CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

- Acrylonitrile is well absorbed following inhalation and oral exposure; approximate absorption rates are 50 and 90%, respectively. Data are not available to estimate dermal absorption rates.
- It is widely distributed throughout the body, with higher levels in the liver, kidneys, lungs, and stomach.
- The primary metabolic pathway is conjugation with glutathione. It is also metabolized by the microsomal enzyme system to form 2-cyanoethylene, which is metabolized to thiocyanate or thiodiglycolic acid.
- Acrylonitrile is primarily excreted in the urine as conjugates or thiocyanate. A small percentage is excreted in air as carbon dioxide.
- Physiologically based pharmacokinetic (PBPK) models of rats and humans have been developed for predicting internal doses of acrylonitrile and cyanoethylene oxide.

3.1.1 Absorption

In a well-controlled and conducted study with volunteers, Jakubowski et al. (1987) reported that an average of 52% of the inhaled dose of acrylonitrile (5 or 10 mg/m^3) is absorbed by the lungs. Similar results were reported by Rogaczewska and Piotrowski (1968), who found that 46% of inhaled acrylonitrile is retained by the lungs of humans.

Pilon et al. (1988b) demonstrated in rats exposed to 4 mg/kg acrylonitrile (2,3-^{14}C) in a closed-circuit inhalation chamber that the absorption of acrylonitrile was biphasic, characterized by a rapid dose-dependent phase that was followed by a slower dose-independent phase.

Results of studies in laboratory animals with [^{14}C]-acrylonitrile indicate acrylonitrile is rapidly and extensively absorbed by the oral route. Radiolabeled acrylonitrile is detected in blood within 30 minutes after administration of an oral dose and peak plasma concentrations are reached 6 hours after administration (Farooqui and Ahmed 1982). Extensive absorption is indicated by the fact that only 2–10% of administered radioactivity is recovered in the feces (Ahmed et al. 1982, 1983; Farooqui and Ahmed 1982; Young et al. 1977).

In studies in volunteers conducted by Rogaczewska and Piotrowski (1968), absorption by skin was estimated to be 0.6 mg/cm^2/hour. Although no quantitative estimates of dermal absorption could be made, absorption of acrylonitrile via the dermal route by humans was demonstrated in a case study by Vogel and Kirkendall (1984). Accidental spraying of a man with acrylonitrile resulted in marked symptoms of acrylonitrile toxicity, indicating that significant amounts of acrylonitrile had been absorbed, primarily through the skin.

3.1.2 Distribution

Acrylonitrile is rapidly distributed throughout the body after inhalation exposure. Measurable amounts of acrylonitrile derived radiolabel were present in the brain, stomach, liver, kidney, lung, and blood of rats within 1 hour of initiation of exposure (Pilon et al. 1988b).

Tissue distribution of radioactivity in rats after a single oral dose of [^{14}C]-acrylonitrile indicates that acrylonitrile and its metabolites are rapidly distributed to all tissues (Ahmed et al. 1982, 1983; Burka et al. 1994; Silver et al. 1987; Young et al. 1977). Species differences are apparent. In mice, cyanide levels in the blood peaked at 1 hour, while in rats, peak levels were not reached until 3 hours after administration (Ahmed and Patel 1981). The highest levels of radioactivity were recovered in the gastrointestinal tract, in particular in the stomach. The retention of acrylonitrile and its metabolites in the stomach appears to be due, at least in part, to covalent binding (Ahmed et al. 1982; Silver et al. 1987). Following intravenous administration of [^{14}C]-labeled acrylonitrile, radiolabel was distributed to the gastrointestinal tract, suggesting enterohepatic circulation of acetonitrile or its metabolites (Ahmed et al. 1996; Jacob and Ahmed 2004; Young et al. 1977).

Distribution studies by whole-body autoradiography in rats and monkeys revealed accumulation of radiolabel in the liver, kidney, lung, adrenal cortex, and stomach. In fetuses exposed *in utero*, only the eye lens accumulated radiolabel at a higher concentration than that observed in maternal blood (Sandberg and Slanina 1980).

No studies were located regarding distribution in humans or animals following dermal exposure to acrylonitrile.

3.1.3 Metabolism

Proposed pathways for the metabolism of acrylonitrile are presented in Figure 3-1 (Ahmed et al. 1983; EPA 1980b; Langvardt et al. 1980; Linhart et al. 1988; Muller et al. 1987; Pilon et al. 1988a; Roberts et al. 1989, 1991; Sumner et al. 1997, 1999). Studies indicate that the metabolism of acrylonitrile in animals proceeds by the same pathways whether exposure is by the oral (Ahmed et al. 1983; Langvardt et al. 1980; Pilon et al. 1988a) or the inhalation route (Gut et al. 1985; Muller et al. 1987; Tardif et al. 1987). No data were located regarding the metabolism of acrylonitrile following dermal exposure.

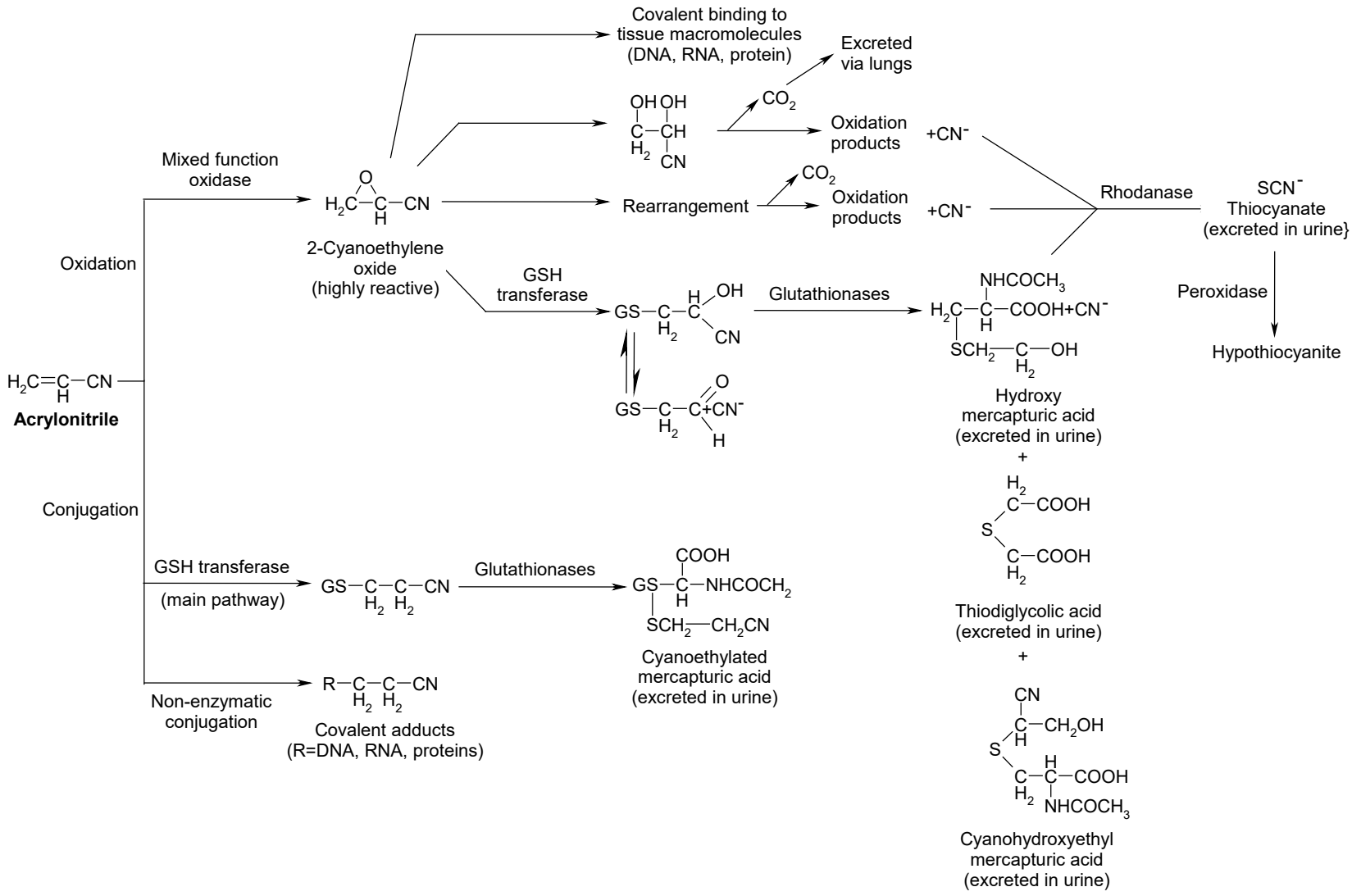
Both enzymatic and nonenzymatic biotransformation of acrylonitrile occurs. Acrylonitrile is capable of covalently binding to proteins and other macromolecules such as lipids or nucleic acids, or acrylonitrile can also be directly conjugated to glutathione and excreted in urine as cyanoethylmercapturic acid.

Alternatively, acrylonitrile is metabolized to 2-cyanoethylene oxide by the microsomal enzyme system. Cytochrome P450 2E1 is the major contributor in the microsomal pathway (Subramanian and Ahmed 1995; Sumner et al. 1999). Cytochrome c peroxidase has also been shown to oxidize acrylonitrile (Chinchilla et al. 2014). 2-Cyanoethylene oxide can react directly with tissue macromolecules, or it can be further metabolized to oxidation products that release cyanide. Cyanide is converted to thiocyanate and excreted in the urine. 2-Cyanoethylene oxide is also conjugated with glutathione and metabolized to 2-hydroxyethylmercapturic acid, which is excreted in the urine.

Acrylonitrile is also metabolized to CO_2, which is eliminated through the lungs. Carbon dioxide is produced when acrylonitrile is metabolized to ethylene oxide and degraded to oxidation products and cyanide via the epoxide hydratase pathways (Farooqui and Ahmed 1982; Young et al. 1977).

Studies indicate that acrylonitrile conjugation with glutathione is the preferred pathway for metabolism (Ghanayem and Ahmed 1982; EPA 1978; Pilon et al. 1988a). However, if glutathione is depleted or the pathway is overloaded (as may be the case at high doses), microsomal metabolism to the thiocyanate via 2-cyanoethylene oxide is increased. Following an oral dose of acrylonitrile to rats (0.09–28.8 mg/kg) or mice (0.09–10.0 mg/kg), excretion of urinary metabolites from the microsomal pathway increased linearly with dose, whereas excretion of metabolites from the direct glutathione conjugation pathway plateaued, suggesting saturation of the glutathione pathway (Kedderis et al. 1993). Increased thiocyanate excretion with glutathione depletion or increased dose was demonstrated by Pilon et al. (1988a). Glutathione depleted rats excreted 58% of an orally administered dose as thiocyanate, while

Figure 3-1. Proposed Metabolic Scheme for Acrylonitrile



Sources: Ahmed et al. 1983; Albertini et al. 2023; Linhart et al. 1988; Muller et al. 1987; Roberts et al. 1989, 1991; Sumner et al. 1997, 1999

normal rats (glutathione sufficient) given the same dose (4 mg/kg) of acrylonitrile excreted only 16% as thiocyanate. Normal rats (glutathione sufficient) given acrylonitrile at 10 mg/kg excreted 23% of the dose as thiocyanate.

The increased metabolism of acrylonitrile to 2-cyanoethylene oxide has significant implications in acrylonitrile toxicity. 2-Cyanoethylene oxide has been shown to react with cell macromolecules (including nucleic acids) both *in vivo* and *in vitro* (Guengerich et al. 1981; Hogy and Guengerich 1986). This metabolite may be responsible for the carcinogenic effects of acrylonitrile.

Urinary excretion patterns of thiocyanate suggest that there are quantitative species differences in acrylonitrile metabolism (Ahmed and Patel 1981). Thiocyanate was identified as a metabolite in rats, mice, rabbits, and Chinese hamsters. About 20–23% of the administered dose was excreted as thiocyanate in rats, rabbits, and Chinese hamsters, while 35% was excreted as thiocyanate in mice (Gut et al. 1975). A larger portion of the urinary metabolites were derived from the microsomal pathway in mice compared to rats (Fennell et al. 1991; Kedderis et al. 1993). It has also been observed that mice metabolize acrylonitrile more rapidly than rats (Ahmed and Patel 1981; Gut et al. 1975; Jacob and Ahmed 2004). Maximum blood cyanide concentrations were observed 1 hour after dosing in mice, but 3 hours after dosing in rats (Ahmed and Patel 1981). In mice, thiocyanate was present in the urine within 4 hours of dosing, while in rats, thiocyanate was present in urine only at time intervals >4 hours (Gut et al. 1975).

In humans, metabolites of acrylonitrile have been identified in urine following occupational exposure (assumed to be by the inhalation route) and in controlled exposure studies. Metabolites identified in humans were the same as those in animals (Jakubowski et al. 1987; Sakurai et al. 1978). Acrylonitrile and thiocyanate were quantified in urine of workers exposed to acrylonitrile. Dose-related increases in thiocyanate were observed, indicating that cyanide is liberated with the metabolism of acrylonitrile. In a study with volunteers under controlled conditions, N-acetyl-S-(2-cyanoethyl)-L-cysteine (2CyEMA) was monitored in urine as an indication of exposure. On average, 22% of the absorbed acrylonitrile was metabolized to 2CyEMA; however, considerable individual variability was observed. The 2CyEMA excretion ranged from 13 to 39% of the absorbed dose (Jakubowski et al. 1987).

In a case study of a human male accidentally sprayed with acrylonitrile, recurring signs of cyanide poisoning were seen over a 3-day period (Vogel and Kirkendall 1984). This indicates that acrylonitrile is also metabolized to cyanide following predominantly dermal exposure.

3.1.4 Excretion

Studies on workers in an occupational setting showed a dose-response relationship between the concentration of acrylonitrile of inspired air and the recovery of metabolites in the urine (Houthuijs et al. 1982; Sakurai et al. 1978). In a controlled study using volunteers, urinary metabolite data suggested that the elimination of acrylonitrile followed first-order kinetics, with a half-life of 7–8 hours (Jakubowski et al. 1987).

The predominant route of excretion in rats exposed by inhalation is via urine (Gut et al. 1985; Tardif et al. 1987; Young et al. 1977). In rats exposed to 5 ppm of [$^{1\text{-}14}$C]-acrylonitrile for 6 hours, 68% of the absorbed radioactivity was excreted in the urine within 220 hours, with 3.9% in the feces, 6.1% in expired air as $^{14}CO_2$, and 18% of the radioactivity being retained in the body tissues. Following exposure to a higher concentration (100 mm), a larger fraction of the dose was recovered in urine (82%) and a smaller fraction (2.6%) was retained in the body (Young et al. 1977), indicating that urinary excretion is dose-dependent. Percent fecal excretion was similar at both doses.

Following oral exposure, the major route of excretion of acrylonitrile in rats is via the urine, either as thiocyanate or as other products of conjugation. Within the first 24 hours of a single oral dose, 40–60% was recovered in the urine (Ahmed et al. 1983). Farooqui and Ahmed (1982) reported that 10 days after the administration of a single dose, 61, 3, and 13% of the dose had been accounted for in the urine, feces, and expired air, respectively. Approximately 25% was retained in the body covalently bound to tissues.

A study by Young et al. (1977) showed that retention and excretion of acrylonitrile are not directly proportional to dose. The data suggest a saturation process, perhaps due to covalent binding to tissue macromolecules. Seventy-two hours after administration of single oral doses of either 0.1 or 10 mg/kg, the proportion of the dose retained in the carcass was 37% at the low dose (0.1 mg/kg) and 27% at the high dose (10 mg/kg).

A study by Jacob and Ahmed (2004) compared elimination of acrylonitrile in mice and rats following intravenous doses of [^{14}C]-labeled acrylonitrile (mice, 3.4 mg/kg; rats, 11.5 mg/kg). In mice, 74% of the radiolabel was eliminated in 48 hours: 4% in expired air, 16% in urine, and 54% in feces. In rats, 26% of the radiolabel was eliminated: 2% in expired air, 4% in urine, and 20% in feces.

No studies were located regarding excretion in humans or animals following dermal exposure to acrylonitrile.

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Models are simplified representations of a system with the intent of reproducing or simulating its structure, function, and behavior. PBPK models are more firmly grounded in principles of biology and biochemistry. They use mathematical descriptions of the processes determining uptake and disposition of chemical substances as a function of their physicochemical, biochemical, and physiological characteristics (Andersen and Krishnan 1994; Clewell 1995; Mumtaz et al. 2012a; Sweeney and Gearhart 2020). PBPK models have been developed for both organic and inorganic pollutants (Ruiz et al. 2011) and are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Mumtaz et al. 2012b; Ruiz et al. 2011; Sweeney and Gearhart 2020; Tan et al. 2020). PBPK models can also be used to more accurately extrapolate from animal to human, high dose to low dose, route to route, and various exposure scenarios and to study pollutant mixtures (El-Masri et al. 2004). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints (Clewell 1995).

Gargas et al. (1995) Rat Model

Description. Gargas et al. (1995) developed a model to simulate the kinetics of acrylonitrile and its microsomal metabolite, cyanoethylene oxide, in the rat. The model consists of two modules: one representing acrylonitrile and the other representing cyanoethylene oxide. Each model includes compartments representing arterial and venous blood, brain, fat, liver, lung, and two lumped compartments representing rapidly perfused and slowly perfused tissues. The acrylonitrile and cyanoethylene oxide modules are connected by conversion of acrylonitrile to cyanoethylene oxide in the liver compartment. Acrylonitrile absorbed from the gastrointestinal tract is assumed to undergo first-order transfer to the liver ($hour^{-1}$). Exchange between blood and each tissue compartment is assumed to be flow-limited and governed by the tissue blood flow rate and the arterial-venous concentration difference. The concentration of acrylonitrile or cyanoethylene oxide in tissue is assumed to be in equilibrium with tissue venous blood concentration, determined by tissue:blood partition coefficient. Two pathways for metabolism of acrylonitrile are represented in the liver: a saturable pathway that

converts acrylonitrile to cyanoethylene oxide (V_{max}, K_m) and an unlimited first-order pathway that conjugates acrylonitrile with glutathione ($hour^{-1}$). Cyanoethylene oxide is assumed to be eliminated by conjugation with glutathione in brain, liver, rapidly perfused tissues, and slowly perfused tissues. Both acrylonitrile and cyanoethylene oxide are assumed to undergo unsaturable first-order binding to blood sulfhydryls and hemoglobin ($hour^{-1}$).

A fixed fraction (88%) of the acrylonitrile metabolized through the cyanoethylene oxide pathway or through the direct glutathione or sulfhydryl binding pathway is assumed to be excreted in urine. The model includes a pathway for elimination of acrylonitrile and cyanoethylene oxide in exhaled air.

Calibration and Evaluation. Tissue:blood partition coefficients were calculated from measured tissue:air partition coefficients (Gargas et al. 1995). Rate constants for reaction of acrylonitrile and cyanoethylene oxide with hemoglobin were determined from observations of the time course for [^{14}C] binding to hemoglobin isolated from rat erythrocytes and incubated with [^{14}C]-labeled acrylonitrile (Gargas et al. 1995). Parameters for conversion of acrylonitrile to cyanoethylene oxide, conjugation of acrylonitrile and cyanoethylene oxide with glutathione, and binding of cyanoethylene oxide to blood sulfhydryls were optimized against observations of the time course of blood acrylonitrile and cyanoethylene oxide concentrations following a single intravenous dose of acrylonitrile (3.4–84 mg/kg) or a single oral dose of cyanoethylene oxide (0.6 or 5.3 mg/kg) administered to male Fisher 344 rats.

The model was evaluated against data from studies conducted in rats that were not included in the model calibration. These data consisted of observations of the fraction of an oral dose of [^{14}C], administered as [^{14}C]-labeled acrylonitrile, excreted in urine and identified as being derived from either the cyanoethylene oxide pathway or from the direct conjugation of acrylonitrile with glutathione (Kedderis et al. 1993). The comparison between the observations and predictions are presented in plots without measures of variance in the observations; however, the model appeared to predict the observed dose-response relationship for both urinary metabolite pathways. The model also predicted the observed dose-response relationship for covalent binding of [^{14}C] to rat hemoglobin, following an oral dose of [^{14}C]-labeled acrylonitrile (Fennell et al. 1991).

Kedderis et al. (1996) Rat Model

Description. Kedderis et al. (1996) modified the Gargas et al. (1995) model to include a stomach compartment and parameters to simulate the kinetics of the chemical reaction of acrylonitrile with glutathione in brain, liver, stomach, rapidly perfused tissues, and slowly perfused tissues.

Calibration and Evaluation. The rate of reaction of acrylonitrile with glutathione was measured in incubations of acrylonitrile and glutathione at concentrations above and below the non-protein sulfhydryl concentration measured in rat liver (mean 8.83±0.49 nmol/L) (Kedderis et al. 1996). Parameters for the oral absorption rate coefficient, conversion of acrylonitrile to cyanoethylene oxide, conjugation of acrylonitrile and cyanoethylene oxide with glutathione, and binding of cyanoethylene oxide were optimized against observations of the time course of blood acrylonitrile and cyanoethylene oxide concentrations following a single intravenous dose of acrylonitrile (3.4–84 mg/kg administered to male Fisher 344 rats) (Gargas et al. 1995), or following a single oral dose of acrylonitrile (3 or 30 mg/kg) (Kedderis et al. 1996).

The model was evaluated against data from studies conducted in rats that were not included in the model calibration (Kedderis et al. 1996). Rats were exposed (whole-body) to acrylonitrile in air (186, 254, or 291 ppm) or were administered a single oral dose of acrylonitrile (10 mg/kg). The model predicted the observed post-inhalation exposure time course for concentrations of acrylonitrile and cyanoethylene oxide in venous blood, brain, and liver, with most predictions within ±2 standard deviations of the observed means. The model predicted the observed time course for the concentrations of acrylonitrile in brain and liver, and cyanoethylene oxide in liver following the oral dose of acrylonitrile; however, it overpredicted the observed concentrations of cyanoethylene oxide in brain.

Applications to Dosimetry Extrapolation. Kirman et al. (2000) used the Kedderis et al. (1996) model to predict various internal dose metrics achieved in rat inhalation and oral bioassays of acrylonitrile in which brain tumors were assessed. Internal doses (peak concentrations of acrylonitrile or cyanoethylene oxide blood or brain) were predicted for several inhalation and oral bioassays, and the predicted internal doses and observed brain tumor responses for each route of exposure were pooled across studies. The pooled data were then used in dose-response models to estimate oral or inhalation exposure concentrations (mg/L drinking water, $\mu g/m^3$ air) corresponding to a $1x10^{-6}$ extra risk of brain tumors.

Sweeney et al. (2003) Human Model

Description. Sweeney et al. (2003) used the structure and parameters of the Kedderis et al. (1996) model to develop a corresponding human model. The human model included estimates of variation in human parameter values, represented by the coefficients of variation of normal distributions of the parameters.

Calibration and Evaluation. The rat liver V_{max} for conversion of acrylonitrile to cyanoethylene oxide was scaled to the human liver based on the observed ratio of the V_{max} observed in *in vitro* preparations of rat and human liver microsomes, the microsomal protein content of rat and human livers, and mass of rat and human livers (Lipscomb et al. 2003; Ploemen et al. 1997). The rate coefficient for cyanoethylene oxide hydrolysis was scaled to humans, assuming the same scaling factors used for microsomal conversion of acrylonitrile to cyanoethylene oxide. Rates of conjugation of acrylonitrile and cyanoethylene oxide with glutathione were scaled from the *in vivo* rates in the rat adjusted for differences in rates measured *in vitro* in rat and human liver, liver mass, and liver glutathione levels. Human tissue:blood partition coefficients for acrylonitrile and cyanoethylene oxide were calculated from a measured human blood:air partition coefficient and rat tissue:air coefficients (Teo et al. 1994).

Evaluations of the model against observations in humans were not reported. Sweeney et al. (2003) compared predicted concentrations of acrylonitrile and cyanoethylene oxide in blood and brain of rats and humans, performed sensitivity analyses of these internal dose metrics to parameter values, and estimated the contribution of parameter variability to variability in predicted internal dose metrics (acrylonitrile or cyanoethylene oxide blood and brain concentration area under the curve).

3.1.6 Animal-to-Human Extrapolations

The Kedderis et al. (1996) and Sweeney et al. (2003) PBPK models provide a theoretical basis for dose-response extrapolation from rats to humans, based on predicted internal doses of acrylonitrile or cyanoethylene oxide. The Kedderis et al. (1996) rat model has been evaluated for predicting observed levels of acrylonitrile and cyanoethylene oxide in blood, brain, and liver of rats. However, the Sweeney et al. (2003) human model has not been evaluated with observations made in humans.

As reviewed by Albertini et al. (2023), there are species differences in the metabolism of acrylonitrile. *In vitro* studies examining the oxidation by cytochrome P450 by liver microsomes have found greater rates in the formation of 2-cyanoethylene oxide in mice and rats than in humans; the rates were 4 times higher

in mice and 1.5 times higher in rats. The rate of 2-cyanoethylene oxide hydrolysis was significant in humans and undetectable in rats and mice, although it is inducible in all three species. Additionally, the rate of conjugation of 2-cyanoethylene oxide with glutathione is 1.5 times faster in humans than in rats or mice. Species differences have also been observed in the ratio of the oxidative urinary metabolite, N-acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine (CHEMA), and the conjugated metabolite, N-acetyl-S-(2-cyanoethyl)-L-cysteine (CEMA). The ratios of CHEMA:CEMA were 0.3–0.4 in rats, 0.4–0.9 in mice, 0.26 in humans exposed to acrylonitrile, and 0.19 in the general population. These findings suggest that the oxidative pathway has a much larger role in rodents and that the glutathione conjugation pathway plays a larger role in humans.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to acrylonitrile are discussed in Section 5.7, Populations with Potentially High Exposures.

There are limited data on potential differences in the toxicity or toxicokinetics of acrylonitrile between children and adults. Developmental effects, including malformations, decreased fetal body weight, and decreased pup viability have been reported in laboratory animal studies (Friedman and Beliles 2002; Murray et al. 1978; Nemec et al. 2008); it is noted that these effects typically occurred at doses associated with maternal toxicity. Szabo et al. (1984) found possible age-related differences in toxicity between young and adult rats. The levels of plasma corticosterone and aldosterone were significantly lower in the young rats, as compared to the adult rats.

Several polymorphisms have been evaluated to assess whether they increase the susceptibility to acrylonitrile. A study of workers handling low levels of acrylonitrile found no relationship between N-(cyanoethyl)valine, an acrylonitrile hemoglobin adduct, and the genetic states of polymorphic glutathione transferases, GSTM1 and GSTT1 (Thier et al. 1999). Similar findings were reported for CYP2E1 polymorphisms (Thier et al. 2002).

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 2006).

The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for acrylonitrile from this report are discussed in Section 5.6, General Population Exposure.

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 2006). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to acrylonitrile are discussed in Section 3.3.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 2006). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by acrylonitrile are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

The parent acrylonitrile molecule and its metabolites have been measured in blood and urine. Measurement of thiocyanate, CEMA, and 2CyEMA have been used as biomarkers of exposure to acrylonitrile; however, thiocyanate and CEMA are not specific to acrylonitrile.

Factory workers exposed to an average of 0.1, 0.5, or 4.2 ppm of acrylonitrile in the air during an 8-hour workday averaged 3.9, 19.7, and 360 µg/L acrylonitrile in the urine, respectively, and 4.5, 5.78, and 11.4 mg/L thiocyanate in the urine, respectively (Sakurai et al. 1978). No acrylonitrile was detected in the urine of a control group, but an average of 4.00 mg/L of thiocyanate was found in the urine. The presence of thiocyanate in the urine of workers not exposed to acrylonitrile has been related to cigarette smoking (Houthuijs et al. 1982; Sakurai et al. 1978). Houthuijs et al. (1982) reported post-shift acrylonitrile values of 39 µg/L when the mean acrylonitrile concentration in the air was 0.13 ppm.

2CyEMA is formed by glutathione conjunction and is excreted in the urine. 2CyEMA is considered an adequate biomarker of acrylonitrile exposure (de Jesús et al. 2021) and has been used to monitor acrylonitrile exposure in the U.S. general population (see Section 5.6 for monitoring data).

Increased levels of the hemoglobin adduct N-(2-cyanoethyl)valine have been found in acrylonitrile workers and in smokers (Thier et al. 1999, 2002); the levels in smokers were much lower than in the acrylonitrile workers (Thier et al. 2002). Two studies have evaluated exposure to high levels of acrylonitrile resulting from a train derailment in Wetteren, Belgium using the hemoglobin adduct, N-2-cyanoethylvaline, as a biomarker of exposure. De Smedt et al. (2014) reported that 53% of the nonsmoking residents living in the evacuation zone had N-2-cyanoethylvaline levels that exceeded the reference value of 10 pmol/g globin, as compared to1% in controls. In smokers, 22% exceeded the reference value of 200 pmol/g globin versus 8% of controls. The mean N-2-cyanoethylvaline levels were 206.7 and 212.1 pmol/g globin in the nonsmokers and smokers, respectively. A study of emergency responders found that 25.7 and 55% of nonsmokers and smokers had N-2-cyanoethylvaline levels

exceeding the reference values (Van Nieuwenhuyse et al. 2014). Several investigators have used N-2-cyanoethylvaline levels to estimate individual body burdens (Huizer et al. 2014; Leng and Gries 2014). Huizer et al. (2014) used a BioNormtox PBPK model and N-2-cyanoethylvaline levels to predict initial exposure levels in four workers rescuing a colleague exposed to high levels of acrylonitrile at a train depot. The predicted air concentrations ranging between 5.6 and 17.9 ppm were similar among the workers; however, the results could not be validated with measured concentrations. Another study of these workers estimated an elimination interval of 148 days (Bader and Wrbitzky 2006). In a study in rats exposed to various doses of acrylonitrile (3–300 ppm) in drinking water for 105 days, a dose-related increase in N-(2-cyanoethyl)valine levels were found (Osterman-Golkar et al. 1994). At doses of 0.74 mg acrylonitrile/kg (10 ppm in drinking water) and lower, there was a linear relationship between dose and hemoglobin adduct levels. A sublinear relationship, indicative of saturation, was observed at higher doses.

3.3.2 Biomarkers of Effect

A variety of effects have been demonstrated following acrylonitrile exposure in humans and animals. These effects show a close similarity to an underlying cyanide effect, particularly for acute-duration exposures. Effects can be detected in groups of exposed individuals by monitoring signs and symptoms such as increased salivation, dizziness, and labored and irregular breathing. In some cases, convulsions and coma may occur. Because the release of cyanide for producing toxic effects is common for other compounds, measuring these effects is not specific for acrylonitrile exposure. These effects do identify potential health impairment. It should be noted that the toxicity of acrylonitrile resides not only in the cyanide radical, but also in the entire molecule. The latter structure explains various chronic-duration exposure effects such as cancer that result from acrylonitrile, as opposed to cyanide for which effects are more relevant for acute-duration toxicity. Studies that identify subtle physiological changes that can be used to detect or predict risk of disease following long-term exposure to acrylonitrile are not available.

3.4 INTERACTIONS WITH OTHER CHEMICALS

The interaction between acrylonitrile and other chemicals has not been thoroughly studied. O'Berg (1980) noted that out of eight workers exposed to acrylonitrile who developed lung cancer, seven were smokers (smoking history was not available for the eighth individual). This suggests that smoking might increase lung cancer risk from acrylonitrile exposure, but the data are too limited to draw any firm conclusions on this point.

Radimer et al. (1974) described four cases of severe epidermal necrolysis in individuals who had been exposed to the residual fumes of a mixture of acrylonitrile and carbon tetrachloride used to fumigate their homes. Three of the people died. The study authors thought that this was most likely due to the effects of acrylonitrile but noted that an interaction between carbon tetrachloride and acrylonitrile was possible.

In animals, the hemorrhagic effects of acrylonitrile exposure on the adrenals may be reduced by prior exposure of the animals to adrenergic blockers or chemicals that deplete the adrenal cortex of catecholamines (Silver et al. 1987; Szabo et al. 1980). It is difficult to judge whether adrenergic antagonists would have a similar protective effect in humans, because effects of acrylonitrile on the adrenal have not been described in humans.

Acrylonitrile alone has little tendency to produce duodenal ulcers in animals, but pretreatment with phenobarbital or Aroclor results in a marked increase in the incidence of such ulcers (Szabo et al. 1983, 1984). Although the mechanism of the ulcerogenic effect is not obvious, these data indicate that agents that enhanced mixed-function oxidase activity may also increase the toxicity of acrylonitrile.