Appendix A: Background Information for Strontium

Strontium is a naturally occurring element that exists in the environment mainly as the free metal or in the (II) oxidation state. Because the biological availability and toxicity of strontium are primarily related to the strontium(II) oxidation state, ATSDR (2001e) has focused on that form of strontium. While a number of radioisotopes of strontium exist, the most common are ^{89}Sr and ^{90}Sr. As such, ATSDR (2001e) has focused primarily on radiation from ^{89}Sr and ^{90}Sr when discussing radioactive strontium.

A.1 Toxicokinetics

Following inhalation exposure to strontium compounds, strontium particles are deposited within the respiratory tract based on their aerosol characteristics. Once deposited, absorption is dependent upon the solubility of the compound, with more soluble compounds being readily absorbed (>80% absorption) and less soluble compounds potentially persisting within the lungs (ATSDR 2001e).

Absorption of strontium from the gastrointestinal tract shares a common mechanism with absorption of calcium. Calcium absorption is higher in physiologic states in which there is an increased demand for calcium, such as pregnancy and lactation, suggesting that strontium absorption may also be higher as well. The fractional absorption of ingested strontium has been estimated in healthy human subjects or hospital patients who received an oral dose of strontium chloride ($SrCl_2$) or ingested strontium in the diet (ATSDR 2001e). The results of these studies indicate that approximately 20% (range, 11–25%) of ingested strontium is absorbed from the gastrointestinal tract. Available data suggest no differences in oral absorption of strontium between males and females, nor do available human data suggest a difference in absorption between children and adults (ATSDR 2001e). Studies in rats have suggested that very young animals absorb more strontium than adults (ATSDR 2001e).

There is little evidence for systemic toxicity following dermal exposure to strontium compounds, which would suggest that they are not readily absorbed across the skin of humans (ATSDR 2001e). Absorption of strontium through intact human skin was <1% after 6 hours of exposure, while absorption through scratched and abraded skin was 57% over the same time period (Ilyin et al. 1975).

The metabolism of strontium consists of binding interactions with proteins and, based on its similarity to calcium, probably complex formation with various inorganic anions such as carbonate and phosphate, and

carboxylic acids such as citrate and lactate (ATSDR 2001e). These types of interactions would be expected for all routes of exposure.

Absorbed strontium is excreted in both urine and feces (ATSDR 2001e). Urine appears to be the major route of excretion, with a urine:fecal ratio of approximately 3:1 in humans. The observation of fecal excretion of radioactive strontium weeks to decades after an oral exposure or over shorter time periods after an intravenous exposure suggests the existence of a mechanism for transfer of absorbed strontium into gastrointestinal tract, either from the bile or directly from the plasma (ATSDR 2001e). Evidence for direct secretion of strontium from the plasma into the intestine is provided by studies in animals. The available information does not address the extent to which biliary excretion may also contribute to fecal excretion of strontium. Strontium may also leave the body in breast milk, saliva, or seminal fluid (ATSDR 2001e).

A.2 Health Effects

Stable Strontium. At low exposure levels (below 100 mg/kg/day), ingestion of stable strontium poses no harm to organisms with access to adequate calcium, phosphorus, and vitamin D (ATSDR 2001e). At higher exposure levels, especially under conditions of inadequate calcium, phosphorus, and vitamin D, stable strontium will interfere with normal bone development, causing 'strontium rickets' of variable severity. Human children and weanling animals are the most susceptible populations for this effect. 'Strontium rickets' have been demonstrated in humans (Özgür et al. 1996), and similar results have been found in animal studies (Kshirsagar 1976; Morohashi et al. 1994; Neufeld and Boskey 1994; Reinholt et al. 1985; Storey 1962).

There was one case of anaphylaxis reported in a paramedic who inhaled strontium-containing smoke in an enclosed space (Federman and Sachter 1997). Although other irritants in the smoke may have contributed to the incident, there is supporting evidence that stable strontium can stimulate the release of histamine from mast cells *in vitro* (ATSDR 2001e).

Radioactive Strontium. Following exposure to radioactive strontium compounds, the most severe non-carcinogenic effects seen are the result of incorporation of radioactive strontium, an emitter of beta radiation, into the skeleton, with subsequent irradiation of surrounding tissues (ATSDR 2001e). The cells of the bone marrow are thus highly affected, with pancytopenia being a common effect of high doses of internally deposited radiostrontium. Exposure to young animals also resulted in skeletal effects including

mild trabecular osteopenia, endosteal and periosteal cortical changes (sclerosis and thickening), and mottling or focal osteolytic lesions. Dose-dependent decreases in platelet and erythrocyte counts of 60–90% have been reported in animal studies (ATSDR 2001e). Similarly, the lymphatic tissues are targets of radiostrontium compounds, with profound depletion of lymphocyte numbers seen in exposed animals, as well as impairments in immune function. Radiostrontium compounds can also result in osteonecrosis and abnormal bone development at very high doses (ATSDR 2001e).

Oral treatment of pregnant dams with radioactive strontium compounds appears to have a minimal effect on fetal survival, although one study in rats showed that treatment prior to mating resulted in an increase in fetal mortality (ATSDR 2001e). Studies of exposure to radioactive strontium compounds have not reported increases in developmental effects (ATSDR 2001e).

One study described some delayed effects of external strontium radiation treatment (24-75 Sv over 6-16 months) within patients in one medical practice in Belgrade, Yugoslavia (now Serbia; Bekerus 1970). Eight or 10 years after treatment, about a third of the patients developed delayed reactions to radiation: achromia, excess pigmentation, slight atrophy, and telangiectasis; the author did not specify the exposure levels that resulted in these effects. Acute dermal reactions to radiostrontium have also been described for depilated skin in mice, guinea pigs, and pigs. As a general rule, the progression of symptoms in animals is as follows: after an asymptomatic period, the skin exhibits increasing erythema and pigmentation changes, leading to dry desquamation. Within a few days, exposed skin enters a period of moist desquamation, during which a serum scab forms. Re-growth of the epithelium then commences at the edges of the irradiated field and from surviving hair follicles. Chronic fibrosis as a delayed skin reaction is seen at 3–6 months postirradiation.

Data on the carcinogenic effects of radioactive strontium compounds in humans are limited, and are generally equivocal. However, a large number of studies in animals have shown that exposure to radioactive strontium compounds results in significant increases in cancer incidence and mortality (ATSDR 2001e). As strontium localizes in bone, the most prevalent tumors are those of the bone and bone marrow, including osteosarcoma, chrondrosarcoma, lymphosarcoma, hemangiosarcoma, and leukemia. Inhalation exposure of dogs to insoluble radiostrontium particles has resulted in pulmonary tumors, the most common of which was pulmonary hemangiosarcoma (Snipes et al. 1979). External exposure to strontium radiation has been shown to result in skin tumors, including basal- and squamous-cell carcinoma and fibrosarcoma. In all of these studies, the lifetime radiation doses to the animals were high (4,000–30,000 rad [40–300 Gy]), preventing the extrapolation of these effects to lower doses.

A.3 Mechanisms of Action

The fact that strontium is chemically similar to calcium allows it to exchange for calcium in bone and other cellular compartments that are enriched in calcium (ATSDR 2001e). Many enzymes that are calcium-dependent will function when strontium is substituted, but changes in kinetic parameters may occur. Strontium can interact with secondary cell messenger systems and transporter systems that normally use calcium. Furthermore, synaptic transmission may be variably affected by strontium. Consequently, at high concentrations, differences in the chemical characteristics between strontium and calcium may be the basis for neurotoxic and neuromuscular perturbations associated with stable strontium intoxication.

Variations in the rate of absorption of soluble strontium compounds will affect the severity of their effects. The rate of strontium incorporation into bone may also be influenced by other factors that affect bone mineralization. Some genetic factors include parathyroid hormone receptor, estrogen receptor 1, epidermal growth factor, type I collagen A1, interleukin 1-alpha, and other genes that have not yet been characterized (Audi et al. 1999; Duncan et al. 1999). Persons with chronic kidney failure may be more susceptible to effects of excess strontium because of a reduced ability to excrete strontium (Apostolidis et al. 1998). A study in rats demonstrated that protein deficiency, especially in combination with ethanol consumption, may increase strontium incorporation into bone while reducing fecal and urinary excretion of strontium (Gonzales-Reimers et al. 1999).

Differences in bone physiology suggest that adult rats may have a higher susceptibility to stable or radioactive strontium effects than adult humans. Unlike most mammals (including humans), the epiphyseal growth plate of the long bones of rats never entirely transforms into bone after sexual maturity, so that bone growth continues throughout life (although reduced after the age of 12 months) (Leininger and Riley 1990). Thus, incorporation of strontium into the skeleton is likely to be relatively higher in adult rats compared to other mammals.

Stable Strontium. In animals, excess strontium indirectly suppresses the activation of vitamin D_3 in the kidney, which severely reduces the expression of calbindin D messenger ribonucleic acid (mRNA) and the translation of calbindin D protein in the duodenum (Armbrecht et al. 1979, 1998; Omdahl and DeLuca 1972). As a result, duodenal absorption of calcium is reduced. The reported inverse correlation between the amount of strontium that is absorbed and the levels of parathyroid hormone (Vezzoli et al. 1998) suggest that changes in parathyroid hormone levels mediate this effect. While there are no data on

strontium-binding to the calcium receptor of the parathyroid gland, it is likely that strontium binds in place of calcium, mimicking calcium and thereby suppressing parathyroid hormone levels. A reduction in parathyroid hormone levels will decrease the level of 1-hydroxylase available to activate vitamin D_3.

In addition to its effect on calcium absorption, excess absorbed strontium adversely affects bone development in several ways, leading to the development of rickets in children and young animals. Strontium binds directly to hydroxyapatite crystals, which may interfere with the normal crystalline structure of bone (Storey 1962). In addition, excess strontium may prevent the normal maturation of chondrocytes in the epiphyseal plates of long bones (Matsumoto 1976). Excess strontium apparently interferes with the mineralization of complexed acidic phospholipids that is thought to help initiate the formation of hydroxyapatite crystals in developing bone (Neufeld and Boskey 1994). As a result, affected bone contains an excess of complexed acidic phospholipid and a significantly lower ash weight. Insufficient mineralization reduces the strength of bones, so that the inability to resist compression from increasing body weight results in bone distortion (bowing).

There was one case of anaphylaxis reported in a paramedic who inhaled strontium-containing smoke in an enclosed space (Federman and Sachter 1997). Although other irritants in the smoke may have contributed to the incident, there is supporting evidence that large concentrations of stable strontium can stimulate the release of histamine from mast cells (ATSDR 2001e). Stable strontium stimulates degranulation in several cell types and it has been suggested that it acts by mimicking the receptor-linked rise in calcium that is the usual trigger for such events (Best et al. 1981).

Radioactive Strontium. The adverse health effects of radiostrontium are related to its sequestration in bone, the high energy of its beta emissions, and, in the case of strontium-90, its long biological retention and radioactive half-life (ATSDR 2001e). There is some evidence that body size or skeletal density may affect the outcome of exposures to radiostrontium. It was suggested that two cows that survived large oral doses of strontium-90 owed their survival to their breed characteristics (Cragle et al. 1969). The massive skeletons of Holsteins have wide bone marrow cavities so that tissue in the center of the bone marrow is not within range of the 1-centimeter (in soft tissues) beta emissions from radiostrontium bound to bone. Conversely, mice and rats are more vulnerable than large animals to radioactive strontium because all bone marrow tissues are within beta particle range. This renders rats and mice less useful than larger mammals as models for human exposure to radioactive strontium (ATSDR 2001e). In addition, adult rats are less satisfactory models than adults of other species because of the persistence of the

epiphyseal cartilaginous plate, which will result in the incorporation of larger amounts of radioactive strontium into bone (ATSDR 2001e).

Beta emissions from radiostrontium bound to bone resulted in various bone lesions (trabecular osteoporosis, sclerosis, osteolytic lesions), particularly in animals that were exposed chronically (Book et al. 1982; Clarke et al. 1972; Momeni et al. 1976). In young rats and rabbits exposed orally to strontium-90, necrotic effects on the vasculature of developing bone secondarily disrupted the process of osteogenesis (Casarett et al. 1962; Downie et al. 1959). Disruption in the metaphyseal microvasculature disorganized the transformation of cartilage into bone, so that chondrocytes inappropriately resumed active proliferation.

The severe reduction in hematopoietic tissue results from irradiation of the bone marrow by radio-strontium incorporated into bone. At high exposure levels, thrombocytopenia may lead to platelet loss severe enough to cause hemorrhaging, and the resulting anemia will be exacerbated by destruction of erythropoietic tissue. Impaired immune function results from the genetic damage to lymphocytes.

Radiostrontium is a genotoxic carcinogen. Following exposure *in vivo*, cytogenetic analysis has revealed aneuploidy, chromosomal breaks, gaps, rings, and exchanges, which are manifestations of irreparable changes in deoxyribonucleic acid (DNA). In dogs, acute inhalation of insoluble strontium-90 particles that lodged in the lungs resulted in chronic radiation exposure to the lungs, leading to pulmonary hemangiomas and carcinomas of pulmonary epithelia (Snipes et al. 1979). Other tissues were subsequently affected as the radioactive particles were cleared from the lungs. Following acute inhalation of soluble $^{90}SrCl_2$ aerosols, some dogs developed carcinomas of nasal airway tissues, probably resulting from irradiation of these tissues from the strontium-90 bound to the underlying bone (Gillett et al. 1987). Following oral or inhalation exposures, absorbed strontium-90 was distributed to bone, from which it irradiated the surrounding tissues and induced various kinds of osteosarcomas, as well as malignancies of hematopoietic tissues in bone marrow (ATSDR 2001e).

A.4 Health Guidelines

ATSDR (2001e) did not derive inhalation MRLs for stable strontium due to lack of suitable data.

ATSDR (2001e) did not derive acute or chronic oral MRLs for stable strontium due to lack of suitable data. An intermediate-duration (15–364 days) oral MRL of 2.0 mg/kg/day has been derived based on the study of Storey (1962), which found a no-observed-adverse-effect level (NOAEL) of 140 mg/kg/day for bone mineralization abnormalities in weanling rats that were exposed to dietary strontium carbonate for 20 days. This NOAEL was adjusted by an uncertainty factor of 30 (10 for extrapolation from animals to humans and 3 for human variability; a 3 was utilized because young animals are thought to represent a sensitive population) and a modifying factor of 3 (for short study duration and limited endpoint examination) to give the MRL of 2.0 mg strontium/kg/day.

ATSDR (2001e) did not derive MRLs for exposure to radioactive strontium by any exposure route. The MRLs for ionization radiation presented in the ATSDR Toxicological Profile for Ionizing Radiation (ATSDR 1999) were not cited as being applicable for exposures to radiostrontium. While no rationale for this was cited in the profile, it is likely because the ionizing radiation MRLs are based on external exposures in humans, which is likely to be a less important route of concern for radioactive strontium compounds.

The EPA considers all radionuclides to be cancer classification A (known human carcinogen), and has derived carcinogenic slope factors for inhalation, oral, and external exposure to radiostrontium isotopes (EPA 1997). For the most commonly-occurring isotope, ^{90}Sr, the cancer slope factor for ingestion exposure is $4.09x10^{-11}$ pCi^{-1}.

A.5 Derivation of Target Organ Toxicity Dose (TTD) Values

TTDs for chronic oral exposure to radiostrontium mixtures were derived for endpoints affected by radio-strontium and one or more of the other chemicals in strontium-cobalt-cesium-trichloroethylene-PCB mixture that is the subject of this Interaction Profile. The relevant endpoints for radiostrontium in this mixture include hematological and immunological endpoints. Chronic oral TTDs for these endpoints are derived below, using the methods described in ATSDR (2001a, Section 2.3.2). The derivations are based on data provided in ATSDR (2001e), and in particular, the oral levels of significant exposure (LSE) table.

Hematological Effects

Exposed persons from the Techa River population (see Section 2.2.2 of this document) exhibited alterations in hematological parameters, including thrombocytopenia (Akleyev et al. 1995). These effects

were observed in a portion of the exposed population that received radiation doses to the bone marrow at rates in excess of 30–50 rem (0.3–0.5 Sv) per year. While this population received co-exposure to cesium as well, the bulk of the radiation dose to the affected tissue (bone marrow progenitor cells) is believed to be related to internalized radiostrontium. The 0.3 Sv/year level, utilized as a lowest-observed-adverse-effect-level (LOAEL), converted to $8x10^{-4}$ Sv/day, and an uncertainty factor of 100 (10 for use of a LOAEL and 10 for intrahuman variability) was used to derive a TTD_{HEMATO} of $8x10^{-6}$ Sv/day.

Immunological Effects

Exposed persons from the Techa River population (see Section 2.2.2 of this document) exhibited alterations in immunological parameters, including leukopenia and granulocytopenia (Akleyev et al. 1995). These effects were observed in a portion of the exposed population that received radiation doses to the bone marrow at rates in excess of 30–50 rem (0.3–0.5 Sv) per year. While this population received co-exposure to cesium as well, the bulk of the radiation dose to the affected tissue (bone marrow progenitor cells) is believed to be related to internalized radiostrontium. The 0.3 Sv/year level (utilized as a LOAEL), converted to $8x10^{-4}$ Sv/day, and an uncertainty factor of 100 (10 for use of a LOAEL and 10 for intrahuman variability) was used to derive a TTD_{HEMATO} of $8x10^{-6}$ Sv/day.

Reproductive Effects

Available data on strontium toxicity do not suggest that exposure to radiostrontium compounds results in effects on the testes. No TTD was derived.

Neurological Effects

Available data on strontium toxicity do not suggest that exposure to radiostrontium compounds results in neurological effects. No TTD was derived.

Developmental Effects

Available data on strontium toxicity do not suggest that exposure to radiostrontium compounds results in developmental effects. No TTD was derived.

Hepatic Effects

Available data on strontium toxicity do not suggest that exposure to radiostrontium compounds results in effects on the liver. No TTD was derived.

Summary (TTDs for Radiostrontium)

TTD_{HEMATO} = $8x10^{-6}$ Sv/day (radiation dose localized to bone marrow)

TTD_{IMMUNO} = $8x10^{-6}$ Sv/day (radiation dose localized to bone marrow)

TTD_{REPRO} = Not applicable

$TTD_{DEVELOP}$ = Not applicable

TTD_{NEURO} = Not applicable

$TTD_{HEPATIC}$ = Not applicable

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Appendix B: Background Information for Cobalt

Cobalt is a naturally occurring element that exists in the environment as the free metal, and in the (II) and (III) oxidation states. Because the biological availability and toxicity of cobalt are primarily related to the cobalt(II) oxidation state, ATSDR (2001d) has focused on that form of cobalt. While a number of radioisotopes of cobalt exist (see ATSDR 2001d for a more complete discussion), by far the most common is ^{60}Co. As such, ATSDR (2001d) has focused primarily on radiation from ^{60}Co when discussing radioactive cobalt.

B.1 Toxicokinetics

The absorption of inhaled cobalt particles from the respiratory tract depends on many factors (ATSDR 2001d). The deposition pattern in the respiratory tract is related to particle size and aerodynamic properties. Fractional deposition of inhaled insoluble cobalt particles in humans varied from approximately 50% for particles with a mean geometric diameter of 0.8 μmole to approximately 75% for particles with a mean diameter of 1.7 μmole (Foster et al. 1989). Fractional deposition can be expected to vary considerably with age and breathing patterns. Once deposited, cobalt particles may be absorbed through the alveoli into the bloodstream, dependent primarily on their solubility, while insoluble particles will be phagocytized by alveolar macrophages. Phagocytized particles will eventually be transported to the stomach (ATSDR 2001d).

Absorption of cobalt compounds following oral exposure varies considerably (ATSDR 2001d), generally on the order of 1–10%, though it may range as high as 34% (Bailey et al. 1989; Collier et al. 1989; Patrick et al. 1989), following oral exposure. More soluble forms of cobalt appear to be more readily absorbed (Kreyling et al. 1986). Administration of cobalt chloride labeled with radioactive cobalt-58 and complexed with histidine, lysine, glycylglycine, ethylenediaminetetraacetic acid (EDTA), casein, or glycine resulted in decreased gastrointestinal absorption of cobalt, whereas administration of cobalt chloride (with cobalt-58 tracer) in cows' milk permitted a significantly greater (about 40%) fractional absorption through the gastrointestinal tract than cobalt chloride alone (Taylor 1962). Iron deficiency led to increased absorption of cobalt from the gastrointestinal tract, relative to iron-sufficient animals, and simultaneous administration of cobalt and iron in iron-deficient animals reduced the amount of cobalt absorbed, relative to cobalt alone (Reuber et al. 1994; Schade et al. 1970). Increasing oral doses of cobalt resulted in decreased fractional absorption (Houk et al. 1946; Kirchgessner et al. 1994; Taylor 1962).

Absorption is 3- to 15-fold greater in younger animals (rats and guinea pigs) than in adult animals (Naylor and Harrison 1995).

Four volunteers who placed their right hands in a box filled with hard metal dust (~5–15% cobalt metal, 95–85% tungsten carbide) showed an increase in urinary cobalt levels by an order of magnitude in the post-exposure samples, relative to pre-exposure samples (Scansetti et al. 1994). The levels remained elevated for as long as 48–60 hours. The absorption of 2.2×10^{-5} mg ^{60}Co/kg as cobalt chloride in 1.4N HCl through the intact or abraded skin of guinea pigs was examined by Inaba and Suzuki-Yasumoto (1979). Absorption through intact skin was very small (<1%), while absorption through abraded skin was almost 80% at 3 hours post-exposure. A study in hamsters (Lacy et al. 1996) also reported a low amount of absorption of cobalt through unabraded skin.

Following inhalation exposure of humans to insoluble cobalt compounds (cobalt metal, cobalt oxides), clearance from the body, assessed by both urinary/fecal clearance as well as a reduction in whole-body retention, appears to follow three-phase kinetics, with half-lives of 2–44 hours, 10–78 days, and on the order of years (ATSDR 2001d). Due to generally poor absorption following oral exposure, much of an oral dose of cobalt will be rapidly excreted in the feces. Absorbed cobalt, regardless of route of exposure, is eliminated primarily in the urine, with a small amount (5–30%) eliminated in the bile and feces. As a general rule, large amounts of absorbed cobalt are not retained within the body for long periods of time.

Following injection, animal studies have shown that the chemical form of the cobalt compound can affect its elimination. Subcutaneous injection of cobalt protoporphyrin in rats, in which the cobalt atom is chelated within the porphyrin ring, resulted in a slower elimination from the body than cobalt chloride, with significant cobalt levels (~20% of initial injection) still present in the body 14 days after exposure (Rosenberg 1993). Likewise, intramuscular injection of cobalt mesoporphyrin resulted in primarily in fecal excretion, with a high systemic retention (Feng et al. 1998).

B.2 Health Effects

Stable Cobalt. Results from studies of cobalt-exposed humans and animals indicate that the primary targets for the noncarcinogenic effects of cobalt are the respiratory system, heart, testes, immune system,

and hematological effects (ATSDR 2001d). The critical targets are expected to be the respiratory tract for inhalation exposure, the heart for oral exposure, and the skin for dermal exposure.

Studies of humans occupationally exposed to cobalt have reported respiratory effects, at airborne concentrations as low as 7–15 μg cobalt/m^3; the most sensitive of these appear to be asthma and decreased ventilatory function, with pneumonia and fibrosis occurring at higher levels (Nemery et al. 1992; Shirakawa et al. 1988, 1989). The mechanism behind these changes is not known, but may result from either direct effects of cobalt on the respiratory tissues or from cobalt's known sensitizing properties, which are believed to result in the asthma-like effects seen from chronic occupational exposure to cobalt. Similar effects were seen in short-term (NTP 1991) and chronic (NTP 1998) studies in rats and mice, with exposed animals showing respiratory tract inflammation, hyper- and metaplasia, and fibrosis.

Beer-cobalt cardiomyopathy was observed in people who heavily consumed beer containing cobalt sulfate as a foam stabilizer (Alexander 1969, 1972; Morin et al. 1971). The beer drinkers ingested an average of 0.04 mg cobalt/kg/day (Morin et al. 1971) to 0.14 mg cobalt/kg/day for a period of years (Alexander 1969, 1972). The cardiomyopathy was characterized by sinus tachycardia, left ventricular failure, cardiogenic shock, diminished myocardial compliance, absence of a myocardial response to exercise or catecholamine, enlarged heart, pericardial effusion, and extensive intracellular changes (changes in the myofibers, mitochondria, glycogen, and lipids). However, that the cardiomyopathy may have also been due to the fact that the beer-drinkers had protein-poor diets and may have had prior cardiac damage from alcohol abuse. Approximately 40–50% of the patients admitted to the hospital with cardiomyopathy died within several years. In a follow-up study, 0–43% of the survivors showed a residual cardiac disability and 23–41% had abnormal electrocardiograms (Alexander 1972).

Dermatitis is a common result of dermal exposure to cobalt in humans (ATSDR 2001d). Using patch tests and intradermal injections, it has been demonstrated that the dermatitis is probably caused by an allergic reaction to cobalt. Contact allergy was reported in 22 of 223 (9.9%) nurses who were tested with a patch test of 1.0% cobalt chloride (Kieć-Świerczyńska and Kręcisz 2000). Exposure levels associated with the development of dermatitis have not been identified.

Exposure to stable cobalt can lead to sensitization. In its most serious form, cobalt-sensitization can result in or exacerbate asthma (Shirakawa et al. 1988, 1989). Dermal sensitization and related cobalt-dermatitis have also been described. The mechanism for cobalt sensitization is not completely understood.

Antibodies to cobalt have been detected in individuals sensitized to cobalt, suggesting that a humoral immune response is a component of the sensitization phenomenon (Bencko et al. 1983; Shirakawa et al. 1988, 1989).

Following both inhalation and oral exposure of animals to cobalt, adverse effects on the testes were observed (degeneration, atrophy, decreased weight) (ATSDR 2001d). An increase in the length of the estrous cycle was also reported in female mice following inhalation exposure (NTP 1991). Because no effects on the reproductive system were found in patients who died as a result of beer-cobalt cardio-myopathy, at lower daily doses than those studied in animals, the significance of the animal results to humans is not clear.

Chronic-duration animal studies have shown that exposure to cobalt may cause cancers of the respiratory tract following inhalation exposure (NTP 1998). Available studies in occupationally-exposed humans have suggested an increase in cobalt-related cancers, but have not shown a definitive association between cobalt inhalation and increased cancer incidence or mortality (ATSDR 2001d). Data on the carcino-genicity of stable cobalt following the oral and dermal exposure routes are not available.

Radioactive Cobalt. Studies from exposed humans and animals indicate that radiation from cobalt isotopes can affect a wide variety of tissues, with greater effects occurring in tissues with greater levels of cellular division, such as the cells of the gastrointestinal tract (ATSDR 2001d). Radioactive cobalt isotopes emit both beta and gamma radiations. Given that beta radiation penetrates only short distances in tissues, while gamma radiation is highly penetrating, the majority of the systemic effects seen following exposure to radiocobalt are believed to be the result of gamma emission. While data on effects in humans internally exposed to low doses of cobalt radiation are scarce, animal studies suggest that the developing organism is the most sensitive target for external exposures of cobalt radiation. *In utero* exposure to moderately low doses of cobalt radiation (10–100 rad [0.1–1 Gy]) from external sources has resulted in decreased body weight (Devi et al. 1998; Wang et al. 1993; Zhong et al. 1996), organ weight, including brain weight (Devi et al. 1994, 1998; Hamilton et al. 1989) and delayed or abnormal organ development (Bruni et al. 1994; Devi et al. 1994; Zhong et al. 1996). Beagle dogs exposed *in utero* to 15–88 rad (0.15–0.88 Gy) of radiation from an external cobalt source showed an increased rate of diabetes mellitus (females only), as well as increased death rates from renal disease and neoplasia (Benjamin et al. 1998a, 1998b). Effects on reproductive organs, particularly in males, as well as an increased incidence of cancer are among the other effects noted following exposures to cobalt radiation (ATSDR 2001d). A detailed

description of the health effects of ionizing radiation can be found in the ATSDR Toxicological Profile for Ionizing Radiation (ATSDR 1999).

B.3 Mechanisms of Action

Stable Cobalt. The exact mechanisms by which cobalt exerts its effects on cells are not completely understood. However, a number of potential mechanisms have been identified. One mechanism by which cobalt may exert its effects is through interactions with the immune system. Exposures of humans to cobalt by the inhalation and dermal routes have resulted in sensitization to cobalt (ATSDR 2001d). Exposure to inhaled cobalt chloride aerosols can precipitate an asthmatic attack in sensitized individuals (Shirakawa et al. 1989), suggesting cobalt sensitization as one mechanism by which cobalt-induced asthma may be produced. IgE and IgA antibodies specific to cobalt have been reported in humans (Bencko et al. 1983; Shirakawa et al. 1988, 1989). There is evidence that cobalt sensitivity in humans may to be regulated by T-lymphocytes (Katsarou et al. 1997). A human helper T-lymphocyte cell line specific for cobalt ($CoCl_2$) has been established (Löfström and Wigzell 1986). Cobalt may also interact directly with immunological proteins, such as antibodies or Fc receptors, to result in immunosensitization (Cirla 1994). *In vitro*, cobalt(III) has been shown to reduce the proliferation of both B and T lymphocytes, as well as the release of the cytokines IL-2, IL-6, and IFN-Gamma (Wang et al. 1996). Interrelationships exist between nickel and cobalt sensitization (Bencko et al. 1983; Rystedt and Fisher 1983). In guinea pigs, nickel and cobalt sensitization appear to be interrelated and mutually enhancing (Lammintausta et al. 1985), though cross-reactivity was not reported to occur.

Soluble cobalt has also been shown to alter calcium influx into cells, functioning as a blocker of inorganic calcium channels (Henquin et al. 1983; Moger 1983; Yamatani et al. 1998). This mechanism has been linked to a reduction of steroidogenesis in isolated mouse Leydig cells (Moger 1983). Additionally, soluble cobalt has been shown to alter the inorganic calcium influx in liver cells after exposure to glucagon (Yamatani et al. 1998), and calcium influx into pancreatic β cells (Henquin et al. 1983) and isolated rat islets (Henquin and Lambert 1975). Cobalt may also affect neuromuscular transmission through antagonism with calcium (Weakly 1973).

Cobalt has been shown to have a number of effects on glucose metabolism. Treatment of animals with cobalt results in a depression of serum glucose levels (Eaton and Pommer 1973; Ybarra et al. 1997). In rats made diabetic by pretreatment with streptozotocin, this depression was persistent, whereas it was transient in normal rats (Ybarra et al. 1997). Many of the effects of cobalt on glucose metabolism are

thought to result from alterations in the expression of the *glut* family of glucose transport proteins, a family of facilitative Na+-independent transport proteins thought to mediate non-insulin-dependent transport of glucose. Exposure to soluble cobalt results in increased expression of genes for these proteins, particularly GLUT1, in cells of the liver, kidney cortex, myocardium, skeletal muscle, and cerebrum (Behrooz and Ismail-Beigi 1997; Ybarra et al. 1997). Cobalt also reduces the amount of glucose produced in liver cells following stimulation with glucagon (Eaton and Pommer 1973;Yamatani et al. 1998), as well as reducing insulin release in isolated rat islets (Henquin and Lambert 1975).

Another potentially important mechanism by which cobalt may exert effects is through its effects on heme and heme-containing enzymes. Cobalt is thought to inhibit heme synthesis *in vivo* by acting on at least two different sites in the biosynthetic pathway: synthesis of 5-aminolevulinate and conversion of 5-aminolevulinate into heme (de Matteis and Gibbs 1977). This inhibitory activity might result in the formation of cobalt protoporphyrin rather than heme (Sinclair et al. 1979). Cobalt treatment also stimulates heme oxidation in many organs, due to the induction of heme oxygenase (for review, see Sunderman 1987). Effects on heme synthesis may potentially affect a wide variety of heme-containing proteins, including monooxygenase enzymes (i.e., cytochrome P450), and catalase (Yasukochi et al. 1974). Conversely, cobalt acts, through a mechanism believed to involve a heme-containing protein, to increase erythropoietin, which stimulates the production of red blood cells (di Giulio et al. 1991; Goldberg et al. 1988). The regulatory mechanisms behind this apparent dichotomy have not been fully elucidated.

Another potential mechanism for cobalt toxicity is through oxidant-based and free radical-based processes. Exposure to soluble cobalt increases indices of oxidative stress, including diminished levels of reduced glutathione, increased levels of oxidized glutathione, and free-radical-induced DNA damage (Lewis et al. 1991; Zhang et al. 1998). Cobalt has been shown to generate oxygen radicals, including superoxide, both *in vitro* and *in vivo* (Kadiiska et al. 1989; Kawanishi et al. 1994; Moorhouse et al. 1985). *In vivo* exposure to cobalt in rats resulted in increased lipid peroxidation in the liver (Sunderman and Zaharia 1988). Exposure to cobalt results in accumulation in cardiac tissues, and is thought to stimulate carotid-body chemoreceptors, mimicking the action of hypoxia (di Giulio et al. 1990, 1991; Hatori et al. 1993; Morelli et al. 1994). Cobalt administration to a neuroblastoma/glioma cell line resulted in an upregulation of opioid delta receptors, through a mechanism similar to that of hypoxia (Mayfield et al. 1994). Exposure to cobalt also elicits effects on a number of genes known to be sensitive to oxidant status, including hypoxia-inducible factor 1, erythropoietin, vascular endothelial growth factor, catalase, and monooxygenase enzymes (ATSDR 2001d).

Several studies have demonstrated that hard metal, a metal alloy with a tungsten carbide and cobalt matrix, is considerably more toxic than either cobalt or tungsten carbide alone. A mechanism by which hard metal may exert its effects has been proposed by a group of Belgian researchers (Lasfargues et al. 1995; Lison 1996; Lison et al. 1995). In this proposed mechanism, tungsten carbide, which is a very good conductor of electrons, facilitates the oxidation of cobalt metal to ionic cobalt (presumably Co^{2+}) by transferring electrons from the cobalt atom to molecular oxygen adjacent to the tungsten carbide molecule. The result is an increased solubility of cobalt, relative to cobalt metal alone, and the generation of active oxygen species, both of which have been shown to occur following *in vivo* exposure to hard metal. The cobalt ions formed may be absorbed into the blood and transported throughout the body, where they may elicit effects by the above mechanisms. *In vitro* evidence for this mechanism includes the ability of hard metal particles, but neither cobalt nor tungsten carbide alone, to generate oxidant species and cause lipid peroxidation (Lison et al. 1995; Zanetti and Fubini 1997). Hard metal particles have also been shown to increase the levels of inducible nitric oxide synthase (iNOS), a gene responsive to oxidant stress (Rengasamy et al. 1999).

Radioactive Cobalt. Due to the nature of its ionizing radiation, radioactive cobalt can present a health hazard. Highly-penetrating gamma emissions are the major source of damage to tissues and internal organs following exposure to radioactive cobalt isotopes. If radioactive cobalt is internalized, nearby tissues are at highest risk for damage due to the release of beta particles. In either case, exposure to ionizing radiation results in an increased risk of cellular damage. Ionized molecules within irradiated cells may be repaired quickly to prevent further damage. On the other hand, irreparable damage may be imposed on cellular materials, such as DNA, which might ultimately result in either cell death or the formation of cancerous tumors. Very large acute radiation doses can damage or kill enough cells to cause the disruption of organ systems, resulting in acute radiation sickness or even death. Human and animal data indicate that sufficiently high exposures to cobalt radiation can result in adverse effects such as reduced fertility, abnormal development, genotoxicity, pulmonary fibrosis, gastrointestinal atrophy and fibrosis, hematological and lymphoreticular disorders, cancer, and death (ATSDR 2001d). For a more complete discussion of the mechanisms associated with the toxic effects of ionizing radiation, refer to Chapter 5 of the Toxicological Profile for Ionizing Radiation (ATSDR 1999).

B.4 Health Guidelines

ATSDR (2001d) did not derive acute or intermediate inhalation MRLs for cobalt due to the lack of suitable data.

ATSDR (2001d) derived a chronic-duration inhalation MRL of $1x10^{-4}$ mg cobalt/m^3 based on a NOAEL of 0.0053 mg cobalt/m^3 and a LOAEL of 0.015 mg cobalt/m^3 for decreased ventilatory function in exposed diamond-polishing workers (Nemery et al. 1992). The NOAEL was adjusted for continuous exposure and divided by an uncertainty factor of 10 (for human variability).

ATSDR (2001d) did not derive acute, intermediate, or chronic oral MRLs for cobalt due to the lack of suitable data.

ATSDR (2001d) did not derive MRLs specific for external exposure to cobalt radiation. The MRLs for external exposure to ionizing radiation derived in the ATSDR Toxicological Profile for Ionizing Radiation (ATSDR 1999) are applicable to cobalt. The acute MRL of 0.004 Sv is based on neuro-developmental effects in humans irradiated *in utero* during the atomic bombings of Hiroshima or Nagasaki. The chronic MRL for ionizing radiation is based on the average yearly background dose of ionizing radiation.

The EPA considers all radionuclides to be cancer classification A (known human carcinogen), and has derived carcinogenic slope factors for inhalation, oral, and external exposure to radiocobalt isotopes (EPA 1997). For the most commonly-occurring isotope, ^{60}Co, the cancer slope factor for ingestion exposure is $1.89x10^{-11}$ pCi^{-1}.

B.5 Derivation of Target Organ Toxicity Dose (TTD) Values

As the available data from the sites of concern report that the vast majority of cobalt contamination in these sites consists of radiocobalt, mainly cobalt-60, the TTD values below are based upon radiocobalt and cobalt radiation. TTDs for chronic oral exposure to radiocobalt mixtures were derived for endpoints affected by radiostrontium and one or more of the other chemicals in strontium-cobalt-cesium-trichloro-ethylene-PCB mixture that is the subject of this Interaction Profile. The relevant endpoints for radio-cobalt in this mixture include hematological, immunological, developmental, neurological, and hepatic endpoints. Chronic oral TTDs for these endpoints are derived below, using the methods described in

ATSDR (2001a, Section 2.3.2). The derivations are based on data provided in ATSDR (2001d), and in particular, the oral LSE table.

Hematological Effects

Data are not available on the levels of radiocobalt required to elicit hematological effects in humans or animals after oral exposure. Available data in humans after external exposure encompass only single-exposure scenarios. Whole-body external exposure of dogs to 0.075 Sv/day (assuming a quality factor of 1 for cobalt gamma rays) for up to 700 days resulted in a transient (lasting ~250 days) decrease in hematological parameters, with recovery occurring after 250 days. Using a tissue weighting factor of 0.12 for red bone marrow (ICRP 1993), this corresponds to a minimal LOAEL for hematological effects of radiocobalt of 0.009 Sv/day. To this value, an uncertainty factor of 300 (3 for minimal LOAEL, 10 for animal to human extrapolation, and 10 for intrahuman variability) was applied to give a TTD_{HEMATO} of $3x10^{-5}$ Sv/day.

Immunological Effects

Seed et al. (1989) reported a marked reduction in granulocytes, monocytes, and lymphocytes in beagle dogs exposed to 1.65 Sv/day over a 150–300 day period. To this LOAEL value, an uncertainty factor of 1,000 (10 for LOAEL, 10 for animal to human extrapolation, and 10 for intrahuman variability) was applied to give a TTD_{IMMUNO} of $2x10^{-3}$ Sv/day.

Reproductive Effects

While it is well-known that radiation from cobalt sources can have an effect on the testes, available human and animal data have only examined the effects of acute exposures. In a subchronic study, Searle et al. (1980) exposed female mice to 10 Sv/day of cobalt radiation, and reported a decrease in number of offspring per litter, and an increase in sterility. To this LOAEL value, an uncertainty factor of 1,000 (10 for LOAEL, 10 for animal to human extrapolation, and 10 for intrahuman variability) was applied to give a TTD_{REPRO} of $1x10^{-2}$ Sv/day.

Neurological Effects

Available studies with radiocobalt compounds or cobalt radiation suggest that the nervous system is only affected by cobalt radiation at very high (~5,000 Sv or more) radiation doses, or when exposure occurs during the development of the nervous system *in utero* (ATSDR 2001d). No TTD was derived.

Developmental Effects

ATSDR (1999) has derived an acute-duration (14 days or less) MRL of 0.004 Sv for exposure to ionizing radiation, based on neurodevelopmental effects (decreased IQ scores) in humans exposed *in utero* during the atomic bombing of Hiroshima and Nagasaki. This number is therefore adopted as the TTD for developmental effects of cobalt radiation.

Hepatic Effects

Available data on cobalt toxicity do not suggest that the liver is not a sensitive target following exposure to radiocobalt compounds. No TTD was derived.

Summary (TTDs for Radiation from Cobalt)

$TTD_{HEMATO} = 3x10^{-5}$ Sv/day (dose localized to bone marrow)
$TTD_{IMMUNO} = 2x10^{-3}$ Sv/day (total body dose)
$TTD_{REPRO} = 1x10^{-2}$ Sv/day (total body dose)
$TTD_{DEVELOP} = 4x10^{-3}$ Sv (total body dose)
TTD_{NEURO} = Not applicable
$TTD_{HEPATIC}$ = Not applicable

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Appendix C: Background Information for Cesium

Cesium is a naturally occurring element that exists in the environment as the free metal and in the (I) oxidation state. Because the biological availability and toxicity of cesium are primarily related to the cesium(I) oxidation state, ATSDR (2001c) has focused on that form of cesium. While a number of radioisotopes of cesium exist, the most common are ^{137}Cs and ^{134}Cs. As such, ATSDR (2001c) has chosen to focus primarily on radiation from these two isotopes when discussing radioactive cesium.

C.1 Toxicokinetics

Numerous toxicokinetic studies have been performed in animals exposed to small quantities of the radioisotope cesium-137. The biokinetic behavior of cesium has also been studied in humans either given tracer amounts of radiolabeled cesium or accidently exposed to larger amounts (ATSDR 2001c).

Inhaled or ingested cesium (in soluble compounds) is rapidly absorbed and widely distributed to all major tissues and organs (ATSDR 2001c). Insoluble airborne particles containing cesium may be retained in lung tissues and slowly cleared, but they do not appear to be absorbed in any significant amounts. Insoluble particles that are ingested are mostly excreted in the feces; human and animal studies indicate that only a small percentage of the ingested material is absorbed. Some degree of dermal absorption occurs, as demonstrated by qualitative findings of internalized radioactivity from compounds containing radioactive cesium following dermal exposure in humans and animals.

Following absorption, widespread distribution of cesium to all major soft tissues is observed in humans and animals, cesium levels being slightly higher in skeletal muscle than other tissues (ATSDR 2001c). Distribution patterns in animals have been shown to be similar following exposure by inhalation, oral, and parenteral routes of exposure. Cesium crosses the placenta and can be found in breast milk.

No reports were located regarding metabolism of cesium in humans or animals (ATSDR 2001c). However, cesium behaves in a manner similar to potassium, both elements being more highly concentrated in intracellular fluid. Cesium can replace potassium in biological systems, and cesium retention has been experimentally estimated based on elimination rates of potassium.

Excretory rates of cesium-137 have been studied in numerous populations exposed via fallout following nuclear incidents such as the Chernobyl accident, and models have been developed to describe relationships between intake and elimination (ATSDR 2001c). Experimental human studies have also been performed using tracer amounts of radioisotopes of cesium. Urinary excretion is the primary route of elimination of cesium, independent of the route of exposure. Urinary:fecal ratios in humans have been found to range from 2.5:1 to 10:1. Radiolabeled cesium excretory rates were lower in males suffering from muscular dystrophy than in age-matched controls (ATSDR 2001c).

Elimination of cesium in humans appears to be age- and sex-related and may be principally a function of body mass. Children ages 5–14 exhibited average elimination half-lives for cesium of 20 days, with no significant difference between males and females; elimination half-lives in older groups were significantly higher (47 days for adolescent and adult females; 67 days in 15-year-old males; 93 days in males 30–50 years of age).

C.2 Health Effects

Stable Cesium. No reports were located regarding health effects in humans or animals following inhalation exposure to potentially hazardous concentrations of stable cesium (ATSDR 2001c). Data on exposure to stable cesium by the dermal route are also lacking, with the only available studies being of acute duration and limited in scope.

Neulieb (1984) reported a case of a human who ingested about 34 mg Cs/kg (as cesium chloride) after morning and evening meals (68 mg Cs/kg/day) for 36 days. Self-reported gastrointestinal effects included decreased appetite, nausea, and diarrhea. The human reported experiencing, within 15 minutes of ingestion, general feelings of well-being, heightened sense perception, and tingling sensations in lips, cheeks, hands, and feet. No self-reported adverse effects were noted in performance of mathematical tasks or in automobile driving skill. Other examinations of the effects of stable cesium after oral exposure are limited to acute toxicity studies in animals.

Radioactive Cesium. No reports were located regarding health effects in animals following acute-, intermediate-, or chronic-duration inhalation or dermal exposure to radiocesium. Information regarding adverse effects following oral exposure is limited to two studies in which significantly reduced fertility and temporary sterility were observed in male mice following single or repeated oral administration of

radioactive cesium nitrate; post-mating embryo mortality was associated with increased frequency of dominant lethal mutations (Ramaiya et al. 1994).

Adverse neurological, developmental, reproductive, genotoxic, and cancer effects have been observed in animal studies employing external exposure to radioactive cesium sources. Impaired motor activity, decreased thickness of cortical layers of the brain, and increased aggressive behavior were observed after the birth of rats that had been briefly exposed *in utero* to relatively high (0.75–1.0 Sv) levels of external radiation from a cesium-137 source (Minamisawa et al. 1992; Norton and Kimler 1987, 1988). The most vulnerable developmental period was around gestational days 14–15. In another study, adverse developmental effects in fetal rats irradiated with 400 Sv on gestational day 12 included reduced litter size, smaller head size, retarded odontogenesis, and cleft palate when examined on gestational day 18 (Saad et al. 1991, 1994). Significant increases in the formation rate of micronuclei were seen in blood cells of other fetal rats following irradiation (50 Sv) of pregnant dams via a cesium-137 source on gestational day 14 (Koshimoto et al. 1994). Significantly reduced fertility (including temporary sterility) was reported in male mice exposed to an external cesium-137 source for almost 20 days (300 Sv total dose); an increased frequency of dominant lethal mutations was also indicated by increased post-mating embryo mortality (Ramaiya et al. 1994). Increased lifetime risk of mammary tumors was noted in female rats that were exposed to single whole-body doses of 100 Sv of radiation from a cesium-137 source between the ages of 8 and 36 weeks (Bartstra et al. 1998). Irradiation at 64 weeks, however, yielded fewer carcinomas than unirradiated controls.

C.3 Mechanisms of Action

Stable Cesium. Due to the relatively low abundance of stable cesium in the environment, its limited use in industry, and the lack of cesium-induced toxicity in animal studies, stable cesium is not likely to be of toxic concern to humans exposed to cesium by inhalation, oral, or dermal contact. Although a number of investigators have reported cesium-induced alterations in behavior or cardiac activity in animals systems exposed to cesium chloride via parenteral injection or using *in vitro* methods, the underlying mechanisms are not yet fully understood.

Cesium appears to have both depressant and anti-depressant properties in rodents, attenuating the conditioned avoidance response of pole-climbing (Bose and Pinsky 1983) and reducing vertical and horizontal motor activity (Bose and Pinsky 1981, 1984; Bose et al. 1981; Pinsky et al. 1980), and

enhancement of amphetamine-induced hyperactivity and reducing the locomotor depressive action of reserpine (Messiha 1978).

Increased vertical activity (rearing), but not horizontal activity (locomotion), was observed in mice given repeated injections of stable cesium chloride (Johnson 1972). Rastogi et al. (1980) found no increase in behavioral activity in rats repeatedly injected with stable cesium chloride, but noted a number of biochemical changes in the brain that included a significant rise in tyrosine hydroxylase activity that resulted in a slight but significant increase in tyrosine levels, markedly enhanced levels of the neurotransmitters norepinephrine and dopamine, and increased levels of a norepinephrine metabolite (4-hydroxy-3-methoxyphenylglycol). Cesium appeared to significantly block the uptake of norepinephrine by synaptosomes.

Cesium has been shown to trigger short-lived early afterdepolarizations (EADs) and polymorphic ventricular tachyrhythmias (VTs) in canine myocardial muscle fibers and Purkinje cells (Brachmann et al. 1983; Levine et al. 1985; Murakawa et al. 1997; Patterson et al. 1990), effects that are similar to those observed in humans with congenital and acquired long Q-T syndrome (Bonatti et al. 1983). Although the mechanisms responsible for these effects have not been elucidated, available animal data suggest that cesium-induced EADs and VTs are most likely the result of ionic imbalance due to reduced potassium permeability (Isenberg 1976) and/or imbalances of intra- and extracellular concentrations of calcium and sodium (Szabo et al. 1987).

Radioactive Cesium. Due to its ionizing radiation, radioactive cesium may present a significant health hazard. Highly-penetrating gamma emission is the major source of damage to tissues and internal organs following external exposure to radioactive cesium isotopes (ATSDR 2001c). Once radioactive cesium is internalized, nearby tissues are at highest risk for damage due to the release of beta particles. In either case, exposure to ionizing radiation results in significant risk of cellular damage. Ionized molecules within irradiated cells may be repaired quickly to prevent further damage. On the other hand, irreparable damage may be imposed on cellular materials, such as DNA, which might ultimately result in the formation of cancerous tumors. Very large acute radiation doses can damage or kill enough cells to cause the disruption of organ systems, resulting in acute radiation sickness in developing fetuses, and even death. Limited human and animal data indicate that exposure to radioactive cesium can result in adverse effects such as reduced fertility, abnormal neurological development, genotoxicity, and possibly cancer (ATSDR 2001c). For a more complete discussion of the mechanisms associated with the toxic effects of ionizing radiation, refer to Chapter 5 of the Toxicological Profile for Ionizing Radiation (ATSDR 1999).

C.4 Health Guidelines

ATSDR (2001c) did not derive oral or inhalation MRLs for cesium due to lack of available data.

ATSDR (2001c) did not derive MRLs specific for external exposure to cesium radiation. The MRLs for external exposure to ionizing radiation derived in the ATSDR Toxicological Profile for Ionizing Radiation (ATSDR 1999) are applicable to cesium. The acute MRL of 0.004 Sv is based on neuro-developmental effects in humans irradiated *in utero* during the atomic bombings of Hiroshima or Nagasaki. The chronic MRL for ionizing radiation is based on the average yearly background dose of ionizing radiation (ATSDR 1999).

The EPA considers all radionuclides to be cancer classification A (known human carcinogen), and has derived carcinogenic slope factors for inhalation, oral, and external exposure to radiocesium isotopes (EPA 1997). For the most commonly-occurring isotope, ^{137}Cs, the cancer slope factor for ingestion exposure is $3.16x10^{-11}$ pCi^{-1}.

C.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

As the available data from the sites of concern report that the vast majority of cesium contamination in these sites consists of radiocesium (mainly cesium-137), the TTD values below are based upon radio-cesium and cesium radiation. TTDs for chronic oral exposure to radiocesium mixtures were derived for endpoints affected by radiostrontium and one or more of the other chemicals in strontium-cobalt-cesium-trichloroethylene-PCB mixture that is the subject of this Interaction Profile. The relevant endpoints for radiocesium in this mixture include hematological, immunological, developmental, neurologic, and hepatic endpoints. Chronic oral TTDs for these endpoints are derived below, using the methods described in ATSDR (2001a, Section 2.3.2). The derivations are based on data provided in ATSDR (2001d), and in particular, the oral LSE table.

Hematological Effects

Exposed persons from the Techa River population exhibited alterations in hematological parameters,

including thrombocytopenia (Akleyev et al. 1995). These effects were observed in a portion of the exposed population that received radiation doses to the bone marrow at rates in excess of 30–50 rem (0.3–0.5 Sv) per year. While the bulk of this exposure is believed to have come from radiostrontium, the role of radiation from cesium compounds cannot be ruled out. The 0.3 Sv/year level (utilized as a LOAEL), converted to $8x10^{-4}$ Sv/day, and an uncertainty factor of 100 (10 for use of a LOAEL and 10 for intrahuman variability) was used to derive a TTD_{HEMATO} of $8x10^{-6}$ Sv/day.

Immunological Effects

Exposed persons from the Techa River population exhibited alterations in immunological parameters, including leukopenia and granulocytopenia (Akleyev et al. 1995). These effects were observed in a portion of the exposed population that received radiation doses to the bone marrow at rates in excess of 30–50 rem (0.3–0.5 Sv) per year. While the bulk of this exposure is believed to have come from radio-strontium, the role of radiation from cesium compounds cannot be ruled out. The 0.3 Sv/year level (utilized as a LOAEL), converted to $8x10^{-4}$ Sv/day, and an uncertainty factor of 100 (10 for use of a LOAEL and 10 for intrahuman variability) was used to derive a TTD_{IMMUNO} of $8x10^{-6}$ Sv/day.

Reproductive Effects

Ramaija et al. (1994) identified a NOAEL of 154 Sv and a LOAEL of 385 Sv for temporarily sterility in male mice exposed once per day for 14 days external cesium radiation. To the NOAEL of (154 Sv/14 days = 11 Sv/day), an uncertainty factor of 100 (10 for animal to human extrapolation, 10 for intrahuman variability) to derive the TTD_{REPRO} of 0.1 Sv/day.

Neurological Effects

Available studies with radiocesium compounds or cesium radiation suggest that the nervous system is only affected by cesium or cesium radiation at very high radiation doses or when exposure occurs during the development of the nervous system *in utero* (ATSDR 2001c). No TTD was derived.

Developmental Effects

ATSDR (1999) has derived an acute-duration (14 days or less) MRL of 0.004 Sv for exposure to ionizing

radiation, based on neurodevelopmental effects (decreased IQ scores) in humans exposed *in utero* during the atomic bombing of Hiroshima and Nagasaki. This number is therefore adopted as the TTD for developmental effects of cesium radiation.

Hepatic Effects

Data on the hepatic effects of oral or external exposure to radiocesium compounds are not available. No TTD was derived.

Summary (TTDs for Cesium)

$TTD_{HEMATO} = 8x10^{-6}$ Sv/day (dose localized to bone marrow)
$TTD_{IMMUNO} = 8x10^{-6}$ Sv/day (dose localized to bone marrow)
$TTD_{REPRO} = 0.1$ Sv/day (total body dose)
$TTD_{DEVELOP} = 4x10^{-3}$ Sv (total body dose)
TTD_{NEURO} = Not applicable
$TTD_{HEPATIC}$ = Not applicable

C.6 References

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Appendix D: Background Information for Trichloroethylene

D.1 Toxicokinetics

Studies of humans and rats indicate that inhaled trichloroethylene is rapidly and efficiently absorbed (ATSDR 1997). Initial rates of uptake are high, but decrease as steady state conditions are approached. Studies in humans indicated that 37–64% of inhaled trichloroethylene was absorbed. Ingested trichloroethylene is rapidly and completely absorbed by the gastrointestinal tract (ATSDR 1997). Fasted rats given gavage doses of 5–25 mg/kg trichloroethylene displayed peak blood concentrations within 6–10 minutes and absorbed >90% of the dose within 9 hours (D'Souza et al. 1985). Dermal absorption is rapid as indicated by observations of peak blood and exhaled air concentrations occurring within 5 minutes after a human subject immersed one hand in a trichloroethylene solution (ATSDR 1997). Once absorbed, trichloroethylene is widely distributed to organs throughout the body (including the developing fetus) and, due to its lipophilic properties, can accumulate in fat to a limited degree (ATSDR 1997). For example, 17 hours after a 6-hour/day, 4-day exposure of rats to 200 ppm, trichloroethylene was detected in perirenal fat and blood, but was not detected in other tissues (Savolainen et al. 1977).

Studies of humans and rodents indicate that inhaled trichloroethylene is eliminated from the body predominately in the urine as metabolites and, to a lesser degree, in exhaled breath as the parent chemical or other volatile metabolites such as trichloroethanol and carbon dioxide (ATSDR 1997). For example, following single or sequential daily exposures of human subjects to 50–380 ppm, 11 and 2% of the dose was eliminated as trichloroethylene and trichloroethanol in exhaled breath; 58% was eliminated as urinary metabolites; and 30% was unaccounted for (Monster et al. 1976, 1979). Trichloroethylene was detected in exhaled breath of humans 18 hours after exposure ended due to the relatively long elimination half-life of trichloroethylene in fatty tissue (3.5–5 hours) compared with other tissues. Following inhalation exposure to radiolabeled trichloroethylene, mice excreted 75% of radioactivity in the urine and 9% as carbon dioxide in exhaled breath (Stott et al. 1982). Similar patterns of elimination were observed in mice and rats following oral administration of trichloroethylene (Koizumi et al. 1986).

The principal urinary metabolites of trichloroethylene in humans and animals are trichloroethanol, trichloroethanol-glucuronide, and trichloroacetic acid (ATSDR 1997; Lash et al. 2000). Trichloroethylene is principally metabolized in the liver, but metabolism can also occur in Clara cells of the lungs and in the kidney. The principal pathway in humans and animals involves initial oxidation catalyzed by

CYP isozymes (CYP2E1 and 2B1/2). It has been proposed that this reaction forms an epoxide intermediate (trichloroethylene oxide) that rapidly converts to chloral hydrate, but an alternative proposal indicates that chloral hydrate formation involves chlorine migration in an oxygenated trichloroethylene-CYP transition state (Lash et al. 2000). Chloral hydrate can be oxidized to trichloroacetic acid via chloral hydrate dehydrogenase or reduced to trichloroethanol via alcohol dehydrogenase. Trichloroacetic acid is a strong inducer of peroxisome proliferation and has been associated with hepatocarcinogenicity in rodents. Trichloroethanol can be conjugated with glucuronic acid via glucuronyl transferase to form trichloroethanol-glucuronide. Studies with human hepatic microsomes indicate that CYP2E1 is the predominant isozyme responsible for the initial steps in trichloroethylene metabolism, although there is evidence that CYP isozymes induced by phenobarbital (i.e., CYP2B1/2) may also be involved. At high exposure levels, enzymes involved in the main oxidative metabolic pathway can be saturated, leading to conjugation with glutathione to produce S-(1,2-dichlorovinyl)glutathione (DCVG). DCVG is acted on by γ-glutamyl transferase to remove glutamine, and the glycine is removed by the action of dipeptidases to yield S-(1,2-dichlorovinyl)-L-cysteine (DCVC). Cleavage of the cysteine by β-lyase in the kidney can lead to intermediates with reactive thiol groups that can react with cellular macromolecules leading to renal cytotoxicity and carcinogenicity.

Minor metabolic pathways arising from the initial intermediate, trichloroethylene oxide or the trichloroethylene-CYP transition state, include: (1) transformation to dichloroacetic acid through a dichloroacetyl chloride intermediate (this path appears to be more important in rodents than humans); (2) hydrolytic dechlorinations to form formic acid and carbon monoxide; and (3) hydrolytic dechlorinations to form carbon dioxide via, sequentially, glyoxylic acid chloride, glyoxylic acid and oxalic acid (ATSDR 1997; Lash et al. 2000). Dichloroacetic acid can be conjugated with glutathione followed by sequential removal of glutamine and glycine to form dichlorovinyl-cysteine. Dichlorovinyl-cysteine can be transported to the kidney, where cleavage by β-lyase produces an intermediate with a reactive thiol group that can react with proteins and DNA leading to kidney cytotoxicity and kidney tumor development.

PBPK models have been developed for the disposition of trichloroethylene in mice, rats, and humans, including the prediction of target organ (e.g., liver, lung, brain, kidney) doses of biologically-active metabolites (ATSDR 1997; Clewell et al. 2000; Fisher 2000). The models are being used to aid assessment of noncancer and cancer human health risks based on rodent exposure-response data (Barton and Clewell 2000; Rhomberg 2000).

D.2 Health Effects

Results from studies of trichloroethylene-exposed humans and animals indicate that the primary targets for trichloroethylene noncarcinogens toxicity are the nervous system, liver, heart, and kidneys (ATSDR 1997). The critical target (i.e., the target in which effects occur at the lowest exposure level) is expected to be the nervous system. Studies involving acute- or intermediate-duration inhalation or oral exposures have observed changes in neurobehavior in humans and animals at lower exposure levels (50–200 ppm) than those associated with liver effects (liver enlargement and cellular hypertrophy) and kidney effects (increased kidney weights and cytomegaly and karyomegaly in renal tubular epithelial cells) observed in animal studies (ATSDR 1997). For example, Stewart et al. (1970) found no changes in liver function tests in humans who were exposed to 200 ppm for 7 hours/day for 5 days and reported experiencing headache, fatigue, and drowsiness. Effects on the heart appear to be restricted to cardiac arrhythmias due to trichloroethylene sensitization of the heart to epinephrine and other catecholamines.

Occupational exposure to trichloroethylene has been widespread due to its use in dry cleaning, for metal degreasing, and as a solvent for oils and resins. A recent article (Wartenberg et al. 2000) reviewed over 80 published papers and letters on the epidemiology of cancer in groups of people occupationally exposed to trichloroethylene. Elevated relative risks, ranging from 1.1 to 2.0, have been reported for kidney cancer, liver cancer, and non-Hodgkin's lymphoma in several cohorts of workers repeatedly exposed to trichloroethylene in workplace air (see Wartenberg et al. 2000). Workers in these studies, however, were also exposed to other solvents (e.g., tetrachloroethylene). Accurate adjustment for this and other confounding factors is not possible from the available data. Wartenberg et al. (2000) concluded that there is "moderate support" for a causative relationship between exposure to trichloroethylene and cancer using Hill's criteria of causation. Reflecting this assessment, the International Agency for Research on Cancer (IARC 1995) earlier concluded that the human evidence for trichloroethylene carcinogenicity is limited.

Chronic-duration animal studies have shown that cancer can be caused by inhalation or oral exposure to trichloroethylene, but do not point to a single target organ for increased tumor incidence. Carcinogenic responses have been observed in the liver, kidney, testes, lymphatic system, and lung, but the observed responses are not consistent across studies of different species and strains of animals (ATSDR 1997).

In general, carcinogenic responses to trichloroethylene are thought to involve trichloroethylene metabolites (Bull 2000; Green 2000; Lash et at. 2000). This hypothesis is supported by observations that mice appear to be uniquely susceptible to trichloroethylene-induced liver and lung tumors and display

higher rates of trichloroethylene metabolism than do rats. In contrast, rats appear to be uniquely susceptible to trichloroethylene-induced kidney damage and tumors. Based on its review of available data, ATSDR (1997) concluded that there is adequate evidence to indicate that trichloroethylene is carcinogenic in mice, that the evidence for trichloroethylene carcinogenicity in rats is equivocal, and that further study is required to determine whether or not the processes that induce liver cancer in mice also operate in the human liver. EPA supported monographs on trichloroethylene health risks have been recently published and are being used to develop updated EPA health risk characterizations for trichloroethylene (Scott and Cogliano 2000).

D.3 Mechanisms of Action

Like other solvents, nervous system effects from trichloroethylene are likely to involve disruption of neural membranes by the parent chemical, but trichloroethanol is also involved. In support of this hypothesis, Mikiskova and Mikiska (1966) reported that intraperitoneally administered trichloroethanol was 5–6 times more effective than trichloroethylene in altering electrophysiological variables associated with central nervous system depression in guinea pigs.

Metabolism of trichloroethylene is expected to produce cytotoxic and carcinogenic metabolites, including trichloroacetic acid, dichloroacetic acid, chloral hydrate, and 2-chloroacetaldehyde (ATSDR 1997). Drinking water administration of trichloroacetic acid to rodents has produced carcinogenic changes in the liver that are associated with the proliferation of peroxisomes. Reactive oxygen species produced by peroxisomes are thought to be involved in a sequence of DNA damage, cytotoxicity, regenerative cell growth, and tumor development. Phenobarbital pretreatment and induction of hepatic CYP isozymes appear to be associated with enhancement of acute trichloroethylene hepatotoxicity in rodents (Allemand et al. 1978; Carlson 1973; Moslen et al. 1977; Nakajima et al. 1990), providing further support for the idea that metabolites are responsible for the hepatotoxicity of trichloroethylene. Trichloroethylene-induced liver cancer and peroxisomal proliferation have been associated with rapid metabolism of trichloroethylene to trichloroacetic acid in mice, but this metabolic pathway appears to be limited in rats and humans. Dichloroacetic acid has also been shown to produce liver cancer in mice, but its hepatocarcinogenicity has been hypothesized to involve some other, as yet unspecified, mechanism of action. The mouse liver displays much higher rates of metabolism of trichloroethylene, and is more susceptible to the hepatotoxicity and hepatocarcinogenicity of trichloroethylene, than do the livers of rats and humans. With chronic oral exposure to high doses of trichloroethylene by gavage, increased incidence of toxic nephrosis and renal tumors occurred in male rats, but in female rats the nephrosis was not accompanied

by an increase in kidney tumors. Trichloroethylene-induced kidney damage has been proposed to involve conjugation products of trichloroethylene with glutathione. The conjugated products (e.g., dichlorovinyl-cysteine) can be hydrolyzed by β-lyase in the kidney forming a reactive thiol group that can react with cellular macromolecules and lead to cell damage. In support of this mechanistic hypothesis, chemical agents that inhibit β-lyase protected against dichlorovinyl-cysteine nephrotoxicity in rats (ATSDR 1997).

Trichloroethylene-induced cardiac arrhythmias are thought to involve parent-chemical sensitization of the heart to epinephrine-induced arrhythmias (ATSDR 1997). In animals, chemicals that inhibited the metabolism of trichloroethylene increased the potency of trichloroethylene to induce cardiac arrhythmias, whereas chemicals enhancing trichloroethylene metabolism decreased its potency.

D.4 Health Guidelines

ATSDR (1997) derived an acute inhalation MRL of 2 ppm for trichloroethylene based on a LOAEL of 200 ppm for subjective neurological symptoms such as fatigue and drowsiness in volunteers exposed 7 hours/day for 5 days (Stewart et al. 1970) and an uncertainty factor of 100 (10 for the use of a LOAEL and 10 to account for human variability).

ATSDR (1997) derived an intermediate-duration inhalation MRL of 0.1 ppm for trichloroethylene based on a LOAEL of 50 ppm for decreased wakefulness during exposure, and decreased postexposure heart rate and slow-wave sleep in rats exposed for 8 hours/day, 5 days/week for 6 weeks (Arito et al. 1994), and an uncertainty factor of 300 (10 for using a LOAEL, 3 for extrapolating from rats to humans, and 10 to account for human variability).

ATSDR (1997) did not derive a chronic inhalation MRL for trichloroethylene due to the lack of suitable data.

ATSDR (1997) derived an acute oral MRL of 0.2 mg/kg/day for trichloroethylene based on a LOAEL of 50 mg/kg/day for reduced rearing rate in mice and an uncertainty factor of 300 (10 for the use of a LOAEL, 10 for extrapolating from animals to humans, and 3 for human variability [a full factor of 10 was not used because pups were taken to represent a sensitive population]). The mice were exposed for 7 days beginning at 10 days of age and evaluated for locomotion, rearing, and total activity at 17 and 60 days of age; the effect was seen at 60 days of age (Fredriksson et al. 1993).

EPA's Integrated Risk Information System (IRIS) database (IRIS 2001) does not list a reference dose (RfD), reference concentration (RfC), or a carcinogenicity assessment for trichloroethylene. As reviewed by ATSDR (1997), the EPA Scientific Advisory Board in 1988 offered the opinion that the weight of evidence for trichloroethylene carcinogenicity was on a Group B2/C continuum (i.e., on the border between Group B2 and Group C). EPA has yet to present a more recent position on the weight-of-evidence classification for trichloroethylene carcinogenicity, but is currently evaluating several approaches to extrapolating from the animal tumor data for trichloroethylene to derive estimates of human cancer risks at environmentally relevant exposure levels (Scott and Cogliano 2000). NTP (2001) listed trichloroethylene as reasonably anticipated to be a human carcinogen based on limited evidence of carcinogenicity from studies in humans, sufficient evidence of malignant tumor formation in experimental animals, and convincing relevant information that trichloroethylene acts through mechanisms indicating it would likely cause cancer in humans. IARC (1995) assigned trichloroethylene to Cancer Group 2A, *probably carcinogenic to humans*, based on limited evidence in humans and sufficient evidence in experimental animals. IARC (1995) noted that (1) although a hypothesis linking the formation of mouse liver tumors with peroxisome proliferation is plausible, trichloroethylene also induced tumors at other sites in mice and rats, and (2) several epidemiological studies showed elevated risks for cancer of the liver and biliary tract and for non-Hodgkin's lymphoma.

D.5 Derivation of Target Organ Toxicity Dose (TTD) Values

TTDs for chronic oral exposure to trichloroethylene mixtures were derived for endpoints affected by trichloroethylene and one or more of the other chemicals in strontium-cobalt-cesium-trichloroethylene-PCB mixture that is the subject of this Interaction Profile. The relevant endpoints for trichloroethylene in this mixture include hematological, immunological, reproductive, developmental, neurological, and hepatic endpoints. Chronic oral TTDs for these endpoints are derived below, using the methods described in ATSDR (2001a, Section 2.3.2). The derivations are based on data provided in ATSDR (2000), and in particular, the oral LSE table.

Hematological Effects

Tucker et al. (1982) defined a NOAEL of 393 mg/kg/day and a LOAEL of 660 mg/kg/day for decreased red blood cell counts in CD-1 mice exposed in the drinking water for 6 months. The NOAEL of

393 mg/kg/day and an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for intra-human variability) were used to derive the TTD_{HEMATO} of 4 mg/kg/day.

Immunological Effects

Sanders et al. (1982) reported a NOAEL of 200 mg/kg/day and a LOAEL of 400 mg/kg/day of trichloro-ethylene for suppressed humoral and cellular immunity in mice exposed in the drinking water for 4–6 months. To the NOAEL of 200 mg/kg/day, an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for intrahuman variability) was applied to give the TTD_{IMMUNO} of 2 mg/kg/day.

Reproductive Effects

Available data do not suggest an effect of trichloroethylene on reproductive endpoints. At high doses, systemic toxicity and/or trichloroethylene's narcotic properties may result in secondary changes in measurements of reproductive function, but direct effects have not been noted. No TTD was derived.

Neurological Effects

ATSDR (1997) has developed an acute-duration (14 days or less) MRL of 0.2 mg/kg/day for trichloro-ethylene based on a LOAEL for behavioral changes in mice administered 50 mg/kg/day by gavage for 7 days starting at 10 days of age; effects were seen at 60 days of age. This LOAEL is similar to the LOAEL of 37 mg/kg/day in an intermediate-duration developmental study for behavioral changes at 60 days of age and a decreased number of myelinated fibers in the hippocampus at 21 days of age in rats exposed through their dams (ATSDR 1997). The dams were exposed to trichloroethylene in their drinking water starting 14 days before mating and throughout gestation and lactation. ATSDR (1997), however, also mentions a LOAEL of 23.3 mg/kg/day for behavioral effects and decreased brain myelination in adult rats exposed to trichloroethylene in their drinking water identified by Isaacson et al. (1990). Chronic oral studies in rats and mice have reported overt signs of neurotoxicity, but have only examined higher dose levels. Using the LOAEL of 23.3 mg/kg/day for adult rats and an uncertainty factor of 300 (10 for LOAEL, 10 for species extrapolation, and 3 for human variability because a potentially sensitive subpopulation has been tested) would result in a TTD_{NEURO} of 0.08 mg/kg/day. Because of the short duration of exposure (4 weeks, followed by 2 weeks of nonexposure, and 2 more weeks of exposure), and the lack of investigation of dose-response relationships for sensitive neurological

endpoints in chronic oral studies, an additional uncertainty factor of 10 for extrapolation to chronic exposure is appropriate. The total uncertainty factor of 3000 results in a TTD_{NEURO} of 0.008 mg/kg/day.

Developmental Effects

The lowest oral LOAEL for developmental effects reported by ATSDR (1997) is 37 mg/kg/day for behavioral changes at 60 days of age and a decreased number of myelinated fibers in the hippocampus at 21 days of age in rats exposed through their dams (Isaacson et al. 1989). The dams were exposed to trichloroethylene in their drinking water starting 14 days before mating and throughout gestation and lactation. To this LOAEL an uncertainty factor of 300 (10 for the use of a LOAEL, 10 for species extrapolation, and 3 for human variability because pups represent a sensitive subpopulation) was applied, resulting in a TTD_{DEVEL} of 0.1 mg/kg/day.

Hepatic Effects

Chronic studies of trichloroethylene toxicity have failed to report hepatic effects, even at doses as high as 1,000 mg/kg/day. The greatest subchronic NOAEL that is still below available subchronic LOAEL values is from the study of Stott et al. (1982) who exposed B6C3F1 mice to trichloroethylene by gavage for 5 days/week for 3 weeks. The study identified a NOAEL of 250 mg/kg/day and a LOAEL of 500 mg/kg/day for increased hepatic DNA content/gram of tissue. This NOAEL value and an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for intrahuman variability) were used to derive the TTD of 3 mg/kg/day for hepatic effects.

Summary (TTDs for Trichloroethylene)

TTD_{HEMATO} = 4 mg/kg/day

TTD_{IMMUNO} = 2 mg/kg/day

TTD_{REPRO} = Not applicable

$TTD_{DEVELOP}$ = 0.1 mg/kg/day

TTD_{NEURO} = 0.008 mg/kg/day

$TTD_{HEPATIC}$ = 3 mg/kg/day

D.6 References

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Appendix E: Background Information for PCBs

PCBs were manufactured in the United States between about 1930 and 1977, predominately for use as coolants and lubricants in electrical equipment such as transformers and capacitors due to their general inertness (they resist degradation by acids or alkali) and heat stability (ATSDR 2000). The manufacture of PCBs in the United States was stopped due to the evidence that they accumulate and persist in the environment and can cause toxic effects. Due to their biostability and lipophilicity, PCBs accumulate and concentrate in food chains; PCB concentrations in fatty tissue increase with increasing order of species in a food chain. There are 209 possible congeners of chlorinated biphenyls. PCBs were manufactured as complex mixtures of chlorinated biphenyls that varied in the degree of chlorination. For example, the commercial product Aroclor 1242, was a mixture of mono- through hepta-chlorinated biphenyls with an average chlorine content of 42%. Once released into the environment, commercial mixtures of PCBs undergo slow changes (predominately volatilization and biotransformation), so that patterns of PCBs in samples of food, human milk, or other environmental biota do not resemble any one particular commercial mixture (ATSDR 2000; Brouwer et al. 1998).

PCB congeners that have chlorines at the meta positions (3, 3', 5, or 5' carbons in the phenyl rings) or the para positions (4 or 4' carbons) can assume a co-planar geometry (i.e., the two rings can exist in the same plane), which is important in determining binding to the Ah receptor, a mediator of some of the toxic effects of PCBs. Increasing degrees of chlorination at the ortho positions (2, 2', 6, and 6' carbons) leads to increasing steric hindrance that prevents a co-planar geometry. In general, PCBs with no or only a single chlorine at an ortho position are co-planar, whereas congeners with two or more ortho chlorines are non-co-planar. PCBs without ortho chlorines generally account for only minor percentages of total PCBs in commercial PCB mixtures or samples of environmental biota (ATSDR 2000).

E.1 Toxicokinetics

PCBs can be absorbed via the inhalation, oral, and dermal routes of exposure, and are expected to passively diffuse across cell membranes (ATSDR 2000). Data on absorption of inhaled PCBs are insufficient to estimate rates of absorption, but studies of humans and animals exposed to airborne PCBs provide qualitative information that inhaled PCBs can be absorbed (ATSDR 2000). Ingested PCBs appear to be efficiently absorbed based on studies of infants consuming PCBs in their mothers' breast milk and studies of animals indicating retention percentages ranging from 60 to 100% of ingested doses.

Studies of animals dermally exposed to doses of radiolabeled PCBs for 24 hours reported absorption efficiencies ranging from about 15 to 60% of administered doses based on monitoring of urine for several weeks post-dosing.

Once absorbed, PCBs tend to accumulate in lipid-rich tissues, but PCBs have been detected in other tissues as well (ATSDR 2000). For example, in rats given gavage doses of Aroclors 1254 or 1260, the highest concentrations of PCBs were found in fat tissue, followed by concentrations in kidney, liver, and brain; plasma and muscle tissue showed the lowest concentrations. PCB concentrations in human milk can be high relative to other tissue due to breast milk's high fat content, and PCBs are efficiently transferred to children through breast-feeding. Results from animal studies support the importance of breast-feeding transfer to infants, and further indicate that PCBs can cross the placental barrier and enter the fetus. The amount of PCBs transferred to offspring is expected to be higher during lactation than during gestation. For example, in female rats administered PCBs before gestation, an average of 0.003% of the administered dose was transferred to the fetus, whereas 5% was transferred to sucklings (ATSDR 2000).

Rat studies indicate that different PCB congeners can accumulate to different degrees in different tissues. In rats given gavage doses of Aroclor 1254 (comprised of 2.1% mono-, di-, and tri-chlorinated PCB congeners, 19.1% tetra-, 49.6% penta-, 25.9% hexa-, 2.9% hepta-, and 0.5% octa- and nona-chlorinated PCB congeners), heavily chlorinated congeners (with 6–9 chlorines) accounted for greater percentages of total PCBs in analyzed tissues than in Aroclor 1254 itself (Kodavanti et al. 1998). Most PCBs in Aroclor 1254 have at least one ortho chlorine; PCBs without ortho chlorines account for <3% of PCBs in Aroclor 1254. Hexa- through nona-chlorinated congeners accounted for 29.3% of PCBs in Aroclor 1254, and, in contrast, 70, 66, and 49% of total PCBs in frontal cortical brain, liver, and fat tissues, respectively. Observations that lower chlorinated congeners or congeners with two adjacent unsubstituted carbons (i.e., at the meta and para positions; 3,4 or 3',4' positions) are metabolized more quickly than higher chlorinated congeners or congeners without adjacent unsubstituted carbons (ATSDR 2000; Parham and Portier 1998; Safe 1994) may provide at least a partial explanation of this differential tissue accumulation among PCB congeners.

Hydroxylated PCBs (i.e., phenolic PCBs) are the major metabolites of PCBs in humans and animals, and are formed either by direct catalysis or via arene oxide intermediates by several CYP oxygenase isozymes (ATSDR 2000; Expert Panel 1994; Safe 1994). Phenolic PCBs can be further hydroxylated to form dihydrodiols and catechols, or conjugated with glucuronides or sulfates, which facilitates excretion in bile

or urine. Glutathione conjugates are formed from arene oxide intermediates by glutathione S-transferase catalysis and transported to the intestine in the bile (Safe 1994). In the intestine, cleavage of the carbon-sulfur bond by microbes leads to the formation of thiol intermediates which can be methylated and reabsorbed. Following reabsorption, the methylated thiols can be further oxidized to form methylsulfonyl-PCBs which have been proposed to be involved in respiratory toxic effects from PCB exposure (Bergman et al. 1992; Brandt and Bergman 1987). Non-ortho-substituted PCBs appear to be preferentially metabolized initially by CYP isozymes that are induced by 3-methylcholanthrene (e.g., CYP1A1 and 1A2), whereas PCBs with multiple ortho substitutions appear to be preferentially metabolized by phenobarbital-inducible isozymes (e.g., CYP2B2, 2B1, and 3A) (ATSDR 2000; Expert Panel 1994). Congeners with mono-ortho substitution appear to be metabolized by both types of CYP isozymes.

Comparison of congener concentrations in commercial PCB mixtures with concentrations in adipose tissue from exposed workers indicates that some PCB congeners are more readily transformed by metabolism than others (ATSDR 2000). For example, both 2,2',4,4',5,5'-hexachlorobiphenyl and 2,2',4,4',6,6'-hexachlorobiphenyl are found in commercial PCB mixtures and in environmental samples, but 2,2',4,4',5,5'-hexachlorobiphenyl was detected in the workers' adipose tissue and 2,2',4,4',6,6'-hexa-chlorobiphenyl was not (ATSDR 2000). Results from rat studies indicate that the rate of metabolism decreases as the degree of chlorination on both phenyl rings increase and is dependent on the position of chlorine atoms on the phenyl ring (ATSDR 2000; Parham and Portier 1998; Safe 1984). Higher rates of hydroxylation are expected with PCBs that have two adjacent unsubstituted carbons in a phenyl ring at the 3,4 or 3',4' positions (i.e., meta-para unsubstituted carbons). For example, in humans exposed to PCBs, hexa- and hepta-chlorinated congeners were more slowly cleared from the blood than tetra- and penta-chlorinated congeners, and, among tetra- and penta-chlorinated congeners, those without adjacent unsubstituted carbons were more slowly cleared than those with adjacent unsubstituted carbons. In mice administered one of five tetrachlorobiphenyls, elimination half-lives for the congeners increased in the following order: 2,6,2',6' = 2,3,2',3' < 2,3,5,6 << 3,4,3',4' = 3,5,3',5, consistent with decreasing rate of metabolism in this sequence.

Different PCBs induce different spectrums of CYP isozymes (Connor et al. 1995; Hansen 1998). Commercial mixtures, such as Aroclor 1254 and 1242, induce both types of CYP isozymes. Co-planar PCBs without ortho substitution (e.g., the 3,3',4,4'-, 3,3',4,4',5-, and 3,3',4,4',5,5'-congeners) are among the most potent PCB inducers of CYP1A1/1A2 and have the greatest affinity for the Ah receptor. Mono-ortho PCBs with lateral substitutions (e.g., the 2,3,3',4,4'-, 2,3,4,4',5-, 2',3',4,4',5-, 2',3,4,4',5-,

2,3,3',4,4',5-, 2,3,3',4,4',5'-, 2,3',4,4',5,5'-, and 2,3,3',4,4',5,5'- congeners) induce both CYP1A1/1A2 and CYP2B1/2B2 isozymes and have less affinity for the Ah receptor than the non-ortho PCBs. Some diortho PCBs induce both types of CYP isozymes and have less affinity for the Ah receptor than the mono-ortho congeners (e.g., the 2,2',3,3',4,4'-, 2,2',3,4,4',5'-, and 2,2',3,3',4,4',5-). In contrast, most congeners with multiple ortho chlorines and one or two para chlorines (e.g., 2,2',4,4'-, 2,2',4,4',5-, 2,2',4,5,5'-, 2,3,3',4',6-, 2,2',4,4',5,5'-, 2,3,3', 4',5,6-, 2,2',3,4,4',5',6-, 2,2',3,4',5,5',6-, 2,2',3,3',4,4',5,5'-, and 2,2',3,3',4,5,5',6'-congeners) induce only the CYP2B1/2B2 and 3A isozymes and essentially do not bind to the Ah receptor.

In general, PCB congeners display a wide range of elimination rates that have been demonstrated in several cases to be associated with the rates at which they are metabolized (i.e., more rapidly metabolized PCBs are more rapidly excreted) (ATSDR 2000). Studies with animals given parenteral or oral doses of PCB mixtures or individual PCBs indicate that excretion of PCBs and their metabolites occurs via feces and urine with much greater amounts excreted in the feces (ATSDR 2000). For example, within 42 days of administration of an intravenous dose of radiolabeled 3,3',5,5'-tetrachlorobiphenyl (a PCB that is more rapidly metabolized than other more highly chlorinated PCBs) to rats, 80% of the dose was excreted in the feces and 6.1% was excreted in the urine. Less than 10% of radioactivity in bile, feces, and urine was parent compound. Within 40 weeks of administration of an intravenous dose of a poorly metabolized PCB (2,2',4,4',5,5'-hexachlorobiphenyl), rats excreted 16% of the dose in feces and 0.8% in the urine. Another significant route of elimination is breast milk; it has been estimated that an infant in an industrialized country may accumulate about 7% of its lifetime PCB body burden during 6 months of breast feeding (ATSDR 2000).

E.2 Health Effects

Associations have been noted between occupational exposure to commercial mixtures of PCBs and several health effects including chloracne and other skin changes; various hepatic effects including increased serum levels of liver enzymes and lipids, induction of drug-metabolizing enzymes, and hepatomegaly; decreased birth weight in offspring (of occupationally exposed mothers); and eye irritation (Safe 1994; Swanson et al. 1995).

Studies of cancer mortality in occupationally exposed workers have not found consistent or strong evidence of carcinogenicity, but findings of increased incidence of liver tumors in studies of rats exposed to commercial PCB mixtures suggest that PCBs are probable human carcinogens (ATSDR 2000; Safe

1994). IARC (1987) classified the human evidence as limited, whereas EPA (IRIS 2000) classified the human evidence as inadequate, but suggestive. Some cohort mortality studies of workers exposed during capacitor manufacturing and repair found increased risk for liver, biliary tract, gall bladder, and/or intestinal cancers, but statistically significant increases were not observed in all studies, and clear demonstrations of increasing risk with increasing exposure indices were not found (ATSDR 2000). Most case-control studies examining possible associations between breast cancer in women and concentrations of PCBs in breast tissue or blood found no statistically significant association (ATSDR 2000; Swanson et al. 1995).

Two incidences of consumption of PCB-contaminated cooking oil, one in Japan (the "Yusho" incident) and the other in Taiwan (the "Yucheng" incident), were associated with acne and skin pigmentation in adults and abnormalities in offspring including dark pigmentation of the skin, lower birth weight, and slower development (ATSDR 2000; Safe 1994; Swanson et al. 1995). These incidents are usually cited in discussion of the health effects of PCBs, but it is generally thought that the health effects were due primarily to polychlorinated dibenzofurans rather than PCBs (ATSDR 2000; Expert Panel 1994; Safe 1994; Swanson et al. 1995).

Studies of people and animals with diets containing Great Lakes fish (contaminated with PCBs and other biopersistent chemicals) provide suggestive evidence that frequent dietary consumption of contaminated fish by child-bearing-aged women may be associated with subtle neurobehavioral effects in their children, but no consistent evidence for associations with impaired reproduction, immune capabilities, or physical birth defects (ATSDR 2000). In one prospective study, limited evidence was presented relating maternal PCB exposure levels and deficits in neonatal behavioral development, short-term memory during infancy, and general intellectual ability in early school years (Jacobson 1985; Jacobson and Jacobson 1996; Jacobson et al. 1984, 1990). Statistically significant relationships between maternal PCB exposure levels (cord blood concentrations of PCBs with 7–9 chlorines) and deficits in neonatal behavioral development also were found in another more recent prospective study (Lonky et al. 1996; Stewart et al. 1999, 2000). Studies of people and animals with diets containing contaminated Baltic Sea fish provide suggestive evidence that contaminated fish consumption may be associated with impaired immunological competence or low birth weight, but do not clearly demonstrate dose-response relationships for the potential health hazards (Ross et al. 1995; Rylander and Hagmar 1999; Rylander et al. 1995, 1996, 1998a, 1998b; Svensson et al. 1994). Results from a North Carolina (Gladen and Rogan 1991; Gladen et al. 1988; Rogan et al. 1986a, 1986b, 1987) and a Dutch study (Huisman et al. 1995a, 1995b; Koopman-Esseboom et al. 1994, 1996; Patandin et al. 1998a, 1998b, 1999) of breast-fed children provide some

evidence that exposure to PCBs in human breast milk at exposure levels in the upper range of background levels or exposure to PCBs *in utero* may result in mild neurodevelopmental delays in some children. It is plausible that exposure to PCBs may have contributed to these associations, but these studies of possible health effects from environmental exposure to PCB-containing complex mixtures cannot determine with certainty which chemicals may cause the effects or determine possible interactions that may occur among the components.

Oral exposure to commercial mixtures of PCBs has been demonstrated to produce a wide array of toxic effects in animals including:

1. inhibition of body weight gain or body weight loss in rats, rabbits, monkeys, or minks after acute, intermediate, or chronic exposure (ATSDR 2000; Safe 1994);

2. increased porphyrin levels in liver, urine, or kidneys in rats after intermediate exposure (ATSDR 2000; Safe 1994);

3. dermal effects including acne, alopecia, or finger- and toenail loss in monkeys or rats exposed for intermediate or chronic exposure (ATSDR 2000);

4. induction of hepatic levels of Phase I (CYP oxygenases) and Phase II (e.g., ridine-5'-diphosphate glucuronyltransferases [UDP-GT]) enzymes (ATSDR 2000; Safe 1994);

5. increased liver weight, increased serum cholesterol, or degenerative liver changes (e.g., fatty changes, necrosis) in rats after acute exposure, in monkeys, rats, or mice after intermediate exposure, and in monkeys or rats after chronic exposure (ATSDR 2000);

6. altered thyroid hormone levels (e.g., T_4), histology, or weight in adult rats after acute exposure, in rats or mice after acute *in utero* exposure, and in adult rats after intermediate exposure (ATSDR 2000; Safe 1994);

7. fetal toxicity and decreased fetal survival in rats and hydronephrosis in mice exposed for acute durations *in utero*, fetal toxicity and decreased survival in monkeys, rats, mice, rabbits, guinea pigs, or minks exposed for intermediate durations, or in monkeys exposed for chronic durations (ATSDR 2000);

8. altered neurobehavior and/or brain chemistry in adult rats after acute exposure or adult monkeys or rats after intermediate exposure (ATSDR 2000);

9. altered neurobehavior in rats or mice after acute *in utero* exposure, in offspring of rats or mice exposed for intermediate durations, or in offspring of monkeys exposed for chronic durations (ATSDR 2000);

10. impaired reproductive function or altered reproductive organ weight or structure in adult monkeys, rats, mice, or mink after intermediate exposure, or in adult monkeys after chronic exposure (ATSDR 2000);

11. altered reproductive function or reproductive organ weight or structure in rats after acute *in utero* exposure (ATSDR 2000);

12. decreased immunological responsiveness (e.g., increased mortality from microbial infection or decreased antibody production in response to foreign blood cells) and/or altered organ weights or histopathology of thymus or spleen in monkeys, rats, mice, rabbits, or guinea pigs exposed for intermediate durations and in monkeys exposed for chronic durations (ATSDR 2000); and

13. increased incidence of liver tumors in rats exposed for chronic durations, and promotion (but not initiation) of preneoplastic lesions and tumors in the liver and lung of rats and mice following initiation by other carcinogens such as N-nitrosodiethylamine (ATSDR 2000).

E.3 Mechanisms of Action

Mechanisms by which the broad array of toxic effects observed in animals orally exposed to PCB mixtures develop are incompletely understood, but there is evidence to suggest that PCB congeners differ qualitatitively and quantitatively in biological activities and that multiple and diverse mechanisms are involved in responses to PCB mixtures. Research in the 1970s and 1980s focused on mechanistic similarities between PCBs and chlorinated dibenzo-p-dioxins (CDDs) involving initial mediation of effects by the Ah receptor (Poland and Knutson 1982; Safe 1990, 1994), but research through the 1990s has found increasing evidence for the involvement of alternative mechanisms for several PCB-induced effects (Chauhan et al. 2000; Cheek et al. 1999; Fischer et al. 1998; Hansen et al. 1998; Harper et al. 1993a, 1993b; Safe 1994; Tilson and Kodavanti 1998). An in-depth and all-inclusive review of the many recent and ongoing research efforts regarding PCB mechanisms of action is outside of the scope of this profile; rather, an overview of this large body of research is presented with the intent of providing information relevant to public health issues.

PCB Effects Involving Ah-receptor Dependent Mechanisms

INDUCTION OF HEPATIC CYP1A OXYGENASES AND PHASE II ENZYMES

PCBs induce hepatic Phase I enzymes (CYP oxygenases) and Phase II enzymes (e.g., UDP glucuronyl-transferases, epoxide hydrolase, or glutathione transferase) to varying degrees and specificities (Connor et al. 1995; Hansen et al. 1998; Safe 1994). Demonstration of relationships between PCB molecular structure and induction of CYP isozymes has provided a framework within which much mechanistic research has been conducted. In general, commercial mixtures of PCBs induce both 3-methyl-cholanthrene-type (CYP1A1 and 1A2) and phenobarbital-type (CYP2B1, 2B2, and 3A) CYPs. Strong

structure-activity relationships have been demonstrated between CYP1A1/1A2 induction in rodents and non-ortho and mono-ortho PCBs which can assume a coplanar molecular configuration and bind to the Ah receptor (Connor et al. 1995; Hansen et al. 1998; Safe 1994). In structure-activity studies of CYP1A induction in hepatocytes from Cynomolgus monkeys by 20 PCBs varying in degree and pattern of chlorine substitution (4–7 chlorines), the most potent inducers were without ortho chlorines (van der Burght et al. 1999). Many PCBs with ortho chlorines (mono-, di-, tri-, and tetra-ortho congeners) displayed no CYP1A induction activity, but a few mono-ortho and multiple-ortho congeners displayed activities that were about 1,000- and 10,000-fold less than the most potent non-ortho congeners, respectively (van der Burght et al. 1999). A working mechanistic hypothesis involves initial binding of coplanar PCBs to the Ah receptor in the cytosol of target cells, transport of the ligand-receptor complex to the nucleus, and subsequent changes in gene expression (e.g., induction of CYP1A1/1A2) leading to toxic responses via subsequent molecular mechanisms that are largely unexplored. Support for this hypothesis comes from the similarity in the array of PCB effects compared with the array produced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related halogenated aromatic hydrocarbons via initial Ah-receptor mediation, results from *in vitro* binding studies, and results from congener-specific *in vivo* studies of specific endpoints (e.g., enzyme induction and down regulation, body weight, and immunological responses to sheep red blood cells) in mouse strains and rat genders differing in responsiveness to Ah-receptor mediation (Hori et al. 1997; Safe 1990, 1994).

The complexity of Ah-receptor mediated effects on hepatic enzyme levels is illustrated by results from a study with mouse strains differing in Ah-receptor responsiveness and three PCB congeners (Hori et al. 1997). Ah-responsive (C57BL/6) and Ah-non-responsive (DBA/2) mice were given single intraperitoneal doses of 3,3',4,4',5-pentachlorobiphenyl, a congener with high Ah-receptor affinity, 3,3',4,4'-tetrachlorobiphenyl, a congener with lesser affinity, and 2,2',5,5'-tetrachlorobiphenyl, a low-affinity ligand. Only the high-affinity 3,3',4,4',5-congener produced body weight wasting in the dose range tested (up to 50 mg/kg) in Ah-responsive C57BL/6 mice, and this effect was accompanied by a decrease in selenium-dependent glutathione peroxidase and an increase in θ glutathione *S*-transferase. The effect on levels of these Phase II enzymes was not produced by the other congeners in C57BL/6 mice, and did not occur in DBA/2 mice exposed to any of the congeners, indicating the involvement of Ah-receptor mediation. These Phase II enzymes both play protective roles in scavenging intracellularly generated peroxides and the balance of their activities is likely to influence a cell's ability to withstand damage from peroxides.

BODY WEIGHT WASTING, THYMIC ATROPHY, AND PORPHYRIA

In addition to induction of hepatic levels of CYP1A1/1A2/1B1 and induction or repression of some Phase II enzymes, PCB-induced effects that appear to predominately involve Ah-receptor initiated mechanisms include body weight wasting and thymic atrophy from acute exposure (Hori et al. 1997; Safe 1994) and porphyria and porphyria cutanea tardea (Franklin et al. 1997; Smith et al. 1990a, 1990b). For example, single intraperitoneal doses of 5 mg/kg 3,3',4,4',5-pentachlorobiphenyl, a potent inducer of CYP1A1 and a high-affinity Ah-receptor agonist (relative to other PCBs), produced marked body weight wasting in Ah-responsive C57BL/6 mice, but not in DBA/2 mice, which have a low-affinity Ah-receptor (Hori et al. 1997). Showing a link between Ah-receptor responsiveness and development of uroporphyria, female F344 rats had significantly higher hepatic levels of porphyrins and ethoxyresorufin deethylase activity (an indicator of CYP1A1) in response to exposure to 0.005% Aroclor 1254 in the diet for 15 weeks than did male rats (Smith et al. 1990b). A similar gender-specific correlation between porphyrinogenic response and CYP1A induction was observed in iron-loaded F344 rats exposed to single intraperitoneal doses of 63 mg Aroclor 1254/kg (Franklin et al. 1997). In mice of the Ah-responsive C57BL/6 strain, a single dose of iron-dextran (600 mg Fe/kg), followed by feeding of a diet containing 0.01% Aroclor 1254 for up to 12 months, produced markedly increased hepatic levels of porphyrins and liver enlargement, but this response to iron and Aroclor 1254 was not observed in similarly treated DBA/2 mice (Smith et al. 1990a). Exposure to iron-dextran alone caused a moderate porphyria in C57BL/6 mice, but not in DBA/2 mice, lending support to a postulate that there are constitutive genetic differences between these strains that influence porphyria development and do not involve Ah-receptor mediation (Smith et al. 1990a). One mechanistic hypothesis proposes that induction of CYP1A2 by the Ah-receptor-PCB complex leads to generation of a competitive inhibitor of uroporphyrinogen decarboxylase in the liver and subsequent accumulation of porphyrins (Franklin et al. 1997).

Ah-RECEPTOR TEF APPROACH TO HEALTH HAZARD ASSESSMENT

A toxicity equivalency factor (TEF) approach to evaluating health hazards from exposure to complex environmental mixtures containing PCBs, CDDs, and chlorinated dibenzofurans (CDFs) has been developed and used to some extent to guide public health decisions because humans are exposed to complex and varying mixtures of these halogenated aromatic hydrocarbons and there are limited toxicological data for these complex mixtures and many of their components (ATSDR 1998; Safe 1990, 1994; van den Berg et al. 1998). PCBs were included in this component-based approach because (1) the spectrum of effects in animals exposed to some PCB mixtures and congeners is similar to the spectrum

produced by 2,3,7,8-TCDD (via Ah-receptor initial mediation), and (2) coplanar PCBs display Ah-receptor binding affinities that were related to their potency in producing health effects in rodents such as body weight wasting and inhibition of immunological responses to sheep red blood cells (Safe 1990, 1994). The TEF approach compares the relative potency of individual congeners, based on *in vitro* or acute *in vivo* data, with that of 2,3,7,8-TCDD, the best-studied member of this chemical class, so that the TEF for 2,3,7,8-TCDD is 1. The concentration or dose of each active component in a mixture of concern is multiplied by its TEF to arrive at a toxic equivalency (TEQ), and the TEQs are added to give the total toxic equivalency of the mixture which are compared with reference exposure levels for 2,3,7,8-TCDD expected to be without significant risk for producing health hazards. TEFs have recently been recommended by the World Health Organization for 7 CDD, 10 CDF, and 12 PCB congeners (Van den Berg et al. 1998).

Limitations in using the TEF approach for assessing health hazards from PCB-containing environmental media revolve around the inherent assumptions that the components jointly act in an additive manner through a common Ah-receptor initial mechanism and the evidence that Ah-receptor-binding congeners in PCB-containing environmental mixtures are minor components (Hansen 1998; Safe 1998a, 1998b) Several studies have provided evidence of non-additive interactions between specific PCB congeners and between some PCB congeners and 2,3,7,8-TCDD (Safe 1998a, 1998b) and there is evidence, discussed below, that several Ah-receptor-independent mechanisms may make contributions to toxic effects from PCB mixtures.

PCB Effects Involving Ah-receptor Independent Mechanisms

INDUCTION OF HEPATIC CYP2B OXYGENASES

In contrast to the distinct relationships between CYP1A1/1A2 induction, PCB molecular structures, and Ah-receptor initiation of toxic effects, relationships between potency in inducing CYPs 2B1/2B2/3A, PCB structural properties, and toxic effects are less clear (Connor et al. 1995). For example, some PCBs with two ortho chlorines and lateral chlorines induce both types of CYPs and display a very small affinity for the Ah receptor, whereas other di-ortho PCBs with one or two para chlorines predominately induce CYP2B1/2B2/3A and have no measurable affinity for the Ah receptor (Connor et al. 1995; Hansen 1998). Nevertheless, it is clear that PCB induction of phenobarbital-type CYPs is independent of the Ah receptor and that the most potent inducers of CYP have at least two ortho chlorines and one or two para chlorines.

Other PCB-induced effects involving Ah-receptor independent mechanisms include: neurological and neurodevelopmental effects involving changes in brain dopamine levels (Seegal 1996, 1998; Seegal et al. 1989, 1990; Shain et al. 1991), inhibition of dopamine vesicular uptake (Mariussen et al. 1999), and/or changes in brain cell intracellular calcium homeostasis and related signal transduction processes (Kodavanti and Tilson 1997; Tilson and Kodavanti 1997, 1998; Tilson et al. 1998; Wong and Pessah 1996, 1997; Wong et al. 1997); and tissue injury related to activation of neutrophils (Brown and Ganey 1995; Ganey et al. 1993; Tithof et al. 1995).

BRAIN DOPAMINE LEVELS AND NEUROLOGICAL EFFECTS

Aroclor 1254 decreased cellular levels of dopamine in cultured pheochromocytoma cells which synthesize, store, release, and metabolize dopamine in a manner similar to the intact mammalian central nervous system (Seegal et al. 1989). Daily oral exposure of adult nonhuman primates (*Macaca nemestrina*) to Aroclor 1016, a commercial mixture of lightly chlorinated PCB congeners, for 20 weeks, likewise, produced decreased dopamine concentrations in brain regions including the caudate, putamen, substantia nigra, and hypothalamus (Seegal et al. 1990). In these brain regions, only three PCB congeners were detected (2,4,4'-trichlorobiphenyl and 2,2',4,4'- and 2,2',5,5'-tetrachlorobiphenyl), suggesting that nonplanar PCBs, which are poor Ah-receptor agonists, may have been responsible for the effect. Structure-activity studies of 50 PCB congeners in the pheochromocytoma *in vitro* system found that the most active congeners had two ortho chlorines (e.g., 2,2',4,6-, 2,2',5,5'-, and 2,2',4,5-tetrachlorobiphenyl) and that congeners that were relatively strong Ah-receptor agonists (e.g., 3,3',4,4'-tetrachlorobiphenyl, 3,3',4,4',5-pentachlorobiphenyl) were inactive or had minimal effects on dopamine levels (Shain et al. 1991). However, ortho substitution was not the sole determinant of activity in this system; for example, a congener with four ortho chlorines (2,2',6,6'-tetrachlorobiphenyl) had no effect on dopamine levels in pheochromocytoma cells (Shain et al. 1991). The effect on dopamine levels has been postulated to involve decreased dopamine synthesis via direct or indirect PCB inhibition of tyrosine hydroxylase (Choksi et al. 1997; Seegal 1996) or L-aromatic amino acid decarboxylase (Angus et al. 1997) and/or decreased uptake of dopamine into vesicles (Mariussen et al. 1999). For example, several congeners that were inactive in causing dopamine level changes in pheochromocytoma cells (e.g., 2,2',6,6'- and 3,3',4,4'-tetrachlorobiphenyl) were much less active in inhibiting vesicular uptake of dopamine than other more active congeners (e.g., 2,2',4,6- and 2,2',4,5'-tetrachlorobiphenyl) (Mariussen et al. 1999).

DISRUPTION OF CA^{+2} HOMEOSTASIS AND NEUROLOGICAL EFFECTS

Neurological and/or neurodevelopmental effects from exposure to PCBs also have been hypothesized to involve interference with calcium homeostatic mechanisms and intracellular second messenger systems by PCB congeners that are not effective Ah-receptor agonists (see reviews by Kodavanti and Tilson 1997; Tilson and Kodavanti 1998; Tilson et al. 1998). In agreement with structure-activity relationships observed for PCB effects on dopamine levels in pheochromocytoma cells (Shain et al. 1991), 2,2'-dichlorobiphenyl altered intracellular calcium homeostasis in cultured rat cerebellar granule cells (increased free calcium levels and inhibited calcium buffering systems) at non-cytotoxic exposure concentrations (higher concentrations were cytotoxic) (Kodavanti et al. 1993). In contrast, 3,3',4,4',5'-pentachlorobiphenyl, one of the most effective Ah-receptor agonists among tested PCBs (Safe 1994), was not cytotoxic in the tested concentration range and did not alter calcium homeostasis to as great an extent as 2,2'-dichlorobiphenyl (Kodavanti et al. 1993). Using phorbol ester binding in rat cerebellar granule cells as a measure of protein kinase C translocation (which is thought to play key roles in cellular signal transduction in neurons and be regulated by several intracellular factors including intracellular levels of free calcium), commercial mixtures of PCBs (Aroclors 1016, 1254, and 1260) were shown to increase protein kinase C translocation in a concentration-dependent manner with varying potencies (Kodavanti et al. 1995). Aroclors 1016 and 1254 were more potent than Aroclor 1260. Examination of 24 PCB congeners showed that the most potent congeners (e.g., 2,2'-dichlorobiphenyl, 2,2',5,5'-tetrachlorobiphenyl, and 2,2',4,6,6'-pentachlorobiphenyl) had multiple ortho chlorines, whereas congeners without ortho chlorines tended to have either no or lower activities (Kodavanti et al. 1995). Similar results were found in structure-activity studies of 24 PCB congeners and their effects on *in vitro* Ca^{+2} sequestration by microsomes and mitochondria from freshly isolated rat cerebellar cells (Kodavanti et al. 1996). Structure activity relationships for PCB congeners and protein kinase C translocation in rat cerebellar granule cells and Ca^{+2} sequestration were similar to relationships for PCB congener-induced changes in dopamine levels in pheochromocytoma cells. For example, 2,2',5,5'- and 2,2',4,6-tetrachlorobiphenyl were among the most potent congeners and 2,2',6,6'- and 3,3',4,4'-tetrachlorobiphenyl were inactive in all three systems (Kodavanti et al. 1995, 1996; Shain et al. 1991).

One proposed molecular target for PCB disruption of calcium homeostasis that may be involved in neurological and neurodevelopmental effects is ryanodine-sensitive Ca^{+2} channels. Commercial PCB mixtures with intermediate to high degrees of chlorination (Aroclors 1248, 1254, 1260) enhanced ryanodine binding to calcium release channels in sarcoplasmic reticulum membranes from skeletal or cardiac rabbit muscles, and mixtures with lower (Aroclors 1221, 1232) or higher chlorination

(Aroclor 1268) showed little enhancement (Wong and Pessah 1996). Examination of selected penta-chlorobiphenyls indicated that ortho substitution favored activity; 2,2',3,5',6–pentachlorobiphenyl induced the greatest enhancement of ryanodine binding, whereas the 3,3',4,4',5-isomer did not enhance binding (Wong and Pessah 1996). The 2,2',4,6,6'-isomer with full substitution at the ortho positions produced less enhancement than the 2,2',3,5',6-isomer, indicating that some degree of rotation about the biphenyl bond may be important for full activity. Results from studies with hippocampal slices from freshly dissected rat brains indicated that perfusion with a triortho congener (2,2',3,5',6-pentachlorobiphenyl) enhanced ryanodine binding and inhibited electrophysiological responses to electrical pulse stimulations, but a mono-ortho congener (2,3',4,4'-tetrachlorobiphenyl) showed no enhancement of ryanodine binding and no inhibition of electrophysiological responses to stimulation (Wong et al. 1997). Offspring of rats exposed to gavage doses of 8 or 32 mg/kg/day 2,2',3,5',6-pentachlorobiphenyl on gestation days 10–16 displayed neurobehavioral changes as adults (depressed open field locomotor activity, faster acquisition on a working memory task, and no changes in a delayed spatial alternation task) and changes in ryanodine binding to calcium channels in specific regions of the brain (e.g., decreased in hippocampus and increased in cerebral cortex) (Schantz et al. 1997). Although it is not understood how these changes in ryanodine binding are specifically related to the observed neurobehavioral changes, the results from this series of studies emphasize the potential importance of Ah-receptor independent mechanisms in PCB-induced neurological and neurodevelopmental effects.

NEUTROPHIL FUNCTION AND IMMUNOLOGICAL EFFECTS AND TISSUE DAMAGE

PCB-induced functional changes in neutrophils may be involved in impaired immune defenses against pathogens or enhanced inflammatory responses (e.g., production of reactive oxygen species and cytolytic enzymes) leading to tissue injury. Incubation of quiescent cultured rat peritoneal neutrophils with Aroclor 1242 stimulated neutrophil production of superoxide anion and induced degranulation in a concentration-dependent manner without producing cytotoxicity (Ganey et al. 1993). In neutrophils that were activated for these functions, Aroclor 1242 produced further increases in superoxide anion production, but inhibited the activated degranulation process. Similar effects were observed when neutrophils were incubated with 2,2',4,4'-tetrachlorobiphenyl, a congener that has little affinity for the Ah receptor and induces phenobarbital-type CYPs, but 3,3',4,4'-tetrachlorobiphenyl, an Ah-receptor agonist and inducer of 3-methylcholanthrene-type CYPs, did not affect neutrophil function (Ganey et al. 1993). The effects of 2,2',4,4'-tetrachlorbiphenyl on *in vitro* production of superoxide anion by neutrophils were inhibited when neutrophils were incubated in the absence of extracellular calcium or in the presence of TMB-8, an antagonist of the intracellular mobilization of calcium (Brown and Ganey

1995). In addition, neutrophil degranulation induced by 2,2',4,4'-tetrachlorobiphenyl was enhanced by coexposure with the calcium ionophore A23187 (Brown and Ganey 1995). A mono-ortho congener, 2,3,4,5-tetrachlorobiphenyl, displayed somewhat different effects on neutrophil functions than those from the 2,2',4,4'-congener; it stimulated degranulation in quiescent and activated neutrophils, but only increased superoxide anion production in activated neutrophils, not in quiescent cells. The results from the neutrophil studies suggest the involvement of an Ah-receptor independent mechanism that involves PCB-induced increases in intracellular calcium or PCB effects on a signal transduction pathway that is dependent on calcium availability (Brown and Ganey 1995).

PCB Effects Involving Ah-receptor Dependent and Independent Mechanisms

PCB-induced effects that may involve both Ah-receptor dependent and independent mechanisms include liver hypertrophy (Hori et al. 1997); neurodevelopmental effects or reproduction effects involving changes in steroid hormone homeostasis (Arcaro et al. 1999; Connor et al. 1997; Fischer et al. 1998; Gierthy et al. 1997; Li and Hansen 1997; Nesaretnam and Darbre 1997; Nesaretnam et al. 1996; Seegal et al. 1997) and/or thyroid hormone disruption (Brouwer et al. 1998; Hansen 1998; Li and Hansen 1996a, 1996b, 1997); immunological effects (Harper et al. 1993a, 1993b; Silkworth and Grabstein 1982; Stack et al. 1999); and cancer through non-genotoxic mechanisms involving promotion of oncogenic cells (Cogliano 1998; Safe 1994) and/or genotoxic mechanisms (Robertson and Gupta 2000).

LIVER HYPERTROPHY

Liver hypertrophy in animals is produced by oral exposure to commercial PCB mixtures and appears to involve both Ah-receptor dependent and independent mechanisms. An illustration of this phenomenon is the observation that single intraperitoneal doses of any one of three PCB congeners varying in affinity for the Ah receptor produced liver hypertrophy in Ah-responsive (C57BL/6) and Ah-non-responsive (DBA/2 mice (Hori et al. 1997). The studied congeners were 3,3',4,4',5-pentachlorobiphenyl, a congener with high Ah-receptor affinity, 3,3',4,4'-tetrachlorobiphenyl, a congener with lesser affinity, and 2,2',5,5'-tetrachlorobiphenyl, a low-affinity Ah-receptor ligand.

REPRODUCTIVE EFFECTS

There are several studies examining female reproductive function variables in rats (Brezner et al. 1984; Hany et al. 1999; Linder et al. 1974; Sager and Girard 1994), mice (Welsch 1985), rabbits (Seiler et al.

1994), minks (Aulerich and Ringer 1977; Backlin and Bergman 1995; Kihlstrom et al. 1992), and monkeys (Arnold et al. 1995, 1996; Barsotti et al. 1976) repeatedly exposed orally to commercial PCB mixtures, predominately Aroclor 1254. In general, results from these studies identify minks and monkeys as sensitive species.

In minks, repeated exposure to low doses of Aroclor 1254 or Clophen A-50 (0.4–1.8 mg/kg/day) caused reproductive failure that has been associated with fetal death following embryo implantation (Aulerich and Ringer 1977; Backlin and Bergman 1995; Backlin et al. 1997; Kihlstrom et al. 1992). This effect may predominately involve Ah-receptor mediation, as evidenced by observations that only 1/10 mink exposed to 2.5 ppm Aroclor 1254 in the diet from 1 month prior to breeding through parturition produced offspring, whereas exposure by a similar protocol to 2,2',4,4',5,5'-hexachlorobiphenyl or 2,2',3,3',6,6'-hexachlorobiphenyl at concentrations up to 5 ppm did not influence reproductive performance (Aulerich et al. 1985). In contrast, exposure to dietary concentrations as low as 0.1 ppm 3,3',4,4',5,5'-hexachlorobiphenyl in this study (Auerlich et al. 1985), and 0.05 ppm in another study (Aulerich et al. 1987), caused mortality and prevented the minks for reproducing. Dietary exposure of minks to a fraction of Aroclor 1254, containing only congeners with no ortho-chlorines or a single ortho-chlorine and representing <20% of the total weight of Aroclor 1254, reduced litter size and fetal survival and increased incidence of interrupted pregnancies to a similar degree as doses of the complete Aroclor 1254 mixture (1.3 mg/kg/day) containing the same amount of these congeners (Kihlstrom et al. 1992). These results suggest the importance of Ah-receptor mediation of PCB-induced reproductive impairment in minks.

Another mink study comparing reproductive effects from intraperitoneal doses of 2,2',4,4',5,5'- and 3,3',4,4',5,5'-hexachlorobiphenyl reinforces the idea that congeners with high Ah-receptor affinity are more potent than congeners with low Ah-receptor affinity, but also provides evidence that Ah-receptor independent mechanisms may be involved (Patnode and Curtis 1994). Administration of single 20-mg/kg doses of the 2,2',4,4',5,5'-isomer (a poor Ah-receptor agonist that has been detected in wild mink tissues at concentrations 50-fold greater than the 3,3',4,4',5,5'-isomer) to pregnant minks on the approximate date of implantation did not affect the number of implantation sites (assayed 14 days after dose administration), but significantly decreased the number of embryos and embryonic weight, crown-to-rump length, and head length. The 3,3',4,4',5,5'-isomer (at lower dose levels of 0.4 or 0.8 mg/kg) also did not affect the number of implantation sites, but produced more severe effects on embryo survival and the weight, crown-to-rump length, and head length of surviving embryos (Patnode and Curtis 1994).

The mechanisms involved in PCB-induced reproductive impairment in minks are unknown, but examination of mid- to late-gestation placentae from minks exposed to Clophen A50 by light and electron microscopy revealed degenerative lesions in maternal (endothelial detachment and thrombosis in maternal vessels) and fetal (trophoblastic disintegration and loss of fetal capillary integrity) tissues (Backlin et al. 1998). Jones et al. (1997) postulated that the mechanisms are likely to be multifactorial given the possibility of direct and/or indirect tissue damaging actions of PCBs and the wide range of reported effects of PCBs on steroid hormone synthesis and functions including PCB regulation of CYP oxygenases that activate or deactivate different endogenous steroid hormones, estrogenic and antiestrogenic effects of PCBs, and PCB regulation of estrogen and progesterone receptor levels (see Battershill 1994; Li and Hansen 1997; Patnode and Curtis 1994).

Impaired ability to conceive and decreased fetal survival have been observed following repeated exposure of female Rhesus monkeys to commercial PCB mixtures. Exposure to dietary levels of 2.5 or 5 ppm Aroclor 1248 (approximately 0.1 or 0.2 mg/kg/day) for 16–19 months (including a 7-month period before breeding with non-exposed males) produced resorptions or abortions in 3/8 and 4/6 impregnated female Rhesus monkeys, compared with 0/12 in a control group (Barsotti et al. 1976). In this study, 12/12, 8/8, and 6/8 females became impregnated in the 0-, 2.5-, and 5-ppm groups, respectively. Another study fed encapsulated Aroclor 1254 at dose levels of 0, 0.005, 0.02, 0.04, or 0.08 mg/kg/day to female Rhesus monkeys for 37 months before breeding with non-exposed males and continued dosing through mating and gestation (Arnold et al. 1995). Incidences of abortions, resorptions, or stillbirths were 2/11, 5/10, 3/4, 2/6, and 4/5 in impregnated monkeys in the control through high-dose groups, respectively; respective incidences for impregnation success were 11/16, 10/16, 4/15, 6/14, and 5/15 (Arnold et al. 1995). Mechanisms for these effects in monkeys are unknown, but microscopic examination of tissues from control and exposed monkeys in the second monkey study found no evidence for an association with endometriosis (Arnold et al. 1996).

The plausibility that PCB effects on reproductive function (and other functions such as neurobehavior and immunological competence) may involve PCB effects on endocrine functions has led to investigations of the estrogenic and anti-estrogenic activities of PCB mixtures and individual congeners, and the effects of PCBs or related halogenated aromatic compounds on steroid hormone metabolism via induction of Phase I or Phase II enzymes. How these PCB effects are specifically related to PCB effects on reproductive function is unknown, but the results of these investigations provide further evidence that reproductive effects from PCB mixtures may not be restricted to Ah-receptor mediation alone and are likely to involve multiple mechanisms that have yet to be elucidated.

The estrogenic and anti-estrogenic activities of some commercial PCB mixtures, PCB congeners, and hydroxylated derivatives of PCB congeners have been assayed by examining uterine variables in immature or ovariectomized female rodents, cell proliferation or gene expression variables in cultured cells including human breast cancer or HeLa cells, and *in vitro* binding to estrogen receptor preparations (see Andersson et al. 1999; Arcaro et al. 1999; Battershill 1994; Connor et al. 1997; Gierthy et al. 1997; Hansen 1998; Kramer et al. 1997; Krishnan and Safe 1993; Li and Hansen 1997; Moore et al. 1997; Safe 1998a; Safe 1999 for reviews). In general, PCB-induced estrogenic activities have been characterized as weak compared to the endogenous hormone, 17β-estradiol, a wide variability of responses has been observed across types of PCBs and assays indicating the involvement of multiple mechanisms (e.g., direct binding to the estrogen receptor is not the only way that estrogenic or anti-estrogenic physiological effects may be mediated), anti-estrogenic activities have been most strongly associated with PCBs that are Ah receptor agonists, and hydroxylated metabolites of PCBs are postulated to be at least partly responsible for physiological responses to PCBs that may involve changes in estrogen receptor-dependent physiological processes.

Early studies showed that subcutaneous administration of 8 mg of Aroclors 1221, 1232, 1242, or 1248 increased uterine weight and glycogen content in rats, but similar exposure to Aroclors 1254, 1260, 1262, or 1268 did not produce this estrogenic effect (Bitman and Cecil 1970). More recent studies have provided further evidence that PCB mixtures can produce estrogenic responses (albeit weak) and that PCB congeners with multiple ortho chlorines (or their hydroxylated metabolites) may be at least partly responsible for these responses. Intraperitoneal doses of Aroclor 1242 (8 mg/rat on day 20 or 0.08 or 0.32 mg/rat on days 20 and 21) significantly increased uterine wet weight in immature female rats to about 40% of the increase produced by 0.001 mg 17β-estradiol (Jansen et al. 1993). Similar increases in uterine wet weight were produced by exposure to di-ortho congeners or hydroxylated derivatives (0.640 mg 2,2',5,5'-tetrachlorobiphenyl or 0.250 mg 2,4,6–trichloro-4'-hydroxy-biphenyl on days 20 and 21), but not by exposure to a coplanar congener without ortho chlorines (0.160 mg 3,3',4,4'-tetrachloro-biphenyl). In another study, the tetra-ortho congener, 2,2',6,6'-tetrachlorobiphenyl, displayed similarly weak estrogenic responses in an *in vitro* human breast cancer cell assay and an *in vivo* immature female rat assay (Arcaro et al. 1999). This congener did not competitively bind *in vitro* to recombinant human estrogen receptors α and β, but a hydroxylated metabolite, 2,2',6,6'-tetrachloro-4'-hydroxy-biphenyl, competitively bound to estrogen receptor α and produced proliferative responses in the breast cancer assay at concentrations about 10-fold lower than effective concentrations of the parent molecule (Arcaro et al. 1999).

Combined exposure of immature rats to 0.32 mg Aroclor 1242 and 0.001 mg 17β-estradiol produced a response similar to estradiol alone, indicating no obvious anti-estrogenic activity, but combined exposure to 0.001 mg estradiol and 0.160 mg 3,3',4,4'-tetrachlorobiphenyl markedly diminished the estradiol-induced increase in uterine wet weight (Jansen et al. 1993). Anti-estrogenic effects similar to those from 3,3',4,4'-tetrachlorobiphenyl were observed in rodent uterine tissue (Astroff and Safe 1990) and human breast cancer cells (Krishnan and Safe 1993) by other congeners with no or single ortho chlorines (e.g., 3,3',4,4',5-pentachlorobiphenyl, 2',3,3',4,4',5-hexachlorobiphenyl), but commercial PCB mixtures were not anti-estrogenic in the breast cancer cell assay. Whereas the data collected by Krishnan and Safe (1993) suggest that anti-estrogenic activities of PCBs may be related to Ah-receptor binding affinity, anti-estrogenic activities of hydroxylated PCB congeners with multiple ortho chlorines have been observed in several assay systems (Connor et al. 1997; Moore et al. 1997; Safe et al. 1998a).

Structure-activity relationships for estrogenic activities of PCB congeners or their metabolites are less clear. Some hydroxylated PCBs (2,4,6-trichloro-4'-hydroxy-biphenyl and 2,3,4,5-tetrachloro-4'-hydroxy-biphenyl) have been demonstrated to competitively bind to mouse estrogen receptor preparations and to increase uterine weight and glycogen in immature mice (Korach et al. 1988). In other estrogenic assays, 2,2',4,4',6-tetrachlorobiphenyl, 2,4,4',6-tetrachloro-4-hydroxy-biphenyl, and 2,4,6-trichloro-4'-hydroxy-biphenyl were equally effective in stimulating proliferation of human breast cancer cells, but only 2,4,6-trichloro-4'-hydroxy-biphenyl caused significant induction of vitellogenin in cultured brown trout hepatocytes (Andersson et al. 1999). A structure-activity study of eight hydroxylated PCBs in a series of *in vivo* and *in vitro* estrogenic assays found that structure activity relationships were complex and differed from one assay to the next (Connor et al. 1997; Safe et al. 1998a). For example, all but one of the compounds displayed competitive binding to rat and mouse cytosolic estrogen receptors (affinities ranged from about 10^{-3} to 10^{-5} of 17β-estradiol's affinity), but no estrogenic activities (wet weight, peroxidase activity, progesterone receptor level) were produced in the uteri of immature rats and mice exposed to three consecutive daily doses of the individual hydroxylated PCB congeners at levels of 25, 50, or 100 mg/kg. In contrast, four of the hydroxylated congeners produced estrogenic effects in cultured human breast cells and HeLa cells (Connor et al. 1997; Safe et al. 1998a).

Complex effects on male reproductive organs and functions have been observed in animals exposed to commercial PCB mixtures including reduced testes weight in adult male offspring of guinea pigs exposed during gestation to Clophen A50 (Lundkvist 1990), reduced testes weight in adult male offspring of female rats exposed from 50 days prior to mating through birth of offspring to 4 mg/kg/day Aroclor 1254 or a mixture of PCBs reflective of the composition of human milk samples (Hany et al. 1999), reduced

fertility (without changes in reproductive organ weights, sperm production, or sperm morphology) in adult male offspring of female rats exposed to doses of 8 mg/kg Aroclor 1254 and higher on lactation days 1, 3, 5, 7, and 9 (Sager et al. 1987, 1991), and elevated testes weight and increased sperm production in adult rats exposed to subcutaneous doses of Aroclor 1242 or 1254 (0.4 to 3.2 mg/day) on postnatal days 0–25 (Cooke et al. 1996). Mechanisms involved in these effects on male reproductive organ development are unknown but have been postulated to involve developmentally specific periods of responsiveness such as long-lasting elevation of testosterone-metabolizing enzymes from *in utero* exposure leading to reduced testes weight (Hany et al. 1999) and continued depression of thyroid hormone levels during the neonatal period leading to Sertoli cell proliferation and increased testes weight (Cooke et al. 1996). Whether or not PCB estrogenic and anti-estrogenic effects may be involved in any of these effects is unknown, but decreases in adult testis size and sperm production following early developmental exposure to other estrogenic compounds such as 2,3,7,8-TCDD is well documented (Gray et al. 1995).

DISRUPTION OF THYROID HORMONE HOMEOSTASIS

Concern that the thyroid hormone system may be important in PCB mechanisms of toxicity stems from mainly two important types of observations (Brouwer et al. 1998; Porterfield and Hendry 1998): (1) extensively corroborated findings in experimental animals that exposure to PCBs *in utero* and/or during early development (e.g., through breast milk) can deplete levels of circulating thyroid hormone in the fetus or neonate, which may give rise to a hypothyroid state during development (Collins and Capen 1980; Cooke et al. 1996; Corey et al. 1996; Darnerud et al. 1996; Goldey et al. 1995; Juarez de Ku et al. 1994; Li et al. 1998; Morse et al. 1996; Provost et al. 1999; Rice 1999; Schuur et al. 1998a; Seo and Meserve 1995; Zoeller et al. 2000); and (2) the recognition of the importance of thyroid hormones in normal development of the brain, as evident from neurodevelopmental disorders and deficits associated with hypothyroidism (Boyages 2000). The latter are typified by iodine deficiency (e.g., endemic cretinism), which can produce a wide range of neurodevelopmental deficits, including auditory, motor, and intellectual deficits. These outcomes suggest an importance of thyroid hormones in the normal development of the fetal cochlea, basal ganglia, and cerebral cortex, which begin to develop in humans during the second trimester of gestation. This is also the time in which the fetal thyroid gland becomes functional.

Evidence for a potential thyroid hormone involvement in PCB toxicity rests largely on observations made in experimental animals, including rodents and nonhuman primates. Although the studies differ in

design, the emerging picture from these studies is that, depending on dose and duration, PCBs can disrupt the production and disposition of thyroid hormones at a variety of levels. The major findings include: (1) histological changes in the thyroid gland indicative of both stimulation of the gland (e.g., similar to that induced by thyroid stimulating hormone [TSH] or a hypothyroid state) and a disruption of the processing of follicular colloid needed for normal production and secretion of thyroid hormone (ATSDR 2000); (2) depression of serum T_4 and T_3 levels, which may effectively create a hypothyroid state (ATSDR 2000); (3) increased rates of elimination of T_4 and T_3 from serum (Goldey and Crofton 1998); (4) increased activities of T_4-UDP-GT in liver (Chu et al. 1995; Desauliniers et al. 1997; Morse et al. 1996; Schuur et al. 1998a; Van Birgelen et al. 1995), which is an important metabolic elimination pathway for T_4 and T_3; (5) decreased activity of iodothyronine sulfotransferases in liver which are also important in the metabolic elimination of iodothyronines (Schuur et al. 1998a, 1998b, 1999); (6) decreased activity of iodothyronine deiodinases including brain Type-2 deiodinase, which provide the major pathways for the production of the active thyroid hormone, T_3 (Morse et al. 1996; Schuur et al. 1998a); and (7) decreased binding of T_4 to transthyretin an important transport protein for both T_4 and T_3 (Cheek et al. 1999; Darnerud et al. 1996).

The above observations suggest that PCBs can disrupt the production of thyroid hormones, both in the thyroid and in peripheral tissues, can interfere with their transport to peripheral tissues, and can accelerate the metabolic clearance of thyroid hormones. The most convincing evidence that PCBs can exert toxicity by disrupting the thyroid hormone system derives from two studies in rats. In one study, neurobehavioral deficits in pups that experienced exposures to Aroclor 1254 *in utero* and during nursing, were significantly attenuated by subcutaneous injections of T_4 that increased serum T_4 and T_3 concentrations that were otherwise depressed in the PCB-exposed animals (Goldey and Crofton 1998). While this study examined relatively high doses of Aroclor 1254 ($\geq$1 mg/kg/day), it nevertheless demonstrated neuro-developmental effects that are directly relevant to observations made in epidemiological studies and to neurological sequelae of fetal hypothyroidism, including motor disturbances and hearing.

In the second study, increased testes weight and sperm production in rats that were administered Aroclor 1254 on postnatal days 1–25 were attenuated by injections of T_4 on postnatal days 1–25, which also prevented the depression in serum T_4 concentrations (Cooke et al. 1996). Here again, although produced by relatively large doses of Aroclor 1254 ($\geq$40 mg/kg/day, subcutaneous), similar effects can be produced by other hypothyroid-inducing agents, including 6-propyl-2-thiouracil (PTU). Furthermore, the effects observed may reflect a disruption of the normal sexual maturation process, which is known to be associated with neonatal hypothyroidism in humans (Longcope 2000).

The effects PCBs on thyroid hormone status appear to involve Ah-receptor mediated actions as well as actions that appear to be independent of the Ah receptor. Depressed levels of serum T_4 have been observed in rats given oral doses of coplanar PCB congeners (Desauliniers et al. 1997; Van Birgelen et al. 1994) or di-ortho-substituted congeners that have relatively low affinity for the Ah receptor (Ness et al. 1993; Van Birgelen et al. 1992). At least one potential Ah-receptor mediated mechanism for this effect is the induction of UDP-GT, which catalyzes the metabolic elimination of T_4 to the T_4-glucuronide conjugate (Desauliniers et al. 1997; Van Birgelen et al. 1995). However, the UDP-GT mechanism does not appear to be important in the depression of T_4 levels produced by non-coplanar PCBs. Li and Hansen (1996a) observed depressed serum T_4 levels in rats administered a PCB mixture extracted from soil. Treatment of the mixture with activated charcoal greatly reduced the content of co-planar PCBs in the mixture, substantially decreased the potency of the mixture for inducing UDG-GT and ethoxyresourufin-O-deethylase (EROD), but had little effect on the potency for depressing T_4 levels. This suggests that an Ah-independent mechanism may exist that is not related to UDP-GT induction.

PCBs, including poly-ortho-substituted PCBs, which have a very low affinity for the Ah receptor, inhibit the binding of T_4 to transthyretin, an important transport protein for both T_4 and T_3 (Chauhan et al. 2000; Cheek et al. 1999; Darnerud et al. 1996). Inhibition of binding of thyroid hormones to transthryetin could alter hormone delivery to target tissues, including the brain, and could also result in depressed levels of serum total TT_4 or TT_3 (Brouwer et al. 1998).

IMMUNOLOGICAL EFFECTS

Studies with inbred mice strains differing in Ah-receptor responsiveness indicate that immunosuppression from PCB mixtures involves Ah-receptor mediation (e.g., Silkworth and Grabstein 1982; Harper et al. 1993a), but there is evidence that other mechanisms also may contribute to PCB-induced immunological effects (Harper et al. 1993a, 1993b; Stack et al. 1999). Illustrating the importance of Ah-receptor mediation for some PCB congeners, Ah-responsive C57BL/6 mice given single intraperitoneal doses of 100 mg/kg 3,3',4,4'-tetrachlorobiphenyl showed marked decreases in the number of splenic plaque-forming cells (PFCs) formed in response to immunization with sheep red blood cells (SRBCs, which are T-cell dependent antigens) compared with similarly treated Ah-non-responsive DBA/2 mice (Silkworth and Grabstein 1982). In addition, median effective doses (ED_{50}) values for 2,3,7,8-TCDD, three chlorinated dibenzofurans, and two PCBs without ortho substitution (3,3',4,4',5-pentachlorobiphenyl and 3,3',4,4',5,5'-hexachlorobiphenyl) in this immunotoxicity assay were lower in C57BL/6 mice than in DBA/2 mice, and the order of immunotoxic potency of these six compounds was the same as that for

potency in inducing CYP1A1 (Harper et al. 1993a). In another study, a series of four hexachlorinated biphenyls with differing chlorine substitution patterns displayed varying ED_{50} values in the same immunotoxicity assay as follows: 2, >1,000, 120, and >1,000 μmole/kg for a monoortho (2,3,3',4,4',5'-), a diortho- (2,2',4,4',5,5'-), a triortho- (2,2', 4,4', 5',6-), and a tetraortho-isomer (2,2',4,4',6,6'-), respectively (Harper et al. 1993b). Harper et al. (1993b) concluded that immunotoxic potency decreases (i.e., ED_{50}s increase) with increasing ortho-chlorine substitution of PCBs, but, as shown above, the decrease was not monotonic with increasing degree of chlorination. Furthermore, this relationship did not apply to more highly chlorinated PCBs with three or four ortho chlorines that are inactive as Ah-receptor agonists and only minimally induce CYP1A1 (Harper et al. 1993b). Three nonachlorobiphenyls (2,2,'3,3',4,4',5,5',6-, 2,2,',3,3',4,4',5,6,6'-, and 2,2',3,3',4,5,5',6,6'-nonachlorobiphenyl) and decachlorobiphenyl displayed ED_{50}s for inhibition of the splenic PFC response to SRBC in C57BL/6 mice that were less than those for hexachlorobiphenyl isomers with multiple ortho chlorines reported above: 15, 7, 17, and 35 μmole/kg, respectively. These results are consistent with the hypothesis that some PCBs induce immunotoxicity via Ah-receptor *independent* mechanisms. In an *in vitro* assay of cell proliferation in response to lipopoly-saccharide (a T-cell independent antigen), Aroclors 1221, 1242, 1254, or 1260 inhibited the proliferative response similarly in splenocytes from either C57BL/6 or DBA/2 mice (Stack et al. 1999). Two non-ortho and two mono-ortho PCBs that have been demonstrated to be effective Ah-receptor agonists and CYP1A1 inducers did not inhibit the *in vitro* proliferative response to lipopolysaccharide, but two di-ortho congeners (2,2',3,4,4',5- and 2,2',4,4',5,5'-hexachlorobiphenyl) significantly inhibited the response. These *in vitro* results provide supporting evidence for the existence of mechanisms of PCB immunotoxic actions that are independent of the Ah receptor.

CANCER

Lifetime oral exposure to any one of four commercial PCB mixtures (Aroclors 1016, 1242, 1254, and 1260) has been demonstrated to produce liver tumors in female rats; Aroclor 1260 also induced liver tumors in male rats (Mayes et al. 1998). Mixtures with high chlorination content (e.g., Aroclor 1254) were generally more potent than mixtures with low chlorine content (e.g., Aroclor 1016) (Mayes et al. 1998). Tumor promotion by commercial PCB mixtures following initiation by a variety of chemical agents also has been investigated in a number of animal systems including rat liver, rat kidney, mouse skin, and newborn mouse liver and lung (see Silberhorn et al. 1990 for review). The tumor promoting effect of extended exposure to PCB mixtures was demonstrated principally in the liver of rats; there is some evidence that PCB mixtures also can promote tumors in mouse lung and mouse skin, but not in rat

kidneys. The mechanism of PCB-induced cancer is poorly understood, but there is evidence to suggest that both Ah-receptor dependent and independent mechanisms may be involved.

PCB promotion of tumors does not appear to be solely an Ah-receptor mediated process, since individual congeners that are not Ah-receptor agonists have tumor promotion capabilities in animal systems. For example, 2,2',5,5'-tetrachlorobiphenyl, 2,2',4,4'-tetrachlorobiphenyl, and 2,2',4,4',5,5'-hexachlorobiphenyl were shown to promote liver tumors in female Sprague-Dawley rats (Hemming et al. 1993; Preston et al. 1985). In addition, 2,2',5,5'-tetrachlorobiphenyl, 2,2',3,3',4,4'-hexachlorobiphenyl, and 2,2',4,4',5,5'-hexachlorobiphenyl were potent inhibitors of *in vitro* gap junctional cellular communication, an assay that is indicative of tumor promotion capacity (Bager et al. 1997; De Haan et al. 1996). A general working mechanistic hypothesis for PCB promotion of liver tumors involves indirect stimulation of cell proliferation following cell or tissue injury by reactive metabolites of PCBs (Silberhorn et al. 1990). Alternatively, the cell injury could be caused by increased intracellular concentrations of other reactive species (e.g., superoxide anion or other reactive oxygen species) caused by an overall imbalance from PCB-induced perturbations of cellular biochemical processes, including induction of CYP oxygenases and glutathione S-transferases, repression of selenium-dependent glutathione peroxidases, and/or disruption of calcium homeostatic processes and signal transduction pathways (Silberhorn et al. 1990).

PCB mixtures have not shown consistent tumor initiating activity in animal initiation-promotion protocols (Silberhorn et al. 1990), but demonstration that chronic oral exposure to commercial PCB mixtures induced liver tumors in female rats (Mayes et al. 1998) suggests that PCBs may have both tumor initiating and promoting activities. Although PCB mixtures generally have been found to be inactive as mutagens in *Salmonella typhimurium* strains and in several other tests of genotoxicity that may be predictive of tumor initiation capability (see Silberhorn et al. 1990 for review), *in vitro* studies with rat microsomes have indicated that metabolism of lower chlorinated PCBs (e.g., 4-chlorobiphenyl, 3,4-dichlorobiphenyl, and 3,4,5-trichlorobiphenyl) can lead to covalently modified macromolecules including proteins and DNA (see Robertson and Gupta 2000 for review). Studies demonstrating the Ah-receptor dependence or independence of this potential genotoxic effect from PCBs were not located. The available data indicate that PCBs are not potent genotoxicants, but the possible involvement of genotoxic mechanisms (involving covalent modification of proteins and/or DNA) in the development of PCB-induced cancer is not without some experimental support.

The relative contribution that Ah-receptor dependent and independent mechanisms may make to carcinogenic responses to PCB mixtures is unknown. Safe (1994) compared carcinogenic responses of female

rats to 2,3,7,8-TCDD in the diet with responses of female rats of the same strain to Aroclor 1260 in the diet using the TEF approach. TCDD at a TEQ feed concentration of 2,100-ppt induced hepatic adenocarcinomas in 11/50 (22%) rats, whereas a TEQ of only 1,040 ppt from Aroclor 1260 induced adenocarcinomas in 24/47 (51%) rats. For this situation, the TEF approach markedly underestimated the carcinogenic response to Aroclor 1260. A possible explanation is that PCB congeners that are not Ah-receptor agonists and are abundant in Aroclor 1260 make significant contributions to the mixture's carcinogenicity. Although this comparison suggests that the TEF approach may underestimate cancer responses to complex PCB mixtures, another study of the tumor promotion activity of a simpler mixture of two CDDs, one CDF, and three PCBs in female rats found that the TEF approach overestimated the observed response by a factor of about 2 (van der Plas et al. 1999). The mixture contained 2,3,7,8-TCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin, 2,3,4,7,8-pentachlorodibenzofuran, 3,3',4,4',5- and 2,3',4,4',4-pentachlorobiphenyl, and 2,3,3',4,4',5-hexachlorobiphenyl at relative levels found in Baltic Sea herring. The rats were initiated with an injection of diethylnitrosoamine, 24 hours after a partial hepatectomy and were administered weekly subcutaneous injections of the mixture for 20 weeks starting 6 weeks after initiation. The volume and volume fraction of glutathione *S*-transferase-positive altered hepatic foci were taken as indicators of tumor promotion activity in this study (van der Plas et al. 1999). Although the composition of this mixture reflected relative concentrations and accounted for >90% of total TEQs in Baltic Sea herring, it did not contain PCBs with multiple ortho chlorines which comprise the predominant bulk of PCB weight in most commercial and environmental mixtures. For example, non-, mono-, and di-ortho congeners accounted for <1, 18, and 82% of PCB weight per gram of fat in human milk samples from Italy (Larsen et al. 1994). Another group of rats was similarly treated with the same synthetic mixture plus a di-ortho PCB congener (2,2',4,4',5,5'-hexachlorobiphenyl), which is one of the predominant PCB congeners in environmental mixtures and has minimal Ah-receptor agonist activity (van der Plas et al. 1999). Mean foci volume and foci volume fraction were increased in rats treated with the supplemented mixture compared with the mixture without the di-ortho congener, but the observed responses were still less than that predicted by the TEF approach. Better understanding of the relative contributions of Ah-receptor dependent and independent mechanisms to the carcinogenicity of PCB mixtures awaits further research.

E.4 Health Guidelines

ATSDR (2000) derived an intermediate oral MRL for PCB mixtures of 0.03 µg/kg/day based on a LOAEL of 0.0075 mg/kg/day for neurobehavioral alterations in infant monkeys that were exposed to a PCB congener mixture representing 80% of the congeners typically found in human breast milk (Rice

1997, 1998, 1999; Rice and Hayward 1997, 1999). The infant monkeys were given oral doses of 0 or 0.0075 mg/kg/day from birth to 20 weeks of age. The dose level was selected to be equivalent to an approximate daily intake of a nursing human infant whose mother's milk contains 50 ppb PCBs. Treated monkeys showed decreases and variable increases in response latencies across three tasks of nonspatial discrimination reversal, retarded acquisition of a delayed alternation task, increased errors at short delay task responses, and alterations in fixed-interval and fixed-ratio performance tasks. The findings were interpreted to suggest that postnatal PCB exposure resulted in impaired learning, impaired perserverative behavior, and/or inability to inhibit inappropriate responding. To derive the MRL, the LOAEL was divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability).

ATSDR (2000) derived an chronic oral MRL for PCB mixtures of 0.02 µg/kg/day based on a LOAEL of 0.005 mg/kg/day for decreased antibody response to sheep red blood cells in Rhesus monkeys exposed to self-ingested capsules of Aroclor 1254 in a glycerol/corn oil mixture (Tryphonas et al. 1989, 1991). The LOAEL was divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability).

Because consensus has emerged on the inappropriateness of assessing environmental PCBs as if they were Aroclors, EPA has developed an approach for assessing cancer risk from environmental PCBs by considering both toxicity and environmental processes (Cogliano 1998; EPA 1996; IRIS 2000). This approach uses animal studies of commercial PCB mixtures to develop a range of human cancer potency estimates and then considers the effect of environmental processes to determine appropriate values for representative classes of environmental mixtures. Guidance is provided for assessing cancer risks from different exposure pathways, less-than-lifetime and early-life exposures, and mixtures containing dioxin-like constituents.

E.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

TTDs for chronic oral exposure to PCB mixtures were derived for endpoints affected by PCBs and one or more of the other chemicals in strontium-cobalt-cesium-trichloroethylene-PCB mixture that is the subject of this Interaction Profile. The relevant endpoints for PCBs in this mixture include hematological, developmental, neurologic, and hepatic endpoints. Chronic oral TTDs for these endpoints are derived below, using the methods described in ATSDR (2001a, Section 2.3.2). The derivations are based on data provided in ATSDR (2000), and in particular, the oral LSE table.

Hepatic Effects

Several studies of groups of humans exposed to PCBs have reported associations between exposure and changes in indices of hepatic damage (e.g., increased serum levels of aspartate aminotransferase), but limitations in study design such as lack of appropriate controls or adjustment of potential confounding variables preclude establishing a causal relationship from the human data (ATSDR 2000). In contrast, studies of orally exposed animals have reported a broad spectrum of PCB-induced hepatic effects including hepatic enzyme induction, liver enlargement, hepatic porphyria, and histopathologic changes in liver tissue ranging from hepatocellular hypertrophy and vacuolization to fatty degeneration, hepatocellular necrosis, bile duct hyperplasia, and liver tumors (ATSDR 2000). The lowest exposure levels associated with liver changes in available animal studies are 0.04 mg/kg/day (no NOAEL was identified) for decreased serum cholesterol in Rhesus monkeys exposed to Aroclor 1254 for 37 months (Arnold et al. 1993a, 1993b), 0.08 mg/kg/day (with a NOAEL of 0.04 mg/kg/day) for increased relative liver weight in Rhesus monkeys exposed to Aroclor 1254 for 72 months (Arnold et al. 1997), 0.2 mg/kg/day (no NOAEL was identified) for hepatocyte necrosis and biliary tract hypertrophy in Rhesus monkeys exposed to Aroclor 1254 for 12 or 28 months (Tryphonas et al. 1986a, 1986b), and 1 mg/kg/day (no NOAEL was identified) for hepatocellular hypertrophy and increased levels of serum enzymes in male rats exposed to Aroclor 1254 or 1260 for 24 months (Mayes et al. 1998). Applying an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from monkeys to humans, and 10 for human variability) to the LOAEL of 0.04 mg/kg/day for decreased serum cholesterol in Rhesus monkeys (Arnold et al. 1993a, 1993b) yields a $TTD_{HEPATIC}$ of 0.1 µg/kg/day for PCB mixtures.

Neurological Effects

Subtle neurobehavioral changes have been observed in studies of children of mothers consuming large amounts of Great Lakes fish contaminated with PCBs and other biopersistent pollutants (ATSDR 2000). Deficits in measures of neurological development have been associated with increasing indices of PCB exposure, but precise and accurate adjustment for possible confounding variables has not always been possible in these studies. Studies in animals support the human data. Neurobehavioral changes have been observed in rats and monkeys following pre- and/or postnatal exposure to commercial Aroclor mixtures, experimental mixtures of PCBs similar to those found in human breast milk, single PCB congeners, and contaminated fish from the U.S. Great Lakes (ATSDR 2000). As described in Section E.4 above, ATSDR (2000) derived the intermediate oral MRL of 0.03 µg/kg/day for PCB mixtures based on a LOAEL of 0.0075 mg/kg/day (no NOAEL was identified) for neurobehavioral changes in infant monkeys

that were orally exposed from birth to 20 weeks of age to a synthetic mixture of PCBs representing 80% of the PCB congeners found in samples of human breast milk and an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolating from monkeys to humans, and 10 for human variability). The intermediate-duration oral MRL is only slightly above the chronic oral MRL of 0.02 μg/kg/day (based on immunological effects in adult monkeys), and is expected to provide protection against possible neurological and neurodevelopmental effects from chronic exposure.

Developmental Effects

The development of the neurological system appears to be a target of critical public health concern associated with pre- and/or post-natal exposure to PCB mixtures (ATSDR 2000). Subtle neurobehavioral effects suggesting impaired learning or perserverative behavior have been observed in monkeys exposed from birth to 20 weeks to oral doses as low as 0.0075 mg/kg/day (Rice 1997, 1998, 1999; Rice and Hayward 1997, 1999). This dose was estimated to correspond to PCB levels in human breast milk of 50 ppb. As discussed in Section E.4 above, these findings serve as the basis of the intermediate oral MRL of 0.03 μg/kg/day. This value is only slightly above the chronic oral MRL of 0.02 μg/kg/day based on impaired immune response in adult monkeys and is expected to be protective of neurological neuro-developmental effects from chronic oral exposure to PCBs.

Hematological Effects

A number of studies have examined the effects of exposure to PCB mixtures on hematological endpoints (see ATSDR 2000). The study of Arnold et al. (1997), which identified a NOAEL of 0.08 mg/kg/day for hematological effects in female Rhesus monkeys exposed daily to Aroclor 1254 for 72 months, was utilized as the basis of TTD derivation. Using this NOAEL and an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for intrahuman variability) to derive a TTD of $8x10^{-4}$ mg/kg/day (0.8 μg/kg/day).

Immunological Effects

ATSDR (2000) derived an chronic oral MRL for PCB mixtures of 0.02 μg/kg/day based on a LOAEL of 0.005 mg/kg/day for decreased antibody response to sheep red blood cells in Rhesus monkeys exposed to self-ingested capsules of Aroclor 1254 in a glycerol/corn oil mixture (Tryphonas et al. 1989, 1991). The

LOAEL was divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability).

Reproductive Effects

Available studies of the reproductive effects have been inconclusive in their conclusions. Mayes et al. (1998) reported no effects on reproductive endpoints in Sprague-Dawley rats exposed for 24 months to Aroclor 1016, 1242, 1254, or 1260 in the concentration range of 4–11 mg/kg/day in the drinking water. However, Allen and Norback (1976) reported that Rhesus monkeys exposed to 0.1 mg/kg/day of Aroclor 1248 in the food for 17 months showed a decrease in spermatogenesis and libido. Arnold et al. (1995) identified a NOAEL of 0.005 mg/kg/day (5 µg/kg/day) and LOAEL of 0.020 mg/kg/day (20 µg/kg/day) for decreased conception rate in female Rhesus monkeys. To the NOAEL of 5 µg/kg/day established in this study, an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) was applied to give a TTD_{REPRO} of 0.05 µg/kg/day ($5x10^{-5}$ mg/kg/day).

Summary (TTDs for PCBs)

TTD_{HEMATO} = 0.8 µg/kg/day ($8x10^{-4}$ mg/kg/day)
Chronic oral MRL (based on immunological effects) = 0.02 µg/kg/day (2x10-5 mg/kg/day)
TTD_{REPRO} = 0.05 µg/kg/day ($5x10^{-5}$ mg/kg/day)
Intermediate oral MRL (based on neurodevelopmental effects) = 0.03 µg/kg/day (3x10-5 mg/kg/day)
$TTD_{HEPATIC}$ = 0.1 µg/kg/day ($1x10^{-4}$ mg/kg/day)

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