8013-07-8)
Pubmed	"Epoxidized soy bean oil" OR "Oils, soybean, epoxidized" OR "Soybean oil, epoxidized" OR "ESBO" OR "Paraplex G-62" OR "Paraplex G-60" OR "Fatty acid, soybean oil, epoxidized" OR "Epoxidized soy bean oil fatty acid" OR "Flexol EPO"

APPENDIX 2

Explanation of Physico-chemical Parameters

The octanol/water partition coefficient (K_{ow}) is defined as the ratio of a chemical's concentration in the octanol phase to its concentration in the aqueous phase of a two-phase octanol/water system. In recent years, this coefficient has become a key parameter in studies of the environmental fate of organic chemicals. It has been found to be related to water solubility, soil/sediment adsorption coefficients, and bioconcentration factors for aquatic life. Because of its increasing use in the estimation of these other properties, K_{ow} is considered a required property in studies of new or problematic chemicals

(<http://www.pirika.com/chem/TCPEE/LOGKOW/ourlogKow.htm>).

The bioconcentration factor (BCF) is the concentration of a particular chemical in a tissue per concentration of chemical in water (reported as L/kg). This property characterizes the accumulation of pollutants through chemical partitioning from the aqueous phase into an organic phase, such as the gill of a fish. The scale used to determine if a BCF value is high, moderate or low will depend on the organism under investigation. The U.S. EPA generally defines a high potential BCF as being greater than 5,000; a BCF of moderate potential as between 5,000 and 100; a low potential BCF as less than 100 (http://en.wikipedia.org/wiki/Bioconcentration_factor; <http://sitem.herts.ac.uk/aeru/footprint/en/Quest/ecotox.htm>).