October 27, 2003

Lecture 12: Carcinogenicity (Soft Tissue Effects)

I. Mutagenicity, Tumorigenicity, Carcinogenicity
   A. Mutagenicity--change in the genetic material (DNA lesion) in nucleus of cell; usually involves change in bases of DNA or reaction with bases (for ex., alkylation) that could be potentially transmitted to new cells.
      1. DNA lesions are normal and occur quite frequently; however, cell nucleus has repair mechanisms for excising the lesion
         a. Replication (DNA---->DNA)
         b. Transcription (DNA---->RNA)
         c. Translation (DNA---->amino acids/proteins)
      2. If the lesion is not repaired, it can be transmitted to new DNA during cell division
      3. Normal metabolism has been estimated to cause 100,000 and 10,000 lesions per cell per day in rats and humans, respectively (Ames and Gold 1993)
   B. Clastogenicity
      1. Chromosomal breakages and fragments
      2. Not necessarily related to gene mutation; more likely due to binding 0g toxicant or metabolite to histone proteins or other proteins associated with DNA; strains caused by binding could cause abnormal breakage of the chromosome.
   C. Tumorigenicity--uncontrolled cellular proliferation leading to formation of a mass of undifferentiated fast growing cells (tumor)
      1. The mechanisms proposed for tumor formation have been observed to be either due to a mutagenic effect or a nonmutagenic effect associated with cell toxicity; a compound causing a mutagenic effect is called a genotoxin, whereas a nongenotoxic compound associated with tumor formation is said to have an epigenetic effect (an epigenotoxin?)
      2. A tumor actually starts as a neoplasm ("new cell growth") (See Figure 1)
         a. An undifferentiated population of cells in an organ, known as stem cells, divides as part of the organ developmental process and replaces cells that are lost during differentiation (cell death is normal);
         b. The stem cells may be the sites most susceptible to damage from chemical exposure; older more differentiated cells may be comparatively unaffected
            1. When a cell has a mutation, it will normally repair it, but in some cases these mistakes persist.
            2. Normally one mistake will not affect functioning of the cell, but during the next generation of cells (i.e., the ones produced during cell division), other mistakes can accumulate; these mistakes may not affect the normal physiological functions of the cell, but the tissue morphology may begin to change (the neoplasm).
               a. Eventually enough damage accumulates across several cell generations to cause the cells to be transformed and not function as originally programmed.

b. Mutations or other adverse effects in normal development of stem cells lead to problems in the more differentiated intermediate cells; thus, the stem cells can accumulate enough damage (genetic or other physiologic damage) to become transformed to tumor like cells. (See Figure 2)

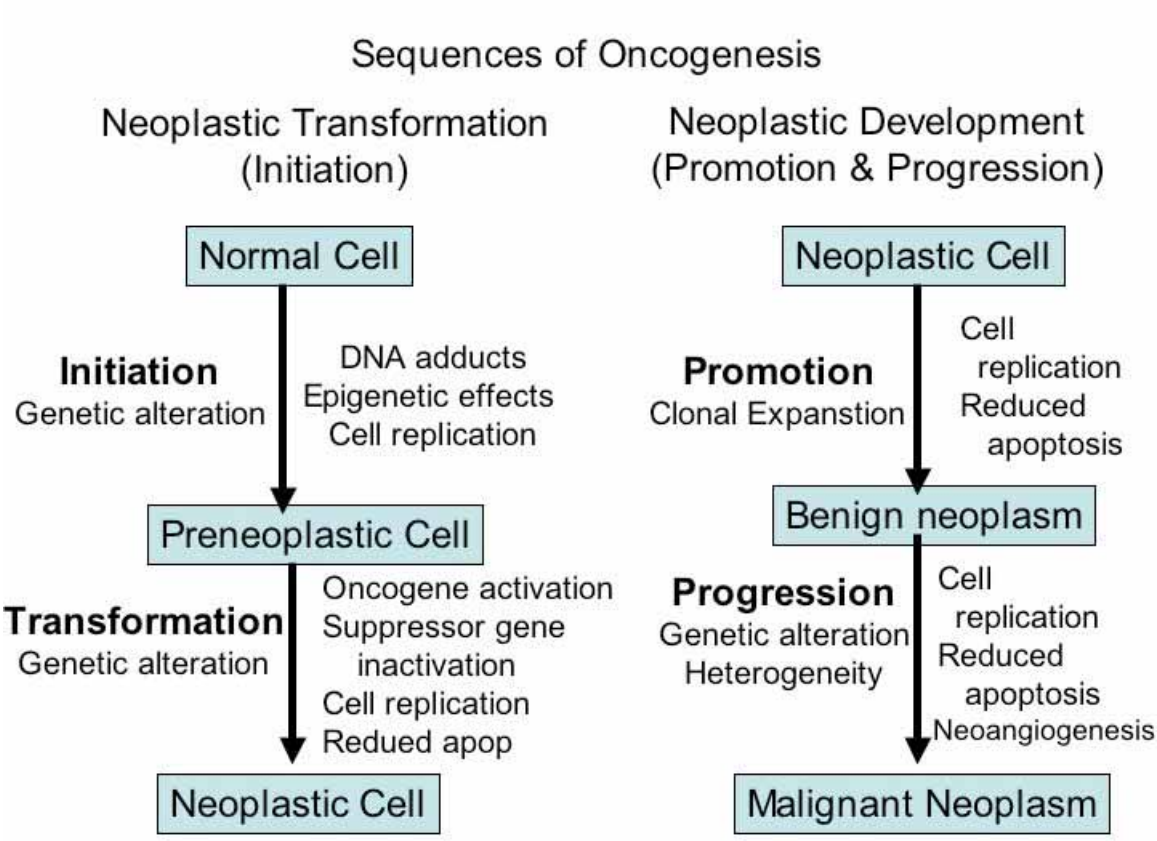

Figure 1. Sequence of oncogenesis (formation of tumor cells/growths). Note the two distinct stages in tumor production: neoplastic transformation (known also as initiation) and neoplastic development (known as promotion and progression). The sequence of processes are functional regardless of whether a compound is directly mutagenic, and thus genotoxic, or it causes "faulty" cells through cellular toxicity or other affects on cell cycling and control (for example, inhibition of apoptosis). Notes that neoangiogenesis is the recruitment of new blood vessels into a tumor (necessary for supplying nutrition to the rapidly dividing cells).

3. High doses probably lead to cell death and chronic cell division in an attempt to replace dead cells; in other words, high doses can overwhelm ability to detoxify contaminant and thus it is more available to cause physiological damage; other possibilities are interactions with specific receptors (see discussion below under carcinogen classification)

a. More probability of mutations because of repair mistakes, especially if cells suffering toxicity (mitogenic theory of tumorigenicity)

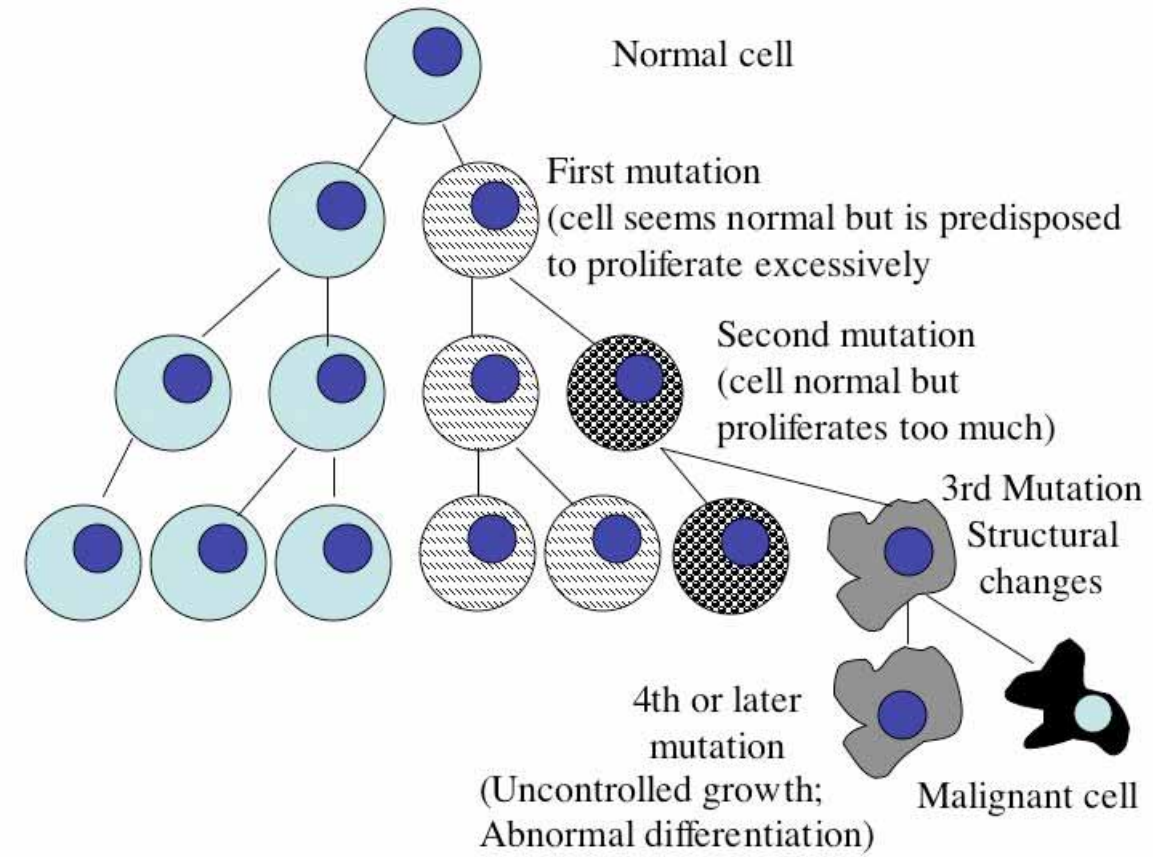

Figure 2. One mutation in a cell, whether due to alkylation by a toxicant mutagen or due to failure to effect repair (as a result of a high dose insult or other form of cellular toxicity) can be passed on to following generations of cells. However, the mutated or affected DNA does not lead to a malignant cancer cell. Several other changes in DNA in subsequent cell generations are required before the cell loses its structure (de-differentiates) and begins to take on the characteristics of a tumor cell.

4. Studies by Cohen and Ellwein (1990) show that exposure of rats to 2-acetyl aminofluorene (2-AAF), a known mutagen, causes increased cell populations of bladder and liver cells that are directly related to duration of exposure (see graph, with exposure at 18, 24, 33 mos.) and dose (see graph in Figure 3 with doses from 45 - 150 ppm)
   a. Thus, even mutagens depend on cell proliferation in the process of tumor formation

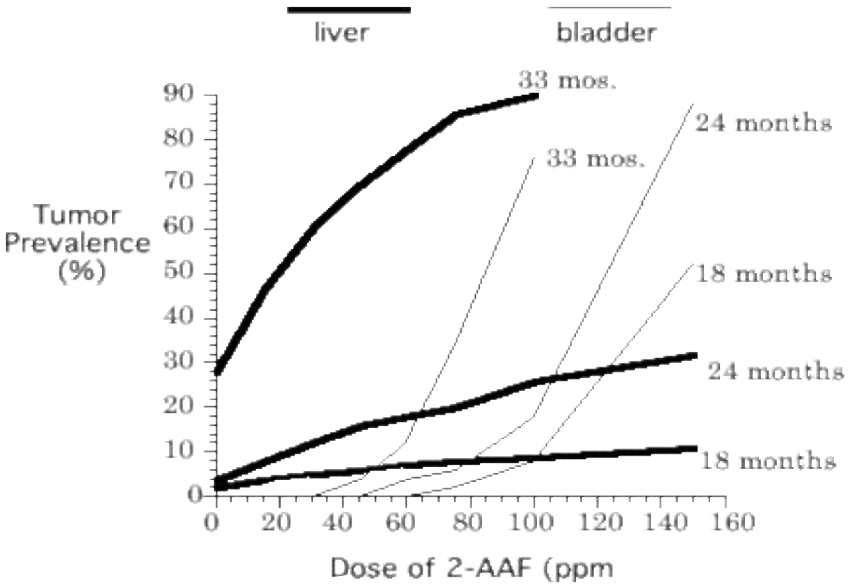

Figure 3. Tumor prevalence in at two organ sites in relation to dose of 2-aminoacetylfluorene. Note that the dose response relationship differs for the two organ sites, suggesting that the mechanism is different (one is genotoxic effect and the other is epigenic).

1. Note that the liver seems to be more sensitive to the effects of 2-AAF when fed to mice for 33 months; however, tumor incidence drops significantly with shorter durations of exposure.
2. On the other hand, the bladder seems more tolerant of doses less than 25 ppm, but it responds quickly to increasing doses and longer durations

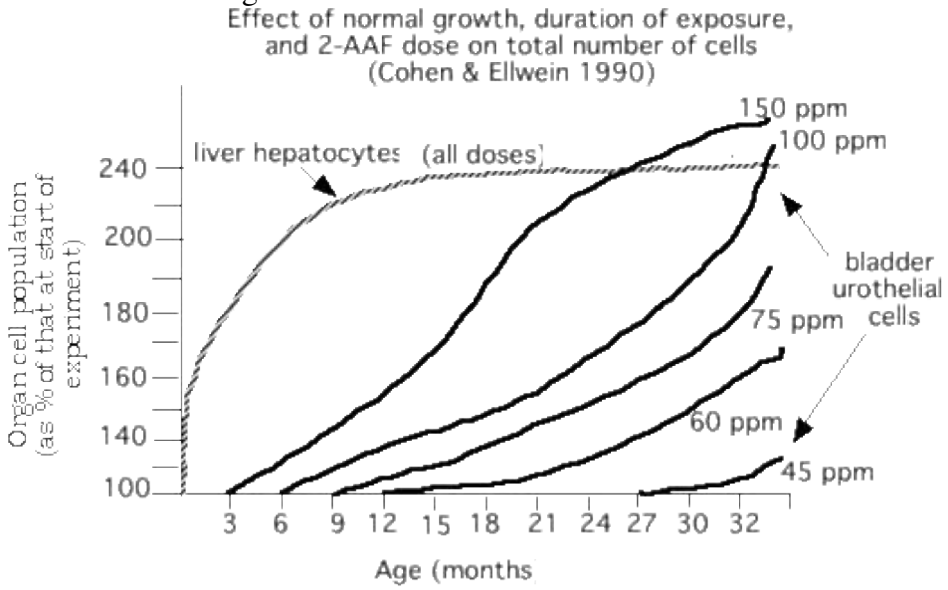

Figure 4. Number of cells (as percent of initial number) for different organs in relationship to age of rodent and dose of 2-AAF.

3. The growth of liver tumor cells parallels the normal growth of the liver, and therefore all doses yield the same growth curve; this observation explains why the liver is sensitive to all doses of 2-AAF (the postulated reason for this observation is explained under carcinogenic mechanisms below)
4. The growth of bladder tumor cells is related to the dose; at the lowest dose (i.e., 45 ppm), even exposure for nearly 3 yrs. produces little change in population of tumor cells; this observation suggests that even for a mutagenic substance, a "practical" threshold for effect may exist.

b. The studies of Cohen and Ellwein indicate that the mechanism of tumorigenicity differ among tissues; they extend their results to the conclusion that carcinogenicity risk assessment must take into account the biological mechanisms of tumor formation

D. Carcinogenicity
1. Whether or not a tumor becomes malignant and therefore considered "cancer" depends on the extent to which the tumor cells can break away from their original mass and invade other tissues; tumors that essentially remain at the site of origin and that do not seem to "damage" surrounding tissue are usually classified as benign; bear in mind, however, that all cancer is really about the process of tumorigenicity.
2. The prevailing wisdom of the EPA is that if a compound causes excess tumor formation (i.e., greater prevalence of tumors in treated animals than in controls) that it should be classified as a carcinogen; the classification scheme of the EPA has two underlying assumptions (Cohen & Ellwein, 1995):
   a. If a chemical causes cancer in rodents, it will cause cancer in humans (interspecies extrapolation)
   b. If a chemical causes cancer at a high dose, it will cause cancer at low doses (dose extrapolation)
3. As mentioned above, Cohen and Ellwein argue for a biologically based classification scheme recognizing that
   a. Genetic alteration is required for cancer to develop (regardless of whether the alteration is caused directly by a mutagenic contaminant or indirectly by a nongenotoxic contaminant);
   b. More than one genetic alteration is required for cancer to develop (known as the multistage model);
   c. DNA replication fidelity is not 100% (in other words, mistakes occur, naturally or as influenced by exogenous mechanisms).
4. Based on the biologically based model, Cohen and Ellwein have proposed a scheme to classify carcinogens; the utility of this scheme is that it would allow low dose extrapolations to man of high dose rodent testing data, and allow a more realistic risk assessment based on known exposures.
   a. **Genotoxic** (DNA is mutated)(Effects likely to persist after dosing ceases)
      1. Theoretically no threshold;
      2. Dose-response may be affected by cell proliferation, but toxicity can be caused at high doses (refer to liver and bladder cell graphs above)

a. In the case of liver cells exposed to 2-AAF, mutation probability in the young stem cells is increased, but not in older intermediate cells (known as foci); thus, during the normal course of cell proliferation in the liver, there is a higher probability of tumor formation with increasing exposures to 2-AAF (in other words, more 2-AAF exposure increases the probability of more mutations)
    1. Interestingly, this mechanism is related to the hydroxylation of 2-AAF, which is highly mutagenic, in the stem cells of the liver. This reaction does not occur in the older more differentiated cells; thus number of tumors in liver is coincident at all doses with the normal proliferative growth of the liver. Liver cells did not proliferate at low and moderate doses of 2-AAF. Thus, the formation of tumors in the liver is caused by the probability of mutations in the stem cells, leading to transformed cells, and then proliferating at the same "normal" rate that the liver grows
b. The bladder cells are exposed to hydroxylated 2-AAF after the 2-AAF has passed through the liver. Another metabolite, a glucuronid conjugate is formed in the liver that also passes to the bladder. This is transformed to N-hydroxy aminofluorene which can mutate any aged cell in the bladder (not just the undifferentiated stem cells). In the bladder, tumors are formed only at doses above 60 ppm owing to a hyperplastic (mitogenic or cell proliferative effect) response. In other words tumors formed only when cell proliferation occurred, and cell proliferation was increased by the presence of doses higher than 60 ppm.

b. **Non-genotoxic** (epigenetic)--effects likely to decline after dosing ceases
    1. Reaction or interference of contaminant with specific cell receptor or growth factor;
        a. Threshold questionable
        b. Usually effective at low doses; however, it depends on the K_m for the binding reaction
    2. Does not react with specific cell receptor
        a. Threshold for effect
        b. Effect strictly related to mitogenesis (i.e., cell toxicity and regeneration)
            1. Contaminant could cause a direct mitogenic stimulus
            2. General cell toxicity and consequent cell division
            3. Interrupt physiological processes

E. The main problem with current testing methods for carcinogenicity (of non-mutagens) is that the shape of the dose-response function at lower, untested doses is unknown. This is why Cohen and Ellwein argue for a biologically based study of carcinogenicity mechanisms for any contaminant.
    1. In the current testing scheme for determining whether a substance is a possible human carcinogen, rodents are fed three doses of toxicant in the diet for a two-year period.

2. The highest dose represents the maximum tolerated dose (MTD), which is the dose causing no more than 10% weight loss in the test animals.
   a. In addition to the MTD, the other doses are typically 1/2 and 1/4 of the MTD, or sometimes the low dose is 10X less than the MTD.
      1. The dose range is normally determined from a subchronic or 90-day dietary exposure study with rodents in which at least three doses are tested.
         a. In the subchronic rodent bioassay,
         b. One of the doses is chosen to be high enough to capture the MTD.
   b. High doses are used to overcome the problem of detecting a positive response of tumors that are typically infrequent in a healthy population. (See Figure 5)
      1. Economics dictate that perhaps 50 animals of each sex are assigned to a dose group.
      2. These animals must than be followed for a period up to 2 years.
         a. After 2 years, or at death (whichever comes first), the animal tissues are prepared for histopathological examination.
         b. Tens of thousands of tissue samples are prepared and analyzed.

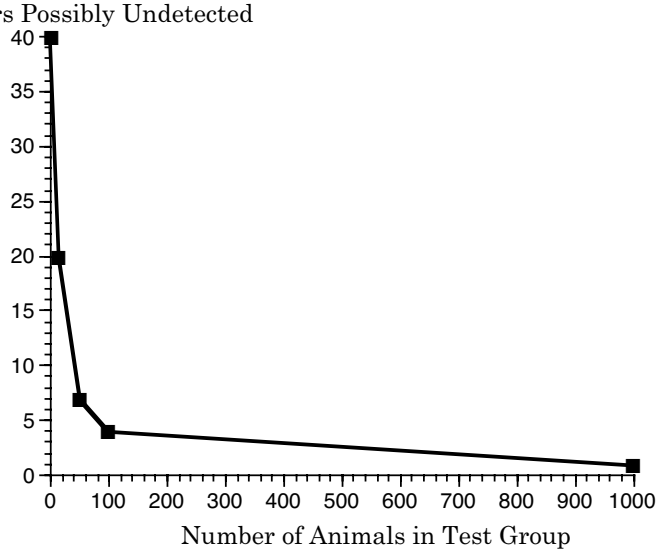

Figure 5. The probability of detecting a tumor, especially a rare one, increases as the number of animals in the test group increases. However, this limitation to the number of optimal animals to test can be overcome by high dose testing, as represented by the use of the Maximum Tolerated Dose.

3. Note that lack of visible signs of toxicity in a rodent, however, should not be assumed to not be causing cellular toxicity or other adverse physiological effects that are not quite manifested as overt symptomology (i.e., you can't ask a rodent how it is feeling today!).

3. The numbers of tumors in different organs or tissues (for example, kidney, bladder, lung, breast, prostate, etc.) are compared to the non-dosed or control group.
4. A model is fit to the data assuming the response is linear down to zero dose; i.e., no threshold exists. (Figure 6)

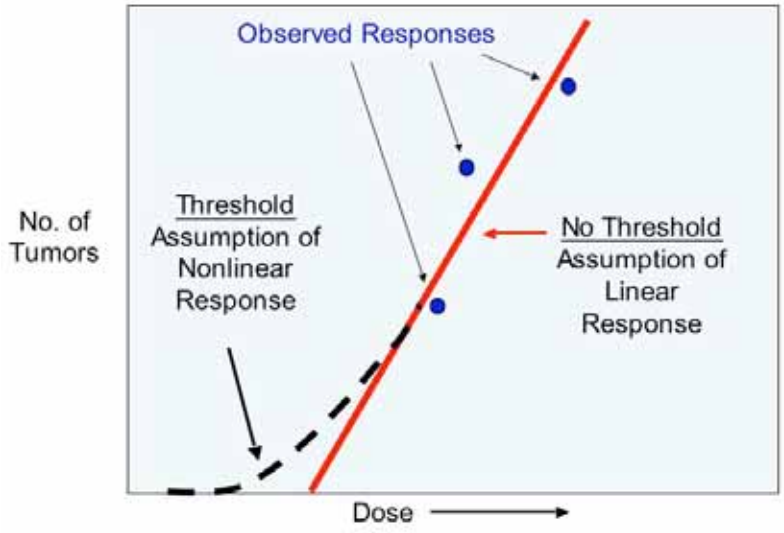

Figure 6. The dilemma of high dose testing. The assumption of a linear model would draw the dose-response curve to the x-axis with no threshold (i.e., any exposure results in observable tumors. However, if there is a threshold (i.e., tumors only develop when a specific dose is exceeded, the dose-response function is curvilinear. The latter model would allow the estimation of a NOAEL (No Observable Adverse Effect Level) and an uncertainty factor to be applied for the estimation of a "safe" dose (Williams 2001).

II. Public Misconceptions about Chemicals and Cancer

A. Ames and Gold (1993) discuss common misconceptions about carcinogenicity, which they list as follows:
   1. Cancer rates are soaring;
      a. Recent articles in the Journal of the National Cancer Institute and data from SEER suggest that incidence rates for some cancers are stable or declining, but some like Non-Hodgkin's lymphoma are inexplicably increasing. (Ries et al. 2000)
      b. Recent research by Weir et al. 2003 indicate the following observations (quoted from the abstract)
         1. "Cancer incidence rates for all cancer sites combined increased from the mid-1970s through 1992 and then decreased from 1992 through 1995. Observed incidence rates for all cancers combined were essentially stable from 1995 through 2000…"
      c. Cancer has been described as a disease of aging. Probability of developing cancer increases as one ages. (Ames 1989) (Figure 7)

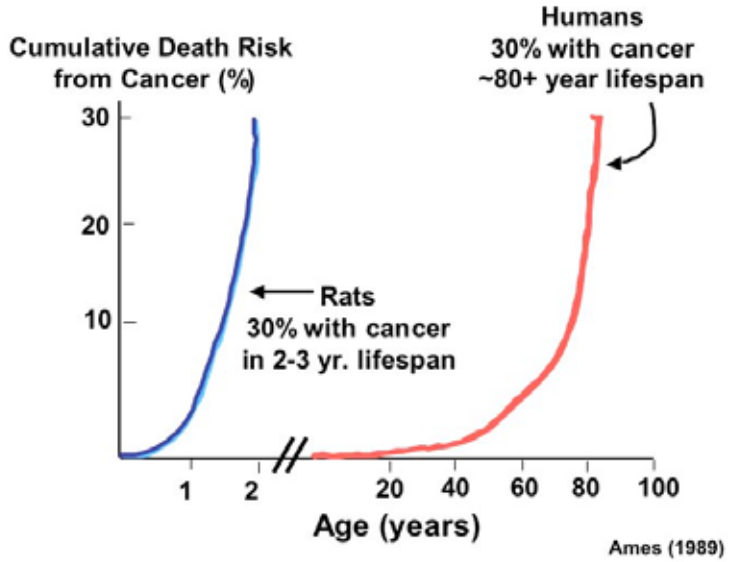

Figure 7. Cumulative probability of developing cancer increases with organism's age. Note that the estimated probability of developing cancer in both rats and humans is the same near the end of the natural life span. These observations suggest that endogenous factors related to aging are the reason for the seemingly high incidence of cancers in our population today. (Ames 1989)

2. Cancer risks to humans at low doses can be assessed by testing chemicals at high doses in rodents;
   a. Ames argues that high doses cause cellular toxicity, leading to cell proliferation of unrepaired DNA damage. (Ames and Gold 1990; Ames et al. 1993)
   b. In a nutshell, Ames believes that high dose testing leads to artifactual tumor production that does not occur at lower, environmentally relevant doses.
3. Most carcinogens and other toxins are synthetic;
   a. In fact, half of all compounds tested for cancer and shown to be positive, are naturally occurring food biochemicals. (Further discussed in Ames and Gold 2000)
4. Synthetic toxins pose greater carcinogenic risks than natural toxins;
5. The toxicology of synthetic chemicals is different from that of natural chemicals.
   a. As a general principle, all chemicals (inorganic, organic, and biochemical) are subject to the laws of thermodynamics and kinetics. In other words the physics of the universe is not different for so-called natural chemicals and anthropogenic chemicals.)

B. To aid in the prioritization of risk management, and to put some perspective on likely risk of human carcinogenesis from exposure to environmental contaminants and natural food biochemicals, Ames and Gold have developed a HERP Index (Human Exposure/Rodent Dose Potency Index)

1. For various chemicals tested in rodent carcinogenicity assays, Ames and Gold have estimated human exposure in terms of mg consumed per day. Mass consumed is then divided by 70 kg (typical toxicological body weight) to yield mg/kg/day of human exposure.
2. The rodent bioassay data is expressed as a TD50 (Tumor Dose 50%), or the effective dose giving an incidence of tumors in 50% of the tested rats.
3. The HERP number is the ratio of human exposure to the rodent TD50, expressed as a percentage. The lower the HERP number, the less the carcinogenic potential.
4. This scheme is useful for determining which exposures should be a priority in controlling (assuming we do not have enough time or money to control everything). (Table 1)

Table 1. The HERP Index: Ranking of carcinogenic hazards of natural compounds and synthetic pesticides based on human exposure and doses causing tumors in 50% of rodents during carcinogenicity bioassays (selected chemicals taken from a more complete list in Ames and Gold 1993; original source is Gold et al. 1992).

Possible Hazard: HERP (%)	Chemical and Form of Human Exposure	Human Dose of Rodent Carcinogen
140	EDB: worker' daily intake (high exposure)	EDB, 150 mg (applicable to exposures pre 1977
16	Phenobaribtial, 1 sleeping pill	Phenobarbital, 60 mg
6.2	Comfrey-pepsin tablets, 9 daily	Comfrey root, 2.7 g
4.7	Wine (250 mL)	Ethyl alcohol, 30 mL
2.8	Beer (12 oz; 354 mL)	Ethyl alcohol, 18 mL
0.3	Lettuce, 1/8 head (125 g)	Caffeic acid, 66.3 mg
0.1	Apple, 1 whole (230 g)	Caffeic acid, 24.4 mg
0.04	Orange juice (6 oz; 177 mL)	d-limonene, 5.49 mg
0.03	Peanut butter (32 g, 1 sandwich)	Aflatoxin, 64 ng
0.005	Coffee, 1 cup (from 4 g)	Furfural, 630 μg
0.002	DDT: daily dietary avg.	DDT, 13.8 μg (before 1972 ban)
0.0006	Well water from Woburn, MA, 1 L contaminated	Trichloroethylene, 267 μg
0.0003	Carbaryl insecticide: daily average	Carbaryl, 2.6 μg (based on 1990 est. daily intake)
0.0002	Toxaphene: daily dietary average	Toxaphene, 595 ng (1990 est. exposure)
0.000001	Lindane: daily dietary avg.	Lindane, 32 ng (1990 est. exposure)
<0.00000001	Chlorothalonil: daily dietary avg.	Chlorothalonil, <6.4 ng (1990 est. exposure)

III. Fish Get Cancer, Too!

A. Despite the de-emphasis on "synthetic" chemicals causing human cancer that has become prevalent in modern biochemical toxicological thinking (note that the emphasis has shifted to neuroendocrine toxicity and possible role of hormonal induced cancer formation), fish do get cancer.
   1. Consider that fish are chronically exposed to environmental concentrations of chemicals
      a. Note that humans are chronically exposed to all kinds of synthetic chemicals also, but our knowledge of the carcinogenic potency of these chemicals is based on high-dose rat testing. Also, our exposure is not continuous and largely comes through our diet.
         1. On the other hand, fish are continually exposed, largely through gill uptake (primarily a partitioning process).

B. During the early 1980's, individual fish in the Great Lakes were noted with tumors, especially on their integument, but tumors or neoplasms were occurring in a variety of tissues.

C. It is known that rainbow trout develop tumors after a 1 ng exposure of embryos to the mycotoxin, aflatoxin (Black and Baumann 1991).
   1. Furthermore, older studies (circa 1941) showed extensive number of brown bullheads had oral tumors (specifically a tumor called papillomas)
      a. Although the link with chemical contaminants in this case is tenuous, it is interesting to note that PCBs were first commercially released in 1929, long before water pollution control laws were effective.

D. Brown bullheads and white suckers appear to be good sentinels for fish neoplasms (Black 1991)
   1. Both fish species are bottom dwellers.

E. Presently, the strongest evidence for a chemical cause of fish tumors are the presence of polyaromatic hydrocarbons (PAHs).
   1. PAHs are both products of incomplete combustion, as well as naturally present in petroleum oils. (Figure

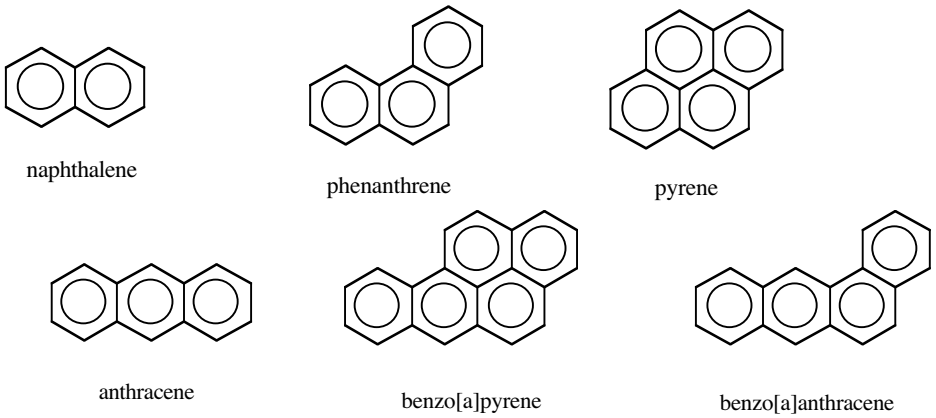

Figure 8. Structures of PAHs. Note that naphthalene is rapidly biodegradable but the larger structures (3 rings and greater) are very recalcitrant compounds.

2. They are highly mutagenic compounds once oxidized by microsomal oxidase P450 cytochrome. (Figure 9)
   a. Presumably, fish are also capable of oxidizing PAHs to mutagenic forms.

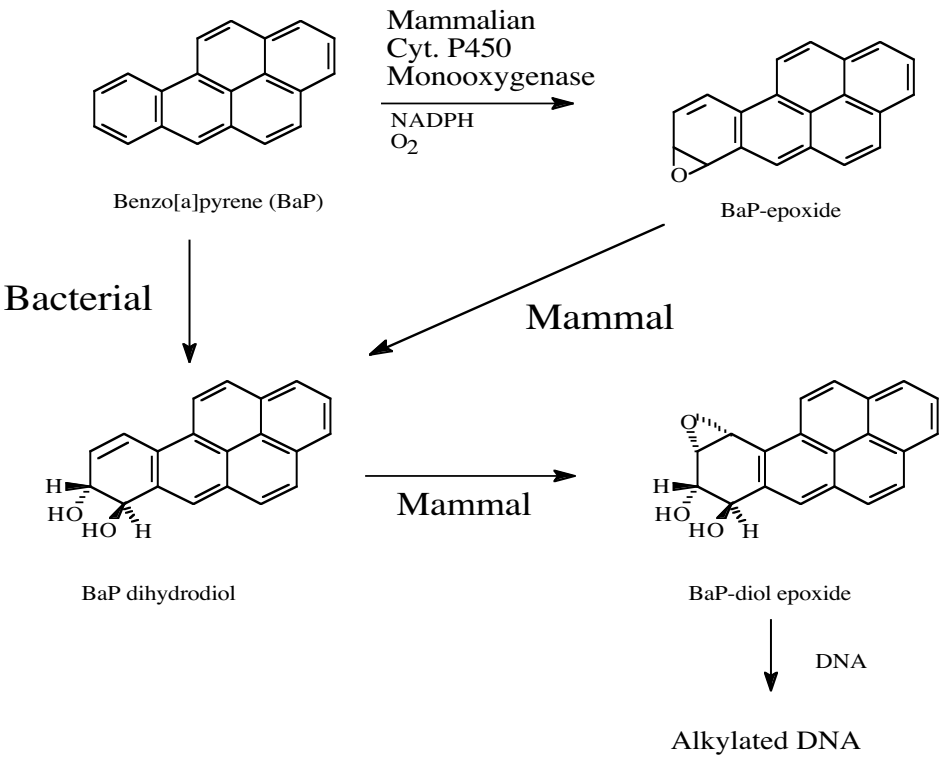

Figure 9. PAHs are oxidized to expoxides that can alkylate DNA, making this group of "naturally" occurring contaminants highly mutagenic.

F. Neoplasms have also been noted in mollusks (Fraley et al. 1991)
   1. "Seasonal and geographic studies of transmissible sarcoma in Maryland softshell clams, *Mya arenaria*, were carried out from 1984-1988. Three major epizootics occurred in the sampling locations, resulting in prevalences as high as 90%, with comparable mortalities in other high prevalence areas. The disease invaded populations of large adult clams first, later spreading to the small juvenile clam populations."
      a. "An apparent 2-year cycle was noted with varying seasonal effects. Affected sites tended to be in the main stem of Chesapeake Bay north of Tangier Sound, primarily in the areas where the major harvesting occurs. Several sites, mostly in upstream locations, were consistently free of disease. The epizootiological study supports the interpretation that the disease is infectious exclusively to this species."
      b. "Regression analysis between sarcoma prevalence and contaminant levels in clam tissues showed a significant correlation between chlordane levels and this disease. No correlations were found with other contaminants that were analyzed."

G. Note that PAHs have been extracted from fish tissue and re-introduced to bred fishes with the resulting induction of new tumors.
   1. This kind of evidence supports strongly the potential of PAHs to induce tuors and is much stronger evidence than the ecoepidemiological evidence cited above in the case of the clam sarcomas.

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