

RISK ASSESSMENT

Risk assessment is an analysis of the potential for adverse effects from chemicals released into the environment or present at hazardous waste sites. Risk is the probability of suffering harm or a negative outcome:

Risk = the probability of negative consequence * value or severity of consequence.

The negative outcome may be measurable (such as years of lost life expectancy) or may not be easily quantifiable (such as death or cancer). When the negative outcome is not quantifiable, the risk is reported as a probability of occurrence, such as the chance of death per million or the lifetime cancer risk.

Risk assessment is still a developing science. It is therefore likely that when the risks posed by a given site are evaluated by more than one qualified person, that a range of risk values will be determined. This is due to the “art” involved, as well as the lack of complete information to conduct the scientific analyses. The primary scientific basis of risk assessment is the science of toxicology. Toxicology is the study of the adverse effects to organisms which result from exposure to toxic chemicals. Risk assessment also requires an evaluation of the routes by which chemical exposures will occur. The uncertainty in risk assessment therefore arises from two distinct areas:

1. lack of information on the effect of a chemical on a specific organism at a specified dose (There may be information from different doses or studies on different target species; especially since there is an inability to use humans in controlled toxicology experiments.)

2. difficulty predicting the transport and eventual fate of chemicals in the environment, and therefore uncertainty in the chemical concentrations to which organisms will be exposed at given locations and times

In spite of imperfect information, risk assessment provides an important tool to assure that site clean-up will ensure the health and welfare of the public and ecosystem. The challenge of regulators is to define an acceptable risk level. Historically, U.S. regulations have been based more on public perception of risk than scientific risk assessment. Perceived risks tend to be higher than true risk levels when the risk-causing agent is involuntarily encountered or poses an unknown but substantial consequence, and there is little apparent benefit trade-off for taking the risk. Because the U.S. is moving toward risk-based remedial strategies, it is important that actual risk levels can be both evaluated and effectively communicated to the public.

This chapter first covers the basics of toxicology, including non-cancer effects, cancer effects, and intake calculations. A special section on endocrine disrupters is then presented. Next, the four steps of quantitative risk assessment for human effects are outlined, with each described. An overview of three case studies at famous Superfund sites is presented. Then, using risk assessment to aid in setting site clean-up goals is discussed. This is followed by a description of the ecological risk assessment (ERA) process. Finally, risk based corrective action (RBCA) as being promoted by EPA for non-superfund sites is presented. The chapter concludes with a summary table of important acronyms commonly used in risk assessment.

1. TOXICOLOGY

The most important foundation of all risk assessments is an understanding of the types of negative effects that will occur upon exposure to a given quantity of a chemical, which is termed toxicology. The presence of a given chemical at a waste site is not important unless it has the potential to cause a negative effect. The types of effects of most concern to humans and animals are:

Death

Cancer (carcinogen = chemical which causes cancer)

genetic mutation (mutagen = chemical which causes a change or mutation in the genetic material of a cell)

Non-cancer effects:

developmental effects: occur between fertilization to sexual maturation of organism

reproductive effects: lack of fertility, fetal death, birth defects,...

teratogen: effect between implantation of embryo through the first 3 months of development

(primary skeletal and organ malformations)

fetotoxicity: developmental problems from 3 months to birth

post-natal developmental effects: birth to adult (for example, lead retards precognitive development in kids)

Endocrine disrupters - interfere with hormone levels in the body

The end-points of these toxic effects are things such as death, reduced birth weight, liver damage, anemia, lung cancer, neurological effects, etc. A single chemical may produce both cancer and a range of non-cancer effects.

Types of toxic effects to plants include reduced growth, lower root density, chlorosis (discoloration), and death.

One of the critical factors determining the toxic effects a chemical will exert is its route of entry into the body. Three exposure routes of compound intake for humans are: ingestion (by drinking or eating), inhalation (into the lungs of gases and particulates), and dermal absorption (penetration through the skin). The quantity of a compound which gets absorbed into the body is termed the "intake". Depending on how a compound gets into the body, it has a different potential to interact with organs or encounter the body's natural detoxifying defenses.

Ingested compounds are those that enter the gastro-intestinal system. Compounds in drinking water or associated with food, or adsorbed to soil that is incidentally ingested, enter the body via this route. They can be absorbed through the lining of the esophagus or stomach and get into the blood stream; but in the stomach the acidic conditions have the potential to alter some compounds. Once in the blood, the chemicals typically go to the liver first, where further chemical changes to the compounds may occur. Sometimes these alterations decrease chemical toxicity while other times the toxicity is actually increased. Liver damage may have later negative effects, since more chemicals can get distributed throughout the body in an unaltered state. Some toxic compounds may also be excreted from the body in urine or feces. One common indication of exposure to toxic chemicals is to measure the concentrations in blood or urine samples.

Compounds which are absorbed directly through the skin also get directly into the blood stream. However, these compounds have a greater "travel time" before reaching locations where changes in the chemical may occur (such as in the liver). Frequently, once the intake quantity of a compound is known, similar effects to ingested chemicals are assumed.

Inhaled particulates and gases enter the body via the lungs, and may either be deposited within the lung itself and/or gain entry into the blood. Alternatively, some of these compounds may be immediately exhaled and not have the opportunity to negatively impact the body. Only small particulates, so-called PM-10, are of primary concern for inhalation effects.

Also important are the interactive effects between chemicals. Exposure to multiple compounds can result in additive effects, magnification of effects (two chemicals interact to produce negative effects which exceed the added effect), or antagonistic effects (two chemicals could interact together to pose less harm than individual compounds). These interactive effects pose large challenges for predicting total health effects in the body. An example of a well-documented magnification effect in humans is exposure to both tobacco smoke and asbestos. Exposure to both increases the risk of lung cancer far above the additive risk of either alone. Another example is the toxic effects of a pesticide, malathion, mixed with other organic phosphates - toxic effects are up to 50 times as severe as the additive effect of the individual compound. Predicting these mixture effects in a reliable manner is nearly impossible, requiring an extensive database of study far beyond that currently available.

Toxicology not an exact science. Studies cannot be conducted on human subjects, so we are forced to extrapolate potential human effects on the basis of animal studies. In addition, since the effect levels we are concerned with are quite low (such as 1 in a million increase in cancer), studies of large enough animal populations cannot be conducted to observe these low effect levels. Therefore, lab studies are conducted at higher chemical exposure levels which will produce measurable effects, and lower dose effects must be interpolated or extrapolated. (Interpolate = what is going on between measured dose values; Extrapolation: what is going on outside dose levels tested). It is true that "only a limited number of toxic substances have sufficient human data to support the development of a quantitative relationship between dose and incidence of adverse health effects." (LaGrega)

Case Example:

In the town of Woburn, MA (near Boston), wells contaminated with trichloroethylene (TCE) were used for city drinking water supply from 1964 to 1979 (when the TCE contamination was first discovered at concentrations of 267 and 193 ppb in two wells). Residents were found to have experienced a variety of effects from this exposure: higher incidence of leukemia (especially among children), skin rashes, immune system effects (helper T to killer T cell ratios in the blood were elevated, indicating the immune system "alert"), heart arrhythmias (irregular heartbeat), and neurological effects (short term memory, motor control, and slowed blink reflex). This TCE exposure occurred not only from ingesting the water, but also through inhalation of volatilized TCE in the shower (some residents noted a burning sensation in their eyes during showers) and direct absorption of the TCE through the skin. One drawback to these results was the small number of people tested: the entire city had a population of only 36,000; the contaminated water wells were not used by the entire town - the test group consisted of only 33 persons. However, the similarity of the health effects patterns across all of these people (in comparison to "normal" ranges) were strikingly consistent. Based on the Woburn residents, there appeared to be a body of evidence which pointed to a variety of effects from chronic exposure to low levels of TCE. (A Civil Action, by Jonathan Harr, 1995.)

1.1 Dose-Effect Relationships for Non-carcinogenic Effects

For many non-carcinogenic compounds, there is a level of exposure below which no adverse effects will occur. In fact, many of these compounds may be required in small quantities for survival and cause beneficial effects. Examples of these include salt, For these compounds there is a so-called “threshold” effect. While it is theorized that a threshold exists, it is difficult to precisely measure this value. Therefore, estimates of threshold values are termed “no observed adverse effect level” (NOAEL). [NOAEL is the dose at which no observed effect occurred] Alternatively, a “lowest observed adverse effect level” (LOAEL) might be defined from a laboratory study, from which a safe dose will be extrapolated. On the basis of NOAEL and LOAEL data, toxicologists will set “acceptable daily intake” (ADI) quantities for the compounds. The ADI reflects a best-estimate of a “safe” level of exposure, and will therefore be somewhat below the predicted NOAEL. The EPA regulatory equivalent of the ADI is the “reference dose” (RfD). The RfD is calculated as:

$$RfD = NOAEL / UF / MF$$

where UF = uncertainty factor(s) (see table below)

MF = modifying factor, 1 to 10, to add in “best judgment” of experts

Since for most chemicals LOAEL and NOAEL data only exists from animal studies, extrapolation to human ADI or RfDs requires the use of a variety of uncertainty factors. Common uncertainty values used are listed below:

Factor	Reason
100	estimating effects from multiple compound exposures as additive which could be magnified
10	estimate effects to a sensitive person in the human population on the basis of the average effect to humans
10	estimating human effects based on animal studies
10	estimating long-term effects based on short-term studies
10	estimating an ADI or NOAEL from LOAEL value

An example of the results from toxicological studies on the effects of alachlor which are summarized on the EXTTOXNET (<http://ace.orst.edu/info.exttoxnet>) is given below.

Example Results from Alachlor Studies

Test Animal	Exposure Route	LD-50, mg/kg	NOAEL	Carcinogen Studies
Rat	oral inhalation	930-1,350 mg/kg 5.1 g/m ³ over 4 hrs 23.4 g/m ³ over 6 hrs	2.5 mg/kg	stomach tumors nasal tumors
Mouse	oral	1,910-2,310 mg/kg		26-260 mg/kg/d F lung tumors
Rabbit	dermal	7,800-16,000 mg/kg		
Dogs	oral		1 mg/kg/day	
Birds		2,000 mg/kg 5,000-10,000 ppm LC50		
Fish	ecological	2.4-6.5 mg/L 96-hr LC50		

Note: the average mouse, rat, and hamster lifespans are 2 years; values for scaling intake from animal studies:

Test Animal	Body Weight, kg	Food Intake, g/day	Water Intake, mL/d	Air Intake, m ³ /day
Mouse - M	0.03	5	5	0.04
Mouse - F	0.025	5	5	0.04
Rat - M	0.5	20	25	0.2
Rat - F	0.35	18	20	0.2
Hamster - M	0.125	12	15	0.09
Hamster - F	0.110	12	15	0.09

As an example, the regulatory limits for alachlor, which are supposedly set on the basis of the results of toxicological studies, are:

ADI (EPA) = 0.0025 mg/kg/day; RfD = 0.01 mg/kg/d, TLV-TWA 5 mg/m³, MCL 0.002 mg/L;

EPA Class B2 carcinogen

Do these values appear to make sense on the basis of what we know of toxicology and risk assessment?

Today, computer programs have been developed to help toxicologists compute human reference doses from animal toxicity data. These programs have a variety of built-in toxicity models, such as the benchmark dose method or power mean response regression model. These models include THWC and THRESHW by R.B. Howe of K.S. Crump company.

To determine if exposures to a toxic chemical causing non-carcinogenic effects are within a “safe” level, the “dose” of the chemical received is compared against the reference dose (RfD) or the “intake” of the chemical is compared to the acceptable daily intake (ADI). If the dose is less than the RfD or ADI, then there should not be a hazard (working on the threshold concept). The ratio of the dose to RfD is termed the hazard index (HI):

$$HI = \text{dose} / \text{RfD} = \text{intake} / \text{ADI}$$

The intake of a toxic compound is typically measured in units of mg toxic compound per kg body weight per day. The basic intake formula is:

$$I = (C \text{ CR EF ED}) / \text{BW AT}$$

where I = intake in mg/kg-day

C = concentration of toxic, mg/L or mg/kg

CR = contact rate, L/d or kg/d

EF = exposure frequency, days/yr

ED = exposure duration, yrs

BW = body weight, kg

AT = averaging time, equal to the exposure duration, yrs

The intake calculation will vary slightly, depending on the exposure route of interest. For inhalation, the retention rate and absorption amounts into the body must be included.

$$I = CA * IR * RR * ABS * ET * EF / \text{BW AT}$$

where CA = concentration in air, IR = inhalation rate, RR = retention rate; ABS = absorption fraction; ET = exposure time; EF = exposure frequency;

A special case for inhalation is the exposure to volatile compounds present in water during a shower. The intake in this case is best estimated by:

$$I = CW * [(ET1/2VS) + (ET2/ VB)] IR RR VW ABS EF ED / \text{BW AT}$$

where CW = concentration of volatile compound in water

ET1 = exposure time in the shower

VS = air volume in the shower

ET2 = exposure time in bathroom (in addition to shower time)

VS = volume of bathroom

VW = volume of water used during shower

(due to the fine spray, temperature, etc. assume most of volatile in the water will in fact escape into the air; a conservative assumption).

Dermal absorption may occur from contaminants associated with soil and from contaminants in “swimming water” or “bathing water”. For soil, of importance is area of skin exposed (SA), skin absorption amount (ABS), soil:skin adherence (SSA), and soil matrix effects (competitive binding of toxics to soil organics versus the skin) should be included.

$$I = SSA * SA * CS * ABS / \text{BW}$$

For water: $I = CW * SA * WS * ABS / \text{BW}$

where CS = concentration of toxic in soil, mg/kg

WS is the water contact with skin in L/cm²-d

Ingestion of toxic compounds can occur via drinking water, incidental soil ingestion, and from contaminated food. In all cases, gastrointestinal absorption (ABS), the ingestion rate (IR, water, soil, or food), and the concentration of the toxic in the media (CW, CS, CF).

$$I = CW * IR * ABS / \text{BW} \text{ or } I = CS * IR * ABS / \text{BW} \text{ or } I = CF * IR * ABS / \text{BW}$$

For soils, ingestion in adults is incidental; however, for very young children (< 6 yrs old) “pica behavior” may be exhibited, which is the intentional eating of mouthing large quantities of dirt or other objects. However, the concentrations associated with foods vary widely. If the food is a plant crop that was grown on contaminated soil, $CF = CS * \text{root uptake factor}$. If the food is meat or dairy products from livestock grown on contaminated food,

$$CF = \text{concentration in livestock food} * BCF * F$$

where BCF = bioconcentration factor, F = fat content of tissue eaten

Common “average human” values which are useful in intake analysis are shown in Table.

Parameter	Average Adult	Adult Male	Adult Female	Child 6-12 yrs	Child 2-6 yrs
Body Weight, kg	70	75	60	29	16
Lifespan, yrs	70	70	78	70	70
Food Intake, g/day	1500	1500	1500		
Water Intake, L/day	2.5	2.5	2.5	2	1
Air Intake, m3/day	22	23	21	11	6
Soil Ingested, mg/d	100	100	100	100	200
Skin Surface Area, cm ²	18150	20000	17000	10500	7000
Skin Adherence of Dust, mg/cm ²	0.75	0.75	0.75	0.75	0.75

For intake of one compound through multiple routes, the overall HI for the compound is:

$$HI = (CDI\text{-inhal} / RfD\text{-inhal}) + (CDI\text{-ingest} / RfD\text{-ingest}) + (CDI\text{-derm} / RfD\text{-derm})$$

Example A. Calculate the intake of non-carcinogenic chlorine dioxide from air containing 5 ppm-v by a female adult on-site worker during a 1 year period.

First, ppm-v should be converted into a mass per volume

(MW of ClO₂ = 67.5; 22.4 L/mol air)

$$5 / 1E6 \text{ mol/mol air} * 67.5 \text{ g/mol} * 1 \text{ mol air} / 22.4 \text{ L} = 0.015 \text{ mg/L}$$

Next, assume (to be conservative) that 100% of the air inhaled is retained, and that 100% of the ClO₂ in the air is absorbed.

$$I = C CR EF RR ABS / BW$$

$$I = 0.015 \text{ mg/L} * 21000 \text{ L/d} * 8 \text{ hrs} / 24 \text{ hrs} * 5 \text{ day} / 7 \text{ day} * 1 * 1 / 60 \text{ kg}$$

$$I = 1.25 \text{ mg/kg-d}$$

Example B. Calculate the intake of naphthalene from soil by an 8 year old child who plays outside 4 hours per day during the summer (June, July, August) on a field with exposed soil containing 100 mg/kg naphthalene.

For this case, we need to know how much dust adheres to the child’s skin and how much of the naphthalene in that soil is available for contact, and of that, how much is actually absorbed.

ASSUME dust adherence 0.5 mg/cm² exposed skin; exposed skin is 60% of the total skin area of the kid (wearing shorts, t-shirt, and shoes); 10% of the naphthalene in the soil is available, and 80% of that is absorbed.

$$I = C CR EF ABS SM / BW$$

$$I = 100 \text{ mg/kg} * (0.5 \text{ mg/cm}^2 * 0.6 * 10,500 \text{ cm}^2 * 1 \text{ kg} / 1E6 \text{ mg}) * 4 \text{ hr} / 24 \text{ hr} * 0.1 * 0.8 / 29 \text{ kg}$$

$$I = 0.00014 \text{ mg/kg-d}$$

HOWEVER, additionally the ingestion intake should also be considered...and maybe dust inhalation, too! However, each intake value should be calculated separately since a comparison with “risk” ADI’s will be route-specific. But the “overall” HI as a result of intake from all sources should be calculated.

Using the HI values allows determination of acceptable risk due to exposure to a mixture of compounds. If more than one compound induces toxic effects by the same mechanism and/or targets the same organs in the body, the HI values calculated for each compound should be added.

$$\text{Total site HI} = HI\text{-compoundA} + HI\text{-compoundB} + \dots$$

If the overall sum of the HI is <1, then no hazard should result. (Remember that an important part of this analysis is an accurate calculation of the dose of chemical exposure. The dose should be the amount of chemical actually adsorbed into the body.)

Example:

a) A farm worker applying atrazine as a pesticide to his fields absorbs approximately 0.3 mg of the compound each day he works with the chemical. EPA has determined that the dermal RfD for atrazine is 5 E-3 mg/kg-d. Does the farmer’s exposure to atrazine pose a hazard to his health?

First, calculate the DOSE of atrazine to the farmer:

Assume that the farmer weighs 70 kg

$$\text{DOSE} = 0.3 \text{ mg}/70 \text{ kg} \cdot \text{d} = 0.0043 \text{ mg/kg-d}$$

$$\text{DOSE} = 0.0043 \text{ mg/kg-d} < \text{RfD} = 0.005 \text{ mg/kg-d}$$

THEREFORE, NO HEALTH HAZARD

b) If the farmer is also applies alachlor (EPA RfD 0.01 mg/kg-d) to his fields, and absorbs approximately 0.3 mg each day, does the pesticide combination pose a hazard to his health?

First, calculate the DOSE of alachlor: $0.3 \text{ mg/d} / 70 \text{ kg} = 0.0043 \text{ mg/g-d}$

$$\text{DOSE} = 0.0043 \text{ mg/kg-d} < \text{RfD} = 0.01 \text{ mg/kg-d}$$

Therefore, alachlor alone does not pose an unacceptable risk.

Without any information, and to be conservative, assume that both atrazine and alachlor may pose a toxic risk due to similar action or target organs. Then, ADD the HI:

$$\text{HI atrazine} = 0.0043 / 0.005 = 0.86$$

$$\text{HI alachlor} = 0.0043 / 0.01 = 0.43$$

$$\text{TOTAL HI} = 0.86 + 0.43 = 1.29$$

Since the overall HI > 1, there may be a health risk to the farmer.

In addition to this range of studies conducted to establish the health effects of toxic chemicals on laboratory animals, the SARA regulations required that the Agency for Toxic Substances and Disease Registry develop a list of hazardous substances most commonly found at CERCLA NPL sites and establish minimal risk levels (MRLs) for these compounds. These MRLs are health guidance levels for daily exposure levels of compounds which should not pose “an appreciable risk of adverse non-cancer health effects.” The MRLs have been established for different classes of exposure duration: acute (1-14 days), intermediate (15-364 days), and chronic (>365 days); and on the basis of an oral or inhalation exposure. MRLs established as of March 1997 are summarized in the Appendix.

1.2 Dose-Effect Relationships for Carcinogenic Effects

A cancer is a tumor, or mass of cells which have mutated from a normal state and therefore divide uncontrollably and can spread to distant locations in the body. A carcinogen is a compound which is involved in the cancer-forming process. There are three steps in cancer development: initiation where a normal cell is affected, promotion where the initiated cell becomes a cancer cell, and progression when the cancer begins to spread to other cells. A so-called carcinogen may be the agent that initiates the initial alteration of the normal cell OR it may be a promoter which causes the initiated cell to develop into a cancer cell.

Carcinogenic effects may include: induction of cancer-types not commonly observed, earlier induction of tumors, or an increase in the number of tumors. EPA has a general classification system for carcinogens on the basis of the weight of evidence that the compound causes cancer in humans. These classes are:

EPA Carcinogen	Category	Scientific Evidence
Class A	Human Carcinogen	sufficient human epidemiologic studies
Class B1	Probable Human Carcinogen - High	limited human epidemiologic studies, sufficient animal evidence
Class B2	Probable Human Carcinogen - Low	inadequate human epidemiologic studies, sufficient animal evidence
Class C	Possible Human Carcinogen	absence of human data, limited animal evidence
Class D	Not Classified	inadequate animal evidence
Class E	Human Non-Carcinogen	sufficient negative animal studies (minimum 2 species) or adequate negative animal (1) and human (1) studies

How are these “sufficient”, “limited”, and “inadequate” classifications given to the test data? Guidelines from the EPA are summarized in Table:

	Evidence Classification	Scientific Evaluation of Data
HUMAN	Sufficient	causal relationship established
	Limited	causal relationship credible, but confounding factors not excluded
	Inadequate	few pertinent data or available studies did not adequately exclude chance, bias, or confounding factors
ANIMAL	No evidence	negative, well-designed studies
	Sufficient	increase of malignant tumors a) in multiple species or strains (>2) b) in multiple exposure with different routes or doses c) unusual degree in single exposure, unusual type, or early age of onset
	Limited	suggest carcinogenic effect except: a) single species, strain, experience b) inadequate doses, exposure duration, poor survival, or too few animals; c) increase only in benign tumors
	Inadequate	due to major qualities or quantitative limitations studies cannot be interpreted as + or -
	No evidence	no increase incidence of neoplasm in at least 2 well-designed animal studies in diff. species

Be careful to remember that these EPA Carcinogen Classes are a weight of evidence classification, and do not directly relate to the toxicity potency of the compounds. For example, for some newly developed compounds which are carefully handled, there may never be any human exposure and therefore the compound could never reach Class A, even if it appears STRONGLY carcinogenic on the basis of animal studies.

Bioassays indicate that effects in animals are generally applicable to man, since human cancers generally have animal counterparts. All multicellular organisms get cancers, and laboratory studies show that multiple species are usually effected by the same chemicals (for example, rats, mice, rabbits, and pigs all experienced carcinogenic effects from vinyl chloride exposure; although, different magnitude of effects in each).

Types of data used to determine toxicology information includes:

Test Method	System	Time	Basis
Structure/Activity	paper, basic lab tests	days	chemical structure indicates type of interaction with DNA
Short-term Tests	bacteria, cultured cells, intact animals	1 d - 8 mo.	detect effects in biological systems, similar effects in humans
Bioassay	intact animals	2 - 5 yrs	chemicals causing effects in animals will cause effects in humans
Epidemiology	humans	months to lifetimes	study human populations directly

The most common and famous short-term test is the Ames Assay. This test measures the potential of a chemical to cause genetic mutations in a bacterium, *Salmonella typhimurium*. Most compounds capable of causing tumors in animal cells (carcinogens) are also mutagens. The test in simple: first, strains of the bacteria which have a mutation which does not allow them to produce a specific enzyme and therefore are unable to grow with histidine are mixed with the potential mutagen. This mixture is then plated on agar with and without histidine. If the chemical causes a mutation, the bacteria will revert to the wild type and have the ability to grow without histidine addition. The number of colonies growing on the plate without histidine is proportional to how efficiently the mutagen reverts the original mutation. In addition, a “control” of cells without the potential mutagenic chemical is also plated on media without histidine, to evaluate the background reversion rate.

Several different types of his mutants are used to test for different classes of mutagens -- for example, frameshift mutagens will revert a frameshift mutation in his, etc. The *S. typhimurium* his mutants used have three additional properties that make them more sensitive to mutagens and therefore a good strain of test bacteria.

1. They have a mutation that makes the outer membrane more permeable to large molecules.
2. They have a mutation that eliminates the ability of the bacteria to excision repair DNA damage.

3. They carry a plasmid which increases error-prone repair of DNA damage. Some chemicals (called pro-mutagens) are not mutagenic unless metabolized to more active derivatives. Liver enzymes that normally detoxify harmful metabolic intermediates are often responsible for the activation of pro-mutagens into mutagens. For example, benzo(a)pyrene is not mutagenic but it is converted by liver enzymes to diolepoxides which are potent mutagens and carcinogens. Therefore, to test for such pro-mutagens, an extract of rat liver enzymes is included in the reversion assay.

An example of some real data is shown in the table below.

Mutagen	Number of reverent colonies per plate without histidine	
	his base substitution mutant of Salmonella typhimurium	his frameshift mutant of Salmonella typhimurium
None (control)	11	25
Diethyl sulfate	14,762	0
ICR - 191	13	499

Results are from McCann, J., N. Spingarn, J. Kobori, and B. Ames. 1975a. Detection of carcinogens as mutagens: Bacterial tester strains with R Factor plasmids. Proc. Natl. Acad. Sci. USA 72 979-983.

Other short-term tests include an acute test conducted at a single dose and observing immediate effect, or a sub-chronic test approximately 3 months of exposure and looks for signs of toxicity and non-death endpoints.

An example of a good chronic bioassay study would involve >960 test animals, and 2 year minimum. The way these numbers were derived is that is has been statistically determined with cost:benefit analysis that the ideal test group size is 50 animals per test condition. This number is usually increased to 60 so that 10 animals can be killed off early in the study period to look for early tumor formation. It is generally necessary to test both male and female animals of the same species, and two different species for completeness. Common test species are rats and mice (which are the cheapest and most readily available), pigs (have some of the best complements to some human organs), rabbits, and monkeys. Typically, will estimate a maximum tolerated dose, which is the highest dose that will not kill the animals from toxic effects other than cancers. The MTD is generally established on the basis of a preliminary toxicity test. Want to then test doses at 90%MTD, 50% MTD, 10-30% MTD, and zero (control group). The two year study period is selected on the basis of the rat/mice lifespan. It is important to establish the proper exposure pathway for correlation to the pertinent environmental condition - breathe, eat, skin absorption. At the end of the study, will observe pathological slides from various organs in the body - 21-23 sections through the test organism, look at brain (3 sections), heart, lung, esophagus, stomach, kidney, liver, pancreas, spleen, urinary bladder, adrenal glands, pituitary gland, thyroid, lymph node, etc. The information obtained includes the total number of animals with tumors, total number of organs with significantly increased tumor incidence, number of tumors per animal, early onset of tumors, etc. It becomes clear how this can be a very expensive process!

$$60 \text{ animals/gp} \times 2 \text{ sexes} \times 4 \text{ dose levels} \times 2 \text{ types of animals} = 960 \text{ test animals}$$

For example, a Daphnia Reproductive Bioassay (WARF: P96080US) provides a 6-day Daphnia reproductive bioassay for detecting and confirming the presence of a toxic substance in an aqueous sample, and/or for screening the substance as an endocrine disrupter. According to the assay, a test sample is brought into contact with at least three adult, oviporous Daphnia of a single clone under conditions of crowding and suboptimal growth conditions to cause stress and stimulate sexual reproduction. The preferred clone for use in the assay is Daphnia galeata-mendotae Wingra clone CDF-1. The bioassay is based upon the measurement of five endpoints that convey quantitative information about the biological activity of the substance: survivorship, numbers of female offspring, numbers of male offspring, number of resting eggs, and number of offspring that display developmental deformities. This is a test for which kits are available, making it quite simple. A patent of this test has been applied for by the inventors, Stanley I. Dodson, Jonathan B. Shurin, Kristin Girvin, Thomas C. O'Keefe, Pamela Van Der Puy, and Takayuki Hanazato. (<http://www.wiscinfo.wisc.edu/warf.boi/P96080US.html>)

Epidemiology studies can be used to determine effects in humans. Epidemiology is a systematic form of case cluster analysis with attempts to control for confounding factors in the data or statistical analysis. In a cohort study, one follows a particular population and looks for differences in disease rates between groups differently exposed to the suspect chemical. In contrast, in case-control studies the researchers picks out diseased cases and disease-free cases and looks for differences in the environmental conditions that may have caused the disease incidence. In a third method, the case-cohort study, one follows a well-defined sub-population which is compared with control cases. Since these studies deal directly with humans, it is generally considered the best way to

determine health risks caused by chemicals. However, confounding factors and the scale of study group and observed incidence rates can complicate data interpretation.

Unlike most non-carcinogens, it is generally assumed that there is no “threshold” for carcinogenic effects. That is, any level of exposure has some negative effect. A variety of biological mechanisms have been proposed, with no consensus. Each biological mechanism can be described by a mathematical representation or equation. These cancer “models” are discussed below:

1. One Hit

This is the most basic model which assumes that only one critical cellular interaction is required to transform a normal cell into a cancer cell. The “hit” is the interaction that must occur before a toxic effect is produced. Then, the probability of developing cancer is the same as the probability of at least one “hit, and the number of hits should be proportional to the dose of the agent received.

2. Multi Hit, Single Target

Here it is assumed that a series of hits are required on a specific target cell before that cell becomes an altered tumor cell.

3. Multi Target, Multi Hit

Here it is assumed that multiple target cells in close proximity must be hit before a tumor will form. This model allows for non-linearity at low doses.

4. Multi-Stage

This hypothesizes that a series of events must occur before a normal cells can form a tumor cell.

EPA most generally supports the use of a linearized multi-stage model.

Computer programs have also been developed to estimate human carcinogenic risks on the basis on animal studies. For example, in the program “Tox_Risk” (version 2.0), the user can even select the type of “model” they want to use, such as multistage, multihit, single hit, etc.

Since the exposure to toxic compounds increases the risk that cancer can occur over a lifetime, the “intake” of carcinogenic compounds is calculated differently than for non-carcinogens. The “averaging time” used for carcinogens is the lifetime, while the averaging time inherent in non-carcinogenic effects was simply the exposure duration. The basic intake equation for carcinogens is:

$$I = (C \text{ CR EF ED}) / \text{BW LT}$$

where I = intake in mg/kg-day

C = concentration of toxic, mg/L or mg/kg

CR = contact rate, L/d or kg/d

EF = exposure frequency, days/yr

ED = exposure duration, yrs

BW = body weight, kg

LT = lifetime, used to average carcinogenic effects, yrs

Based on compound intake, the carcinogenic risk can be calculated from calculated “slope factors” (SF) or carcinogenic potency factors (CPF). For low intake quantities, a linear low-dose model is typically assumed, so

$$\text{Carcinogenic Risk} = R = \text{probability of cancer} = \text{CDI} * \text{SF}$$

where CDI = the chronic daily intake. Again, if the carcinogenic compound can be taken into the body via multiple exposure routes, the total carcinogenic risk must be calculated as:

$$R = (\text{CDI-inhal} * \text{SF-inhal}) + (\text{CDI-ingest} * \text{SF-ingest}) + (\text{CDI-derm} * \text{SF-derm})$$

However, if this R value turns out to be greater than 0.01, this is clearly a “high” dose and an alternative equation to calculate risk based on the 1-hit model should be used:

$$R = [1 - \exp(-\text{CDI} * \text{SF})]$$

1.3 Ecological Toxicity

Ecological toxicity includes the adverse effects of chemical exposure to individual organisms, populations of organisms, and the organism’s environment. The principles of exposure, absorption, metabolism, and effects applied to humans are similar to ecological toxicity. The largest difficulty of the lack of good data.

One important concept for ecological toxicity is “biomagnification”. This occurs as a chemical which bioaccumulates increases in concentration within the organisms as it is transferred up the food chain. Typically, compounds with higher Kow will have a greater tendency to partition into the fat and tissues of animals. Of

particular importance is the concentration in plankton and fish, which bioconcentrate many contaminants out of the water in their environment.

Some examples of “ecological toxicity” effects of two pesticides, alachlor and phosmet, are:

Alachlor: moderately toxic to aquatic invertebrates and to fish. Fish 96-hr LC-50 values are 2.4 mg/L trout, 4.3 mg/L sunfish, 6.5 mg/L catfish, 4.6 mg/L carp, and 19.5 mg/L crayfish. Slightly toxic to practically non-toxic to wildfowl, with 5-d LC-50 of >5000 ppm in mallards and quail, >10000 ppm in pheasants; LD-50 in mallards 2000 mg/kg. Does not pose a problem for bees, non-toxic to earthworms.

Phosmet: Highly toxic to aquatic invertebrates and crustaceans with LC50 values of 2 to 6 µg/L. Highly toxic to fish, depending on species; 96-hr LC50 56 µg/L for trout, 70 g/L for bluegill, and 9 mg/L for minnows.

Bioconcentration factor in fish 6 to 37, little significant bioaccumulation. Large variation in toxicity to birds.

Example toxicity data for 4 heavy metals by juvenile seawater organisms, on the basis of a 96-hr test

Metals and Species	LC-5, mg/L	LC-50, mg/L	LC-95, mg/L
Cu+2			
Bay scallop	0.018	0.029	0.046
Surf clam	0.032	0.051	0.080
Blue Mussels	0.070	0.122	0.21
Se			
Bay scallop	0.168	0.255	0.39
Surf clam	1.35	1.90	2.70
Zn			
Bay scallop	1.20	2.25	4.2
Surf clam	1.80	2.95	4.8
Pb			
Bay scallop	3.30	8.60	22.0
Surf clam	3.35	5.40	8.6
Blue mussels			
Hg	0.122	0.161	0.248
Ag	0.011	0.159	0.249
Cd	0.48	0.96	2.4

Nelson, Miller, and Calabrese. 1988. Arch. Environ. Contam. Toxicol. 595-600.

1.4 Sources of Toxicology Data

There are a variety of sources where toxicology information can be obtained. The easiest to access are the number of sites on the world wide web where information is available. Sites are summarized below:

<http://ace.orst.edu/info/extoxnet>

EXTOXNET is the Extension Toxicology Network that contains toxicology information on pesticides, as funded by the USDA and developed by Cornell University, Michigan State University, Oregon State University, and University of California at Davis.

charlie_xintaras@atsoaa1.em.cdc.gov

ASTDR ToxFAQs - Agency for Toxic Substances and Disease Registry

TRI database (Toxics Release Inventory database)

IRIS - Integrated Risk Information System: http://www.epa.gov/ngispgm3/iris/Substance_List.html

or via <http://www.epa.gov/ncea/iris.htm>

2. ENDOCRINE DISRUPTERS

Endocrine disrupters are chemicals that interfere with the levels of hormone chemicals present in the body. Disruptions in the endocrine system, which regulates human hormone levels, can: increase the incidence of testicular, prostate, and breast cancer; cause defects during fetal development; and decrease sperm counts. The link between synthetic chemicals, specifically pesticides, and imbalances in hormone levels in the body first became a public concern when the book Our Stolen Future by Theo Colburn, John Peterson Myers, and Dianne Dumanoski

was published. Vice President Al Gore compared the book with Rachel Carson's Silent Spring, which was published in 1962 and warned against the threats posed by pesticides like DDT. Gore wrote in the book's foreword: "Our Stolen Future takes up where Carson left off and reviews a large and growing body of scientific evidence linking synthetic chemicals to aberrant sexual development and behavioral and reproductive problems."

Research has suggested that the average human male sperm count has plunged by almost half in the past 50 years. This drop is hypothesized to be due to exposure to synthetic chemicals which interfere with the human hormone system. Skeptics point out that infertility rates have stayed fairly constant over this time period. The debate on the magnitude and importance chemical effects on the endocrine system is spurring research into the problem. Specifically, researchers are studying the exact role that chemicals play in producing the observed adverse effects. Much of this research currently focuses on studying these effects in animal systems, such as fish, birds, and invertebrates. An international conference on Endocrine Disrupters was held in October 1996 in Washington D.C, and brought together experts from the scientific, industrial, and regulatory arenas.

The body has three main networks which maintain human health: the nervous system, immune system, and endocrine system. The endocrine system is composed of glands and the hormone chemicals they produce and release into the bloodstream. Human glands include the pancreas, adrenal glands, thyroid, parathyroid, thymus, and pituitary gland. Hormones are essential for the regulation of numerous biological processes in the body, acting like messengers within the cell or organ. For example: thyroid hormones are essential for brain development; testosterone, progesterone and estrogen are required for reproductive organ development and functioning; and insulin regulates the level of blood sugar in the body. Only very small concentrations of hormones are needed in the body for adequate functioning since natural hormones are extremely potent. Measurement of these low concentrations is only possible using the most sensitive analytical methods. For example, estradiol is the key estrogen hormone, and operates at concentrations in the part per trillion range.

To exert their effects in the body, hormones first bind with specific cell proteins called receptors. The receptor and its hormone have an intricate fit, like a lock and key. There are hundreds of different kinds of receptors, each one designed for a particular chemical signal, and within one cell there are 10,000 or more of one type of receptor. However, only a small number of receptors generally need to be activated to elicit a response. The hormone-receptor complex binds to specific regions of DNA in the cell nucleus to activate specific genes. Hormones cause the genes in cells to send signals to the body, but they cannot alter or damage genes. If something happens to damage the hormone system, then the wrong messages, or no messages, are sent.

At the cellular level humans are essentially the same as other animals, and in many instances the hormones in animals and humans are chemically identical. Researchers are finding high similarity in hormone function as well as chemical structure across different species. Therefore, in spite of the lack of human data, endocrine-related effects observed in animals can be extrapolated to humans (<http://www.wwfcanada.org/hormone-disruptors/hormdist.htm>) As observed by Rachel Carson in 1962: "What we do to the animals, we do to ourselves."

As a fuller understanding of the range of effects is gained, scientists, policymakers, and the public are drawing conclusions about the potential implications for individuals and entire populations. There are published reports of a broad array of endocrine-disruptor effects in animals, which can be grouped into four main classes: decreased immune function, hormone imbalances, reproductive effects (including both decreased fertility and birth deformities), increased cancer in reproductive organs, and behavioral, neurological and cognitive effects. Examples of each of these effects are described below. (sources: <http://www.wwfcanada.org/hormone-disruptors/effects1.htm>; <http://www.wwfcanada.org/hormone-disruptors/links.htm#genend>)

Decreased Immune Function

The immune system is linked closely with the endocrine system. Studies on experimental animals have provided convincing evidence that exposure to DES before birth can significantly and permanently affect the immune system. Observations on humans exposed to DES suggest immune disruption characterized by an increased prevalence of a rare immunologic hyperactivity, rheumatic fever and subsequent microbial infection (strep throat) in DES daughters. Some scientists attribute recent massive die-offs of a variety of marine life (including seals in the North Sea in 1989 and in the Baltic Sea in 1992, dolphins along the Eastern seaboard of the United States in 1989 and in the Mediterranean Sea in 1994) to immune-system depression which increased susceptibility to infection. Studies in 1987 at the University of Barcelona in Spain revealed levels of PCBs two to three times higher from washed-up dolphins than in healthy dolphins. In addition, studies have shown that seals fed with PCB- and dioxin-contaminated Baltic Sea fish had significantly lower killer cell activity and antibody responses than seals fed a less contaminated source. In monkeys, changes in white blood cells associated with the immune system can be measured

at dioxin levels of 10 ng/kg -- 25 percent below the amount found in general human populations. Mice with body burdens of 10 ng/kg display an increased susceptibility to infection by viruses, presumably because their immune system has been damaged. An Inuit population in Québec has higher rates of respiratory and ear infections, and vaccinations don't work well in these children. These children experience ten to fifteen times more infections than children from southern Québec. Levels of PCBs in Inuit breast milk are seven to ten times higher than levels found in women from southern Québec.

Skewed Hormone Balance & Production

Exposure to hormone disruptors can alter the natural hormone balance. Examination of hormone levels in numerous wildlife species exposed to synthetic chemicals indicate that both females and males have higher than normal ratios of estrogen to testosterone, causing 'feminization' of the male species. Blood sampling for estrogenic contaminants in the endangered Florida panther population revealed one male had nearly twice as much estrogen as testosterone, instead of the normal level of two to three times as much testosterone as estrogen. At least one female had more testosterone than estrogen. Working under the assumption that chemicals that mimic estrogen in the body (principally pesticides) could be a cause, the US Fish and Wildlife Service has prohibited the use of estrogenic chemicals in all federally-managed wildlife refuges in the southeastern US. Since a 1980 spill of dicofol in Lake Apopka in Florida, serious hormonal dysfunction and feminization in the lake's alligator population has been observed. Ratios of estrogen to testosterone in eggs was twice as high as normal.

Vitellogenin is an egg-yolk protein produced by female fish, but studies in the UK have found vitellogenin concentrations in caged male fish exposed to the river outfalls of 28 different sewage treatment plants. In some cases, male trout exhibited vitellogenin concentrations in their bloodstreams typical of mature females during egg production. After just three weeks' exposure to the wastewater effluents, the trout had up to 100,000 times more yolk protein in their blood than was normal in male fish. Researchers have identified nonyl phenols, substances used in detergents and various industrial processes, as the likely xeno-estrogens responsible.

Reproductive Tract Abnormalities

During embryonic development, hormones send signals which initiate the development of sex organs. If hormone disruptors upset the natural balance of hormones, the offspring may have subtle or dramatic abnormalities, including changes to the uterus, oviduct, and testes. Human offspring exposed prior to birth to the estrogenic drug DES have more difficulty in conceiving and daughters have increased miscarriages, spontaneous abortions and suffer three to five times more tubal pregnancies. Sons born to mothers who took the hormone drug DES have a higher incidence of malformed penis, undescended testicles at birth, malformed or abnormal sperm during adulthood, or abnormalities that may render them sterile. One study discovered that levels of PCBs in the blood are higher in women who suffer miscarriages. Studies in rats indicate that PCBs cause a reduction in progesterone, a hormone necessary to maintain pregnancy. PCB has been linked to tumour promotion.

For over ten years there has been no record of reproduction in the pallid sturgeon, an endangered species native to the Missouri and Mississippi rivers, and existing sturgeon are usually 30 to 40 years old. High concentrations of PCBs and DDT found in some fish have led Fish and Wildlife scientists to suspect that hormone disruptors might be jeopardizing their reproductive health. For fifteen years researchers have reported that sturgeon gonads "aren't distinctly male or female anymore." Researchers have reported a 100 percent prevalence of thyroid enlargement in 2-4 year old salmon in the Great Lakes. Some salmon have been found with thyroids over a million times their normal size and the problem is so severe in Lake Erie that they are beginning to rupture and explode. Almost all the salmon observed had hermaphroditic reproductive systems.

Between 1985 and 1990, 67 percent of male Florida panthers were born with one or more undescended testes, a condition known as cryptorchidism. Ten years earlier, this condition existed in only 14 percent of males. In humans, there has been a doubling of the incidence of cryptorchidism in England and Wales between 1962 and 1981, and similar increases have been reported in Sweden and Hungary. These men have a higher risk of testicular cancer and typically have fewer sperm and a higher percentage of abnormal sperm. In addition, enlargement of the prostate gland now afflicts eighty percent of men by the age of seventy in western countries. Prostate cancer is now the most common cancer in American men and has increased 126 percent from 1973 to 1991. Rat studies have found that long-term exposure to estrogen can induce prostate cancer.

Decreased Sperm Count & Motility

It has been suggested that the drop in sperm counts in human men reported in five recent studies may be attributed to an increase in estrogen or anti-androgens during fetal and post-natal development. Lower sperm counts

and reduced sperm motility was seen more often in younger men in two studies, which suggests that something is happening in the womb. Some types of PCBs have been inversely associated with sperm motility in men with fertility problems. A review study reported in 1992 which summarized the results of tests on over 15,000 men (most from the US and Europe, but also from India, Nigeria, Hong Kong, Thailand, Brazil, Libya, Peru and Scandinavia) found that the average human male sperm count had dropped 45 percent between 1938 and 1990. Simultaneously, the rate of testicular cancer had tripled in Denmark between the 1940s and the 1980s. French data suggest a drop of 1 million sperm per millilitre per year. Belgium and Scottish studies have corroborated this trend. More recently, a survey of sperm from 577 men in the UK born between 1955 and 1974 found that younger men had significantly poorer sperm quality. Sperm concentration declined by 2.1 percent per year and the number of motile sperm by 2.04 percent showing that the average sperm count of British men born in the 1970s is 24 percent lower than in men born 20 years earlier. As with all sperm studies, however, some regional variations occur. For instance a survey of a less urbanised population published alongside the UK study found that sperm counts in men from Toulouse, in southern France, were holding steady. The authors suggest that differences in pollution levels may explain the results. Similarly, a Danish study found organic farmers who eat pesticide-free food produced a significantly higher number of sperm than other men - more than double; but given the small size of the sample, researchers were not able to make firm conclusions regarding the link between human-made chemicals and male sperm production. This is because it would necessitate knowing the contaminant levels in the mothers of each male and complete knowledge about each chemical identified and its potential hormone disrupting ability.

Cancer/Cell Proliferation of Reproductive Organs

Many tumours are estrogen dependent -- these cells proliferate in the presence of estrogen. For instance, it is well-accepted that excess exposure to estrogen is a primary risk factor for breast cancer, endometrial cancer which affects the uterus, and for endometriosis. Endometriosis incidence appears to be increasing. There are 5.5 million cases in the US and Canada compared to 21 reported cases in the world 70 years ago, and the National Institute of Child Health and Human Development estimates that endometriosis afflicts 10-20 percent of women of childbearing age in the US.. Female monkeys fed low levels of dioxin were subsequently found to develop endometriosis. It is suggested that dioxin can indirectly modify the way in which the body uses estrogen and/or can alter the function of other hormones and chemicals in the body. Certain types of PCBs have also been shown to induce uterine cell proliferation in rats.

One in eight women in the US and Canada now get breast cancer and one-third of those will die from it. Fifty years ago, a woman ran a one in twenty risk of getting breast cancer. Since 1940 breast cancer deaths have risen steadily by one percent per year and this figure represents genuine trends since they are adjusted for age. Among American women 40-45 years of age, breast cancer is now the leading cause of death. In a survey conducted by Dr. Mary Wolff of New York's Mount Sinai School of Medicine, women with breast cancer had four times the levels of DDE found in non-carcinogenic tumours. Another study that examined levels in Caucasian, African-American and Asian women only found significant correlation when the Asian population was excluded. Speculation exists that diet of these women, traditionally rich in soy may have a counteractive effect.

Other research attempting to understand the mechanism of breast cancer and has found the body can process estradiol in two chemically different ways - a "good" estrogen pathway that produces a weak form of estrogen and a "bad" pathway that produces a more potent form thus potentially increasing the chance of cancer. Indole-3-carbinol, a substance found in broccoli, cauliflower, brussell sprouts and other members of the cabbage family, pushes estrogen metabolism toward the "good" pathway. Similar studies have found DDT, PCBs, kepone and the currently used pesticide, atrazine, increase the production of the more potent estrogen. A wide variety of pesticides and related compounds clearly have effects on estrogen metabolism.

Testicular cancer has increased three-fold in Denmark and other industrial countries have seen similar trends. Precancerous cells usually appear in young boys who will ultimately develop testicular cancer. Some researchers note that these cells develop during fetal life, only to lie dormant until some hormonal surge during puberty triggers their proliferation. Data shows sons born to mothers who took the hormone drug DES have a eight-fold risk of developing testicular cancer.

Behaviourial, Neurological and Cognitive Effects

It has been shown in different animal studies that exposure to organochlorines while in the womb will affect sexual behaviour, aggression, neurological response and learning ability. Hormonal imbalances have changed the behaviour of male bald eagles, herring, and western gulls in the wild that did not mate. Lab studies have shown that the injection of chicken and quail eggs with estrogen produced male roosters that never exhibited mating behavior.

In addition, a comparison of terns nesting in clean and contaminated areas in the Great Lakes region revealed substantial nest abandonment and egg disappearance in the contaminated area but virtually none in the clean colony.

Animal studies also suggest that exposure to synthetic chemicals can increase aggression. Pregnant mice fed relatively low levels of DDT and methoxychlor produced male offspring that demonstrated more aggressive behaviour, such as increased territorial urine marking and fighting, than controls. Other researchers have fed rats and mice with water containing the levels of chemical contaminants found in rural wells and discovered they showed unpredictable outbursts of aggression.

Other behavior effects include: "spinning syndrome", depressed reflexes, and learning deficits in off-spring of rats exposed to organochlorines; motor impairment, hyperactivity, and memory and learning deficits in rhesus monkeys exposed to PCBs in the womb and through breast milk; hyper-reactivity to stress in male rats fed PCB-contaminated fish and offspring of female rats fed the fish during pregnancy.

Recent human studies are also finding links between in utero chemical exposure and behavioural response in children. It is known that dioxin and PCBs affect the thyroid system which is critical to prenatal brain development and can lead to loss of intelligence and behavioural changes. Depressed thyroid levels in mothers during pregnancy has resulted in increased hyperactivity in children. Ingestion of PCB-contaminated fish before and during pregnancy resulted in babies with lower birth weights, smaller skull circumference, and cognitive, motor and behavioural deficits at birth compared with offspring whose mothers did not eat fish. Subsequent studies of the children beginning at 6-7 months revealed delays in psychomotor development and poorer visual recognition compared with controls. When examined at 4 years of age, the children of women who had eaten fish in this study exhibited short-term memory problems and 17 of the children refused to cooperate during testing; they were the children of the mothers with the highest PCB concentrations (measured in their breast milk) in the study. An estimated six to ten percent of children under the age of thirteen in the United States suffer from hyperactivity and attention deficit disorders. Biochemical disturbance, as a result of hormone disruption, is a possible factor. Thyroid research has shown a correlation between hyperactivity in children and mothers who had abnormally low levels of thyroid hormones during pregnancy.

Several studies comparing DES-exposed women with their unexposed sisters or other unexposed women found an association between prenatal exposure and increased homosexuality and bisexuality. Studies on DES sons so far have found no indication of influences the sexual orientation of sons, although one report found that the sons appeared more female- than male-like in their spatial abilities and patterns of which side of the brain they used while completing a task. Researchers have found high rates of major depression in both men and women exposed to DES before birth as well as other psychiatric disorders such as anxiety, anorexia nervosa and phobic neurosis. Parallel studies have reported such differences even when DES sons and daughters were unaware that they had been exposed. In one study researchers found that 40 percent of the women and 71 percent of the men had experienced major depression that physically impaired them.

A journal article in Science reported synergistic effects of chemical mixtures; John McLachlan of Tulane University said, "We found in some cases that one plus one equals a thousand." Although chemicals in the environment are much less potent than natural estrogens, the effects of combinations of the compounds were 10 to 1,600 times more potent than the individual compounds in activating estrogen receptor-mediated transcription. The implications for screening environmental chemicals for estrogen effects are enormous. Research is underway to replicate the results reported in the McLachlan study.

The USEPA has taken several steps to address the issue of endocrine disruptors, stating in a background paper (EPA Activities on Endocrine Disruptors) that "they believe the potential implications of endocrine disruptors for our children and for our future are serious enough to warrant the Agency taking prudent, preventive steps, without waiting for the research to be complete." In response, the American Crop Protection Association and the Chemical Manufacturers Association stated, "As EPA decides how to address endocrine disrupting chemicals, remember pesticides already are subjected to much testing." The Food Quality Protection Act of 1996 added provisions for endocrine testing to current law. Although much more research needs done on this issue, it will play a role in future regulations.

Pollutants with Widespread Distribution Reported to have Reproductive and Endocrine-Disrupting Effects

- Organohalogens: Dioxins, furans, PCBs, PBBs, Octachlorostyrene, Hexachlorobenzene, Pentachlorophenol
- Pesticides: 2,4,5-T, 2,4-D, alachlor, aldicarb, amitrole, atrazine, benomyl, beta-HCH, carbaryl, chlordane, cypermethrin, DBCP, DDT, DDT metabolites, dicofol, dieldrin, endosulfan, esfenvalerate, ethylparathion,

fenvalerate, lindane, heptachlor, h-epoxide, kelthane, kepone, malathion, mancozeb, maneb, methomyl, methoxychlor, metiram, metribuzin, mirex, nitrofen, oxychlorane, permethrin, synthetic pyrethroids, toxaphene, transnonachlor, tributyltin oxide, trifluralin, vinclozolin, zineb, ziram

- Penta- to Nonyl-Phenols: Bisphenol A ,
- Phthalates: Di-ethylhexyl phthalate (DEHP), Butyl benzyl phthalate (BBP), Di-n-butyl phthalate (DBP), Di-n-pentyl phthalate (DPP), Di-hexyl phthalate (DHP), Di-propyl phthalate (DprP), Diethyl phthalate (DEP)
- Styrene dimers and trimers ; - Benzo(a)pyrene ; - Heavy Metals: Cadmium, Lead , Mercury

Widespread pollutants that bind to hormone receptors and therefore suspected to have reproductive & endocrine-disrupting effects: 2,4-dichlorophenol, Diethylhexyl adipate, Benzophenone, N-butyl benzene, 4-nitrotoluene

All of the substances presently identified as hormone disruptors are now widely distributed throughout the environment, some are common constituents of consumer products, and many are now found in human tissues. <http://www.wwfcanada.org/hormone-disruptors/list.htm>

On November 5-10, 1995 a multidisciplinary group of international experts gathered for a work session on "Environmental Endocrine-Disrupting Chemicals: Neural, Endocrine and Behavioral Effects" under the auspices of the International School of Ethology at the Ettore Majorana Centre for Scientific Culture in Erice, Sicily.

Participants at the workshop reached a consensus on the following statements :

Endocrine-disrupting chemicals can undermine neurological and behavioral development and of humans and animals exposed pre-birth. Widespread loss of this in nature can change the character of human societies or destabilize wildlife populations. Because of profound economic and social consequences emerge from small shifts in functional potential at the population level, it is imperative to monitor levels of endocrine-disrupting contaminants in humans, animals, and the environment and reduce their production and release.

The endocrine system is sensitive to perturbation. In contrast to natural hormones, some of the manufactured organic compounds that interfere with the endocrine system are persistent and undergo biomagnification in the food web, which makes them of greater concern as endocrine disruptors.

Man-made endocrine-disrupting chemicals range across all continents and oceans, and, because of their persistence in the body, can be passed from generation to generation. The seriousness of the problems is exacerbated by the extremely low levels of hormones produced naturally to induce appropriate responses. Many endocrine disrupting contaminants, even if less potent than the natural products, are present in living tissue at concentrations millions of times higher than the natural hormones. Animals and humans exhibit adverse health effects at current environmental concentrations of man-made chemicals that act as endocrine disruptors.

Gestational exposure to persistent man-made chemicals reflects the lifetime of exposure of females before they become pregnant, and not just recent maternal exposure.

The developing brain exhibits specific and often narrow windows during which exposure to endocrine disruptors can produce permanent changes in its structure and function. Chemical exposure early in life can lead to significant and irreversible abnormalities in brain development at levels that do not produce permanent effects in adults.

Thyroid hormones are essential for normal brain functions throughout life. Interference with thyroid hormone function during development leads to abnormalities in brain and behavioral development. The results of moderate alterations of thyroid hormone concentrations, particularly during fetal life, include cerebral palsy, mental retardation, learning disability, attention deficit hyperactivity disorder, seizures and other permanent neurological abnormalities. Exposure to man-made chemicals during early development can impair motor function, spatial perception, learning, memory, auditory development, fine motor coordination, and balance.

Sexual development of the brain is under the influence of estrogenic (female) and androgenic (male) hormones. Not all endocrine disruptors are estrogenic or anti-estrogenic. For example, DDE, a breakdown product of DDT that is found in almost all living tissue, is an anti-androgen in mammals. Man-made chemicals that interfere with sex hormones share the potential to disturb normal brain sexual development. Wildlife studies of gulls, terns, fishes, whales porpoises, alligators and turtles link environmental contaminants with disturbances in sex hormone production and/or action. These effects have been associated with exposure to sewage and industrial effluents, pesticides, ambient ocean and freshwater contamination, and the aquatic food web.

Commonalities across species in the hormonal mechanisms controlling brain development and function mean that adverse effects observed in wildlife and in laboratory animals may also occur in humans, although specific

effects may differ from species to species. Most important, the same man-made chemicals that have shown these effects in mechanistic studies in laboratory animals also have a high exposure potential for humans.

The full range of substances interfering with natural endocrine modulation of neural and behavioral development are not currently known. Compounds shown to have endocrine effects include dioxins, PCBs, phenolics, phthalates, and many pesticides. Any compounds mimicking, antagonizing, or altering levels of, neurotransmitters, hormones, and growth factors in the developing brain are potentially in this group.

Similarly, the conference participants stated with confidence that:

Every pregnant woman in the world has endocrine disruptors in her body that are transferred to the fetus and in her milk that are transferred to the infant.

There may not be definable thresholds for responses to endocrine disruptors. For naturally occurring hormones, too much can be as severe a problem as too little. Consequently, simple dose- response curves for toxicity do not necessarily apply to the effects of endocrine disruptors.

Because certain PCBs, dioxins, and pesticides are known to impair normal thyroid function, we suspect they contribute to learning disabilities.

Some endocrine disruptors or their breakdown products have similar potency to natural hormones. Even weak endocrine disruptors may exert significant effects because they can bypass the natural protection of blood binding proteins for natural hormones. Some disruptors also have a substantially longer biological half-life than naturally produced hormones, and as a result accumulate in the body to concentrations of concern. Some man-made chemicals that appear non-toxic are converted by the liver to more toxic compounds. Also, compounds that are not toxic in the mother may be toxic to her developing embryo, fetus or newborn. The exquisite vulnerability of the fetal brain to methylmercury and lead are prime examples of this principle.

Functional deficits are not as easily measured as physical anomalies or clinical disease, in part because they are typically expressed as continuous measures, such as IQ, rather than the number of cases in a population. Conventional population surveys may therefore overlook the extent of these deficits. In addition, effects on the most sensitive members of the population are even harder to quantify.

Large amounts of man-made chemicals capable of disrupting the endocrine and nervous systems are used in, third world countries that lack the resources to properly monitor and control exposure levels. Insufficient training in handling chemicals and ignorance of their health effects leads to the likelihood of very high levels of exposure.

In addition, the conference attendees judged that:

Significant reduction in health care costs could result if exposure to endocrine-disruptors were reduced.

The message that endocrine disruptors are present in the environment and have the potential to affect many people over a lifespan has not effectively reached the general public, the scientific community, regulators, or policy makers; any policy based on ignorance of the facts about endocrine-disruptors would be irresponsible.

Exposure effects are inadequately addressed by population averages; rather, the total distribution of responses in a population should be characterized. The magnitude of the risks depends upon the endpoint that is considered. Many motor, behavioral, and cognitive functions are more sensitive than cancer.

Wildlife have been effective models for understanding endocrine disruption at the molecular, cellular, individual, population, and ecosystem levels. Research with diverse wildlife species at all levels of biological organization must be broadened and adequately supported.

Those responsible for producing man-made chemicals must assure product safety beyond a reasonable doubt. Manufacturers should be required to reveal all chemicals in their products with evidence of developmental safety.

3. QUANTITATIVE RISK ASSESSMENT

Quantitative risk assessment (QRA) involves characterizing the potential adverse health effects of human exposures to environmental hazards. This includes not only the numeric "risk" values, but also a characterization of the **uncertainties** in determining that risk level. Quantitative risk assessment should be used as one TOOL to help understand the hazards associated with wastes. QRA has been used to improve the basis of management decisions at hazardous waste sites, allowing a better balancing of immediate hazards versus longterm remediation approaches. QRA also assists in understanding the health implications contaminated sites and alternative remedial strategies.

Some basic definitions:

Background Risk = the risk based on exposure in the absence of a source or chemical being studied

For example, the background level of cancer incidence in the general population is 1 in 4.

Incremental Risk = risk caused by exposure to the particular source or chemical

Total Risk = Background + Incremental

A hazard is the source of a risk -> a chemical poses a hazard due to its toxicity, persistence, etc.

Risk occurs after exposure or is the possibility of exposure.

Science-Based Risk Assessment has four main steps:

1. Hazard Identification (determine the chemicals which are important at a given site)
2. Exposure Assessment (includes exposure routes with who is exposed and how they are exposed)
3. Toxicity Assessment (gather numbers on toxicity, make dose/response assessments)
 - for carcinogens find CPFs or slope factors, determine the probability of lifetime cancer
 - for non-carcinogens find an ADI or RfD, calculate the hazard index
4. Risk Characterization (estimate the overall magnitude of risk and uncertainty of the estimate)

Risk assessments conducted under EPA require that both human health and ecological impacts (to both on and off-site biota) be considered. When these RISK ASSESSMENTS are combined with VALUE JUDGMENTS on acceptable risk levels and the CONTROL COSTS associated with reducing the risk, then an informed DECISION on a strategy to best clean the site can be made. Each of the four steps in the QRA process are described in more detail below.

3.1 Hazard Identification

The Hazard Identification process involves the following steps”

1. Identify the chemical contaminants at the site

List all types, concentrations (with available information), location, and potential for movement of these chemicals.

2. Select the “chemicals of concern” (COCs) at the site

COCs are those chemicals posing most of risk. The COCs are generally selected to serve as surrogates for a vast range of chemicals which may be present at the site. These surrogates are selected because they are the most toxic, most persistent, most mobile, have the highest concentrations present, or pose the greatest exposure risk. The COCs should include the compounds that make up greater than 99% of the total site risk. In addition, compounds which cause both carcinogenic and non-carcinogenic effects and represent all the different media (contaminated groundwater, soil, surface water, air) should be included.

3. Calculate the Toxicity score (TS)

Two toxicity scores for the site will be computed on the basis of the COCs selected: one which groups all the carcinogenic effects and one which groups non-carcinogenic effects. The TS for carcinogens is computed by summing the (maximum concentration of each COC * its slope factor). This reflects the fact that any level of exposure to carcinogenic compounds have the potential to cause negative impacts, since there is no threshold for cancer. The TS for non-carcinogens is computed similarly, by summing the (maximum concentration of each COC / its RfD). For non-carcinogens, it is assumed that there is a threshold level of exposure below which negligible harmful effects will occur.

3.2 Exposure Assessment

The purpose of the exposure assessment stage is to determine the dose of the chemicals of concern to which humans and the environment (biota) may be exposed. Steps in this phase of the QRA are as follows:

1. Identify the sources and locations of contaminants at the site.
2. Identify how these chemicals migrate to receptors, via both current and potential routes.
 - Be sure to note sensitive populations (for example, children at an on-site day care)
 - Also note the potential for both short and long-term exposures.
 - In addition, this stage should be inclusive, with ALL potential receptors identified.
3. Develop exposure scenarios for all receptors.

These exposure scenarios should clearly explain all of the following elements: the specific source of the contaminants from the site, the chemical release mechanism from the source, the route of transfer of the chemical through the environment to reach the receptor, the exposure point at which the receptor encounters the toxic

chemicals, specifically who the receptors are (number, age range), and the exposure route of the receptors (whether via inhalation, ingestion, or dermal contact with the chemicals).

4. Estimate the concentration of the contaminants at exposure point for all pathways.

This tends to be the most difficult element of the exposure assessment. In order to obtain a reason estimate of chemical concentrations, the engineer should make use of monitoring data from the site and various mathematical models to predict chemical movement (such as an air dispersion model and groundwater models). This step should also include a sensitivity analysis to incorporate uncertainties (such as determine how sensitive the final estimated dose is to wind speed and direction; may determine a probability range of exposure concentrations).

5. Calculate the receptor dose (i.e. quantity of chemical ingested, inhaled, and adsorbed through skin)

There are a variety of "intake" equations that can be used to calculate the receptor dose. One general example is:

Intake = (conc of chemical * contact rate * exposure frequency * exposure duration) / (body weight * ave time).

3.3 Toxicity Assessment

The toxicity assessment defines the dose : response relationship for each surrogate chemical. Frequently, there is not good data, with a lack of slope factors for probable/possible carcinogens and even less data on non-carcinogenic effects. Even when these values are available, there is a LARGE UNCERTAINTY in the numbers, due largely to the lack of human data and appropriate concentration or dose levels. To make up for the lack of data, a number of safety factors are applied to that data which IS available. For example: (1) sensitive sub-populations (ave man -> sensitive man) 10x, (2) chronic animal studies to human effects 10x, (3) "less than chronic" studies to chronic effects 10x, (4) LOAEL to NOAEL 1 to 10x.

The toxicity assessment information can be gathered from the data-bases discussed earlier to derive slope values for carcinogens (IRIS) and reference doses (IRIS) or minimal risk levels (ATSDR) for non-carcinogenic effects.

3.4 Risk Characterization

calculate quantifiable estimate of risks to receptors under all exposure scenarios

carcinogens risk = chronic daily intake * slope factor

non-carcinogens risk = Hazard Index = chronic daily intake / RfD

add values for all carcinogens and non-carinogens, if < 1 then O.K.

* better to sum on organ-specific basis (of compound impact)

* may be interactive effects between diff. compounds, multiply?

Overall Contributions to Uncertainty include:

extrapolation models to get from high dose studies to low dose situations

total dose versus dose rate

average person vs. sensitive person

human sensitivity vs. animal sensitivity

synergism or antagonisms with other carcinogens or promoters

potential exposed population vs. actual exposed population

absorptive rate of internal organs at high animal doses vs. low human doses

dose scaling (body weight, concentration in water or food, surface area)

detection limits vs actual concentrations

additivity vs non-additivity of multiple action sites

intermittent vs continual exposure

4. CASE STUDIES OF THE EFFECTS OF HAZARDOUS WASTE SITES

1. Strong epidemiological evidence

One Superfund site at which there is strong field-based evidence of human health effects from exposure to toxic chemicals at a hazardous waste site was in Hardeman County, Tennessee. Here it was found that wastes leaching from an industrial landfill site had contaminated the groundwater with carbon tetrachloride (CT) and other organics. In the nearby private wells at farms, the drinking water of approximately 100 people contained average CT concentrations of 1.5 mg/L (this far exceeds the EPA maximum contaminant level (MCL) in drinking water of 0.005 mg/L). In the exposed population, transitory liver function anomalies were observed. On the basis of human and animal studies, CT is known to be a hepatotoxin. Therefore, observed human health effects corresponded to those the contaminants from the industrial landfill are known to exert; so-called "strong epidemiological evidence".

In addition to these acute effects, there may also be chronic effects to the exposed population, such as long term liver disease.

2. Suggestive epidemiological evidence

Contaminants from an industrial disposal site in Woburn, MA, resulted in the contamination of the Woburn municipal well field with the chlorinated solvents TCE and PCE. In Woburn a so-called “leukemia cluster” also occurred; on the basis of average occurrence of leukemia there should have been 9.1 cases in Woburn but there were actually 20. However, PCE and TCE have not been associated with leukemia. In addition, phone surveys in town found that perinatal death, 2 of 5 congenital anomalies, and 2 of 9 childhood disorders also occurred in greater frequency in Woburn than would normally be expected. However, reconstruction of symptoms and doses of TCE and PCE in the drinking water found a poor dose response. Also noted was a significant blink reflex deficit in 28 persons who had ingested contaminated drinking water in Woburn. In spite of the poor definitive proof that the “cluster” of leukemia and noted birth effects were related to the contaminants rather than merely a “statistical” variance, 14 families that sued WR Grace Co. received an out of court settlement. (More on this case can be found in the book “A Civil Action” by Jonathon Harr, 1993)

3. Ambiguous evidence of human health effects

One of the most famous contaminated sites in the U.S., which spurred passage of the Superfund Law is the Love Canal site. Between 1943 and 1953 Hooker Chemical and Plastics Company disposed more than 20,000 tons of chemical wastes at the site, largely in a canal approximately 20 m wide, 3 m deep, and 1000 m long. Hooker sold the site to the Niagara Falls School Board for \$1 in 1953, and at the time of the sale the Board was aware that chemical disposal of wastes had occurred at the site. In spite of this information, a school located on the site opened in 1955, and soon homes were built near the school. During the mid 1970s contaminated water ponded on the ground surface after heavy rains flooded the canal. In 1977, it was found that 21 of 188 homes near the former canal contained chemical residues (including PCBs) in their basements. After widespread media attention, an “emergency” was declared and state and national funds used to evacuate 237 families from their homes. Clean-up at the site is on-going and expected to cost a total of \$250 to \$300 million. In spite of the public reaction, the site is only ranked number 158 on the NPL. In addition, despite reported health effects by the residents, the ties of these health effects to contaminants present are tenuous.

During a blood survey in 1980, no significant effects of Love Canal exposure were noted; this sample group included 46 people evacuated with 17 that originally declared they were suffering from negative effects. A 1980 survey for cancers in 4897 residents who lived near Love Canal from 1955 to 1977 found no significantly higher cancer levels compared to other non-New York City residents of New York state, with the exception of respiratory cancers. However, elevated respiratory cancers were true for the Niagara Falls area as a whole, and not significantly higher in near-canal residents.

Of all the reported effects of exposure to toxic chemicals from Love Canal, the most widely publicized were birth effects. Numbers reported include: 20% births with defects (24 of 120) of residents in so-called “Wet areas” compared to 7% (12 of 176) in “dry areas”. However, this information was gathered via a 1978 non-scientific phone survey conducted by untrained volunteers.

In a 1980 questionnaire based on parental recall of doctors diagnoses, malformations were reported in 9.7% and 3.8% of Love Canal births by homeowners and renters, respectively. This compare to “control” responses for those not living near the Love Canal of 5.1% and 3.1% for homeowners and renters, respectively. However, the homeowner controls had significantly less alcohol consumption during pregnancy; the difference in renter values are not statistically significant. In the same 1980 questionnaire, low birth weight reports found no significant differences for “dry” near canal or renter populations compared to control reports; and only slightly higher rates (on a statistical basis) for wet area and homeowners. Finally, the same study found no significant differences in the mean birthweights of Love Canal births versus controls.

In addition to the fairly weak evidence of negative human health effects at Love Canal, there has also been no strong link between the contaminants found at Love Canal and these reported effects.

5. SETTING SITE CLEAN-UP LEVELS ON THE BASIS OF RISK ASSESSMENT

Site specific criteria for “clean” or remediated sites can be established which are protective of human health and the environment. These so-called “alternate concentration limits” (ACL) are set based on risk as an alternative to

traditional “standards” such as using the MCL as the limit for groundwater concentrations on-site. The incorporation of risk assessment when setting standards takes into account dilution and attenuation that may occur between the site and the receptor populations. First, the “action limit” (AL) for the contaminants at the site must be established; the AL is concentration above which a significant risk is posed to human health or the environment.

To calculate the AL in each media (Water, soil, air) for non-carcinogenic effects:

$$AL = RfD * BW * CF / IR ABS$$

where RfD is the reference dose of the compound, BW is the body weight of the receptor, CF is a conversion factor (for unit consistency), I = intake rate of the media (water, air, or soil), ABS = absorption.

For carcinogenic effects:

$$AL = (R BW LT CF) / (SF I ABS ED)$$

where R = the acceptable risk level, LT = lifetime, SF = carcinogen slope factor, ED = exposure duration.

The most common “site” clean-up levels are set for soil. The allowable soil concentrations (ASC), including both dermal and incidental ingestion intake, for non-carcinogens is:

$$ASC \text{ in mg/kg} = (BW * AT * RFD\text{-ingest}) / [CF EF ED \{ (IR FI ABS) + (SA AF ABS SM) \}]$$

where IR = ingestion rate, FI = fraction ingested from contaminated source, ABS = absorption from the soil-ingested or soil-dermal, SA = surface area of skin, AF = adherence of soil to skin, SM = soil matrix effect.

For carcinogens:

$$ASC \text{ in mg/kg} = (BW * AT * RSD) / [CF EF ED \{ (IR FI ABS) + (SA AF ABS SM) \}]$$

where RSD = risk specific dose, RSD = acceptable risk / SF-oral

On the basis of the ASC values, the lowest is selected for a given compound (from carcinogenic and non-carcinogenic values), and then the effect of an aggregate effect from multiple compounds is included to arrive at the recommended soil clean-up level (RSCL):

$$RCSL = \% * ASC$$

where % is the percentage of the contribution of that chemical to the overall cancer risk and/or hazards index.

6. ECOLOGICAL RISK ASSESSMENT

An ecological risk assessment (ERA) is conducted, in theory, using the same steps as human risk assessment. However, due to the complexity of conducting a full-scale risk-assessment for each and every type of organism at risk, and the lack of data to support such an analysis, a modified approach is required. The goal is to estimate both short-term acute effects and chronic toxicological effects on the basis of data derived from studies on the acute effects of toxic chemicals during sensitive life stages. The basic steps of the ERA are:

1. Characterize the baseline ecology, and receptor populations

This includes evaluating the diversity of species currently present in the environment, identifying sensitive populations, and selecting indicator species which will be used to conservatively represent the effects on the entire group of species present.

2. Ecological toxicity assessment -

This toxicity assessment on the selected indicator species can use both qualitative and quantitative endpoints to determine toxic effects, including lethality, reproductive effects, tumor development, etc. Generally this will include values such as the LC50 or chronic no effects threshold.

3. Evaluate potential exposures -

Look at the exposure pathways for the toxic chemicals to interact in the environment of concern (Water, air, soil), and estimate the exposure point concentrations. This part of the analysis is directly related to the human exposure assessment.

4. Risk characterization

By a comparison of the ecological toxicity assessment information with the exposure point concentrations, an evaluation of the effects on the populations in the ecosystem are derived. EPA typically encourages the use of a ratio method, where the toxicological benchmarks such as the LC50 are compared to the exposure point concentration. A high risk is inferred if these ratios are less than 10, with higher ratios implying a higher risk. Actual acceptable risk levels are set by agreement of concerned parties on a site-by-site basis.

Some standard methods for evaluating ecological effects have been developed and promoted by the American Society for Testing and Materials (ASTM). These methods are used as indicators of environmentally acceptable ecological effects, based on the effects of test mixtures to standard organisms. The limitations of these methods is that they ignore site-specific conditions and species, and therefore interpretation of these results should be used with care. Commonly used tests are:

a) Static Acute Toxicity Tests on Wastewaters with Daphnia - ASTM 1986

This tests looks at the mortality effects on Daphnia, small freshwater aquatic organisms, after living in dilutions of “wastewater”. The test can be easily modified to look at “groundwater” or surface-water type systems.

b) Microtox - a bacterial toxicity test, initially designed to look at changes in the bioluminescence of saltwater bacteria after exposure to toxic chemicals. This method has also been used to determine general toxicity effects, irrespective of a marine environment.

c) tests on snails, minnows, mussels, shrimp, earthworms, frogs

Clearly, the test organisms selected should be at least somewhat representative of the species of interest, such as fish in water systems, mussels in near-shore environments, earthworms in soil, and frogs in surface water bodies. Of all the test organisms, the simple bacteria system in Microtox is perhaps the most widely applicable, looking for genetic effects.

General categories of end points used in studies to determine ecological effects are:

a) ecosystem structure - look at total quantity of species and total biomass; typically species diversity drops due to toxic effects.

b) ecosystem function - analysis of energy flow and nutrient cycling are important to understand the functioning of the ecosystem. For example, primary productivity and nitrogen cycling in the “baseline” compared to “impacted” system.

c) population-level effects within an individual species - look for changes in the number, age structure, or gene make-up of impacted systems compared to “background” levels. This should be done for the selected indicator species.

d) physiologic effects on individual organisms - acute mortality, growth and development, reproductive success; however, lab single-species, single factor tests ignore the interaction of chemicals in the environment

A combination of the 4 above categories of end-points is typically the best.

In 1990, the Water Pollution Control Federation (WPCF) Research Foundation published a report in which they evaluated 18 different protocols for aquatic ecological risk assessment, most of which were developed by Federal Agencies such as the EPA. One example of a protocol reviewed is the EPA Office of Research and Development (ORD) PIRANHA model, which is a computer-based system with a focus on synthetic organic chemicals. Included in this model are a terrestrial transport model, aquatic transport and fate model, and a bioaccumulation model to aid in source characterization and pathways analysis. Then, a GIS-linked EcoRisk database is used to identify likely species of biota and plants that may be impacted. Target-species toxicology is then estimated from a database of laboratory study results. Since the computer model was still in development at the time of the report, the ability of the model to general the final quantitative estimates of risk and uncertainty of that risk was not evaluated. The conclusion of the report was that there is a long way to go to develop accurate, meaningful models for aquatic risk assessment, but that PIRANHA is a step in the right direction.

7. RISK-BASED CORRECTIVE ACTION

Risk-Based Corrective Action (RBCA) standards as suggested by the US EPA are being implemented in numerous states for clean-up at petroleum-contaminated sites. The Nov. 1997 issue of Soil & Groundwater Cleanup reported that 14 states have or are in the process of incorporating RBCA approaches into their regulatory programs. Procedures for RBCA which integrate risk and exposure assessments with remedial activities to best protect human health and the environment have been outlined by the American Society for Testing and Materials (ASTM). The guidelines include an evaluation of both human and ecological effects, and uses a tiered approach to accommodate a variety of site-specific conditions. Movement to higher “tiers” of evaluation is only required if the situation failed the lower tiers. The general steps in the procedure are outlined below and shown in more detail in Figure R:

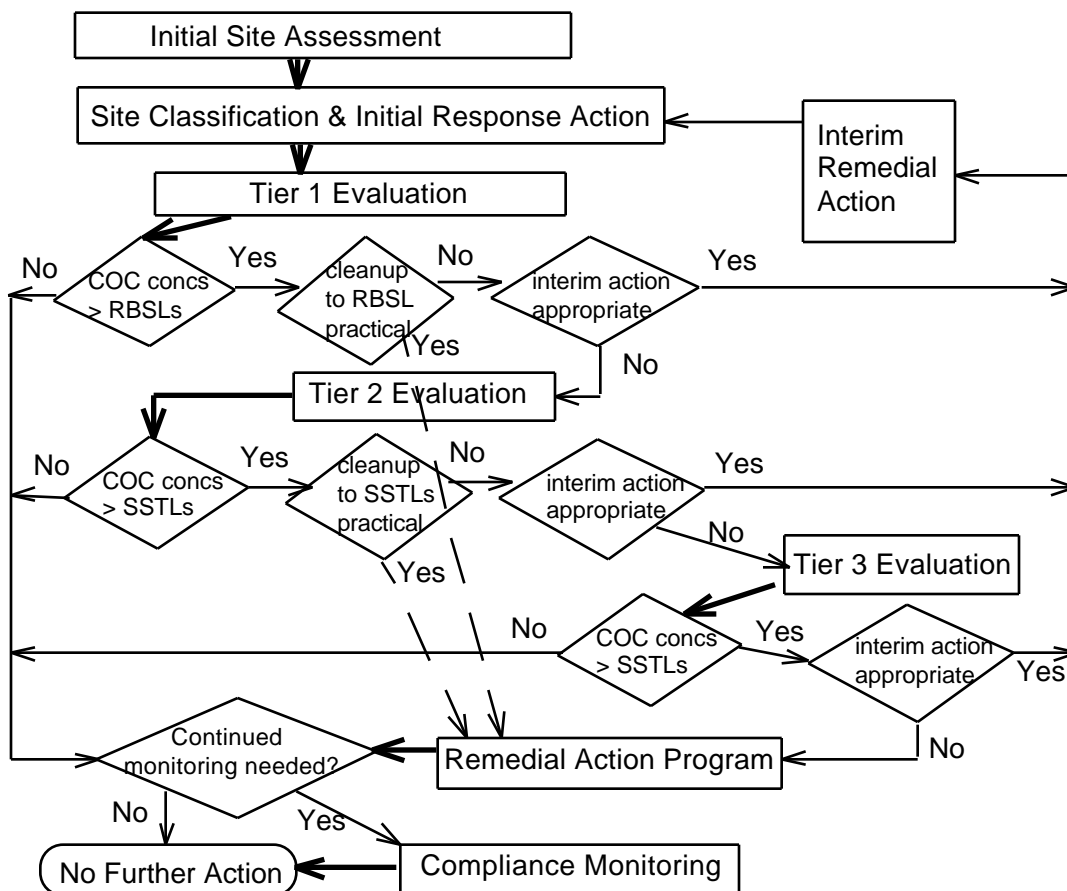
1. Site Assessment
2. Classify site with regard to initial response action, and conduct initial response

3. Tier 1 evaluation - compare compound concentrations at the site with Tier 1 criteria which are non-site-specific (risk based screening levels = RBSLs)
4. Tier 2 - development of site-specific target levels (SSTLs) & points of compliance (POC), which requires more data than Tier 1 and modeling
5. Tier 3 - development of SSTLs and POC, if needed, using more data and modeling
6. Remedial Action to achieve the RBSLs or SSTLs
7. Compliance Monitoring

Each activity is described in more detail below:

1. Site Assessment - gather information necessary for site classification, initial response action, comparison to the risk-based screening levels (RBSLs), and determining the site-specific target levels (SSTLs). This information may be generated from site-specific measurements, or may be estimated from readily available information. The following activities may be included in the site assessment: review of historical records of site activities and past releases, identify chemicals of concern, locate major sources of COCs, locate maximum concentrations of COCs in soil and groundwater, identify points of exposure for receptors and significant exposure pathways, determine current and potential future use of site, determine regional hydrogeological characteristics, and qualitatively evaluate the impacts to environmental receptors.

2. Site classification and Initial Response Action - generally, there is a table of scenarios and initial response actions which can be consulted. Examples of site classification includes: immediate threat to human health, short-term (<2 yrs) threat to human health; longterm threat to human health (>2 yrs); no demonstrable longterm threat. There are guidelines that aid in classifying the site, which relate to explosive vapor concentrations, groundwater contamination and its use, etc. Response actions which correspond to the various classifications include the following (among others): evacuate and ventilate area, start free product recovery, hydraulically control groundwater, remove soils, monitor groundwater, evaluate effect of natural attenuation on plume migration.



3. Compare site conditions with the Tier 1 criteria - Tier 1 generally has a look-up table of RBSLs (see example below). The table has categories corresponding to potential exposure pathways, media (soil, water, air), exposure scenarios (residential, commercial, industrial, or agricultural), and target risk levels for carcinogenic effects or unity hazard index values for non-carcinogens. Each state usually develops its own look-up table, but it is also up to the user to review the assumptions and methodologies used to derive the values in the table to ensure they are valid for the site under consideration. The appropriate RBSLs for the site conditions are compared to concentrations of COCs present at the site. If COC levels exceed any of the RBSLs, proceed to Tier 2 evaluation. Otherwise, determine if further monitoring is needed, but if not there is no further action required at the site.

4. Tier 2 Evaluation - Additional site data may be required. In this phase, indirect exposure scenarios and site specific points of compliance are identified. Then SSTLs for COCs are determined for the source areas and for the points of compliance on the basis of modeled attenuation. A table of SSTL values somewhat similar to the Tier 1 look-up RBSL table may be developed; example shown below. This will include only the media and exposure scenarios of interest, distances to the receptors, and then the SSTLs at the source that result.

5. Tier 3 Evaluation - Additional site data will likely need to be collected. Here, SSTLs for the source area and points of compliance are developed from more sophisticated statistical and contaminant fate analyses. Site specific information is included in both direct and indirect exposure scenarios. Compared to the Tier 1 and 2 evaluations, significantly more data is needed for Tier 3, with much more extensive modeling efforts.

6. Remedial Action - Remedial action designed and implemented, which may include source removal, treatment, and/or containment technologies (for example, soil venting, bioventing, air sparging, pump&treat, or natural attenuation).

Example Tier 1 Table

Exposure Pathway	Receptor Scenario	Target Level	Benzene	Ethylbenzene	Toluene	Xylenes	Naphthalenes	benzo,a, pyrene
outdoor air inhalation, µg/m3	residential	cancer 1E-6 cancer 1 E-4 HQ = 1	0.294 29.4	1040	417	7300	14.6	0.0014 0.14
	commercial	cancer 1E-6 cancer 1 E-4 HQ = 1	0.493 49.3					0.0024 0.235
surficial soil (<3') ingestion/dermal/inhal; mg/kg	residential	cancer 1E-6 cancer 1 E-4 HQ = 1	5.82 582	7830	13300	1.5 E6	977	0.13 13
	commercial	cancer 1E-6 cancer 1 E-4 HQ = 1	10 1000					11500
soil, to protect leaching to GW ingestion, mg/kg	residential	cancer 1E-6 cancer 1 E-4 HQ = 1	0.0172 1.72	575	129	RES	22.9	0.59 RES
	commercial	cancer 1E-6 cancer 1 E-4 HQ = 1	0.0578 5.78					1610
ground water ingestion, mg/L	residential	cancer 1E-6 cancer 1 E-4 HQ = 1	0.0029 0.294	3.65	7.30	73.0	0.146	1.2 E-5 0.0012
	commercial	cancer 1E-6 cancer 1 E-4 HQ = 1	0.0099 0.987					10.2

Example Tier 2 Table

Exposure Pathway	Receptor Scenario	Dist. to Source, ft	SSTLs at Source Sandy Soil, Nat. biodeg, carcinogen risk 1 E-5, HQ = 1				SSTLs at source clay soil, no biodeg; carcinogen risk 1 E-5, HQ = 1			
			Benz	EB	Tol	Xyl	Benz	EB	Tol	Xyl
surficial soil ingestion &	residential	0	22	5100	5400	280	22	5100	5400	280
	commercial	0	120	9600	1.7*	1500	117	9600	1.7*	1500

dermal; mg/kg										
soil, leaching to gw ingestion, mg/kg	residential	100 500	0.32 4.0	88 1200	250 6300	4200 RES	0.20 RES	130 RES	760 RES	RES RES
	commercial	0 100	0.58 1.1	130 250	350 670	6200 1.2*	0.58 0.70	130 380	350 2100	6200 RES
groundwater ingestion, mg/L	residential	100 500	0.05 0.68	6.8 90	14 350	140 >S	0.04 >S	10 >S	43 >S	>S >S
	commercial	0 100	0.10 0.19	10 19	20 38	200 >S	0.10 0.12	10 29	20 120	200 >S

* weight percent; RES - selected risk level not exceeded for pure compound present at any concentration;
>S - selected risk level not exceeded for all possible dissolved levels

7. Monitoring and Site Maintenance - Monitoring is needed to demonstrate the effectiveness of the remedial action and confirm that current conditions persist or improve with time. Some remedial measures, such as caps and hydraulic control, will require maintenance to ensure integrity and continued performance. Upon completion, no further action is required.

8. Remedial Action Closure - When the RBCA RBSLs or SSTLs have been demonstrated to have been achieved at the source areas and points of compliance, as appropriate, and monitoring and site maintenance are no longer required to ensure that conditions persist, then no further action is needed. The exception may be to ensure that institutional controls present remain in place and in good repair.

Underground Storage Tanks (UST) and RBCA:

In the 1980s, to satisfy the need to start corrective action programs quickly, many regulatory agencies decided to utilize cleanup standards for USTs that were developed for other purposes and apply them uniformly to UST release sites. With experience, however, it has become increasingly apparent that applying such standards without consideration of actual or potential human and environmental exposure is an inefficient means to provide adequate protection against the risks associated with UST releases. Similarly, UST regulatory agencies have found that applying identical reporting and review procedures to all corrective actions is inefficient both for them and UST owners. These problems have become increasingly serious as the number of UST release sites has multiplied. As of October 31, 1994, more than 270,000 releases had been reported nationwide. In 1994, 34,000 confirmed releases were newly reported. The upcoming 1998 deadline for upgrading, replacing, or closing UST systems likely will increase that number as owners discover previously unidentified contamination when they are looking at their tank systems to decide whether to upgrade, replace, or close them.

Although the scale of UST releases is daunting, regulators have made tremendous progress over the last six years. All States and territories, as well as a number of local governments, have corrective action programs, and nearly all corrective actions are undertaken by UST owners with State and local oversight. Cleanups have been initiated at more than 209,000 sites (approximately 77% of the reported LUST sites) and completed at more than 107,000 (40%). In spite of this progress, challenges are posed by the more than 163,000 cleanups still underway.

Forty-six States have established State financial assurance funds to help owners satisfy the Federal requirement for evidence of ability to pay the costs of corrective action. These funds serve both to satisfy Federal law and to provide financial assistance to help UST owners pay for corrective actions. These funds together collect more than \$1.3 billion dollars a year, but many are beginning to face solvency issues as reimbursement requests increase. Currently, claims waiting to be paid exceed \$1.3 billion. Unfortunately, when reimbursement is not immediately available, corrective actions tend to slow down.

To help deal with these challenges, EPA provides support for streamlining (simplifying and accelerating) the administrative and field investigation processes; promotes the use of cleanup technologies that offer alternatives to traditional excavation and landfilling (for soils) and pump-and-treat (for groundwater); and assists States in building strong State assurance funds. EPA believes that risk-based corrective action (RBCA) processes are another tool that can facilitate rapid LUST cleanup while still assuring protection of human health and the environment. In November 1992, in its guidance on streamlining of corrective action processes (OSWER Directive No. 9630.13: Streamlined Implementation of UST Corrective Action Requirements), EPA described four situations in which risk factors could be taken into account in corrective action decision-making. The 1997

OSWER Directive 9610.17 states the following:

“Risk-based decision-making is a process UST implementing agencies can use to make determinations about the extent and urgency of corrective action, and about the scope and intensity of their oversight of corrective

action by UST owners. The real value of risk-based decision-making lies in its potential to help UST implementing agencies and UST owners oversee/manage cleanups of UST releases based on relative risks to human health and the environment. In addition, risk-based decision-making can provide a coherent decision-making framework to help keep transaction costs under control. Thus, while risk-based decision-making can be as protective of human health and the environment as other approaches, it offers a scientifically sound and administratively effective way to respond to the pressures for timely action at large numbers of sites and efficient use of both public and private resources. It is important to recognize that risk-based decision-making is not intended to be primarily a money-saving tool, even though its use may save money in many cases. At high-risk sites (which account for only 20 to 30 percent of all sites), risk-based cleanups could cost more than those based on other procedures for establishing cleanup goals.

Risk-based decision-making is a mechanism for identifying necessary and appropriate action throughout the corrective action process. Depending on known or anticipated risks to human health and the environment, appropriate action may include site closure, monitoring and data collection, active or passive remediation, contaminant, or institutional controls. In all cases, the objective is the same, i.e., to ensure that adequate protection of human health and the environment is provided. The availability of options such as allowing contamination to remain in place or using institutional controls to prevent exposure will depend on applicable State and local laws and regulations.”

8. RELEVANT ACRONYM LIST

ABS	absorption into body	LD50	lethal dose for 50% of population
ACL	alternate concentration limit	LUST	leaking underground storage tank
ADI	acceptable daily intake	NOAEL	no observed adverse effect level
AL	action limit	NOEL	no observed effect level
ASC	allowable soil concentration	POC	point of compliance
AT	averaging time	QRA	quantitative risk assessment
BW	body weight	R	carcinogen risk or acceptable risk
CDI	chronic daily intake	RBCA	risk-based corrective action
COC	chemical of concern	RBSL	risk-based screening level
CPF	carcingenic potency factor (= SF)	RSCL	recommended soil clean-up level
ED	exposure duration	RfD	reference dose
EF	exposure frequency	SF	slope factor (= CPF)
ERA	ecological risk assessment	SSTL	site-specific target level
HI	hazard index; for non-carcinogens	TLV	threshold limit value
I	intake of toxic compound	TS	toxicity score
LC50	lethal concentration for 50% of population	UST	underground storage tank

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