U.S. FOOD & DRUG ADMINISTRATION

FDA Genetic Toxicology Workshop

How many doses of an Ames-Positive/Mutagenic (DNA Reactive) Drug can be safely administered to Healthy Subjects?

November 4, 2019

Enrollment of Healthy Subjects into First-In-Human phase 1 clinical trials

- Healthy subjects are commonly enrolled into First-In-Human (FIH) phase 1 clinical trials of new drug candidates.
  - Studies are typically short (few days up to 2 weeks)
  - Treatment may be continuous or intermittent (e.g., washout period of 5 half-lives between doses)
  - Receive no benefits and potentially exposed to significant health risks
  - Patients will be enrolled in longer phase 2 and 3 trials

- Advantages of conducting trials with healthy subjects include:
  - investigation of pharmacokinetics (PK)/bioavailability in the absence of other potentially confounding drugs
  - data not confounded by disease
  - Identification of maximum tolerated dose
  - reduction in patient exposure to ineffective drugs or doses
  - rapid subject accrual into a study

Supporting Nonclinical Pharmacology and Toxicology Studies

- The supporting nonclinical data package for a new IND includes
  - pharmacology studies (in vitro and in vivo)
  - safety pharmacology studies (hERG, ECG, cardiovascular, and respiratory)
  - secondary pharmacology studies
  - TK/ADME studies (in vitro and in vivo)
  - 14- to 28-day toxicology studies in a rodent and non-rodent
  - standard battery of genetic toxicity studies (Ames bacterial reverse mutation assay, in vitro mammalian cell assay, and an in vivo micronucleus assay)

    - Toxicology studies are used to
      - select clinical doses that are adequately supported by the data
      - assist with clinical monitoring
    - Genetic toxicity studies are used for hazard identification
      - Cancer drugs are often presumed to be genotoxic and genetic toxicity studies are generally not required for clinical trials in cancer patients.

Positive Genetic Toxicity Studies

- Generally, most drugs found to be positive for mutagenicity (i.e., Ames-positive) outside of oncology indications are not developed
  - The ICH S2 (R1) Guidance provides follow-up studies for a positive *in vitro* mammalian cell chromosomal aberration assay
  - However, Ames positive test results are thought to indicate DNA reactivity and extensive follow-up testing to assess the in vivo mutagenic and carcinogenic potential would be warranted to assess the potential risk for treatment, unless justified by appropriate risk-benefit analysis

- In the U.S., a drug with positive *in vitro* Ames bacterial mutagenicity test may still be administered to healthy subjects enrolled in a single-dose study
  - trial participants must be made aware of the study results in the Informed Consent.

- Pharmacokinetic studies typically require at least 2 to 4 doses (e.g., cross-over study)
  - Risks with a small number of repeat doses?
    - Intermittent (washout period between doses) or continuous dosing

How Many Doses of a DNA Reactive Drug can be Safely Administered to Healthy Subjects? (continued)

- Does this safety concern of an Ames-positive drug only apply to chronic administration or does it also extend to a small number of doses
  - e.g., 1, 2, 3, or 4 doses [a washout period of 5 half-lives might separate each dose]?
  - the worst case might be 14 daily doses?

- Lack of published scientific literature or guidance documents directed toward the cancer risk or other potential health concerns associated with a small number of doses of a Ames-positive (DNA-reactive) drug in Healthy Subjects.

- Results of rodent carcinogenicity studies with a new drug candidate are typically not available until late in development or with a marketing application.
  - Primarily rely on the results of the standard battery of genetic toxicity studies during IND development.

How Many Doses of a DNA Reactive Drug can be Safely Administered to Healthy Subjects? (continued)

- Several Review Divisions allow a single dose of an Ames-positive drug in healthy subjects; however, others do not allow any dosing and yet some others allow more than 1 dose.

- Several CDER Review Divisions have raised questions regarding the number of doses of an Ames-positive drug that can be safely administered to healthy subjects.

- CDER is seeking advice from a Panel of Experts.

- **QUESTIONS**:
  1. How many doses of an Ames-positive drug (DNA reactive drug) can be safely administered to Healthy Subjects?
     a. 1, 2, 3, or 4 doses

How do other Regulatory Agencies deal with the use of DNA reactive drugs in Healthy Subjects?

Health Canada:

- A clinical trial in Healthy Subjects with an Ames-positive drug would not be allowed to proceed without substantial follow-up testing to demonstrate that the drug is not mutagenic *in vivo*.

Japan:

- Do not allow administration of a clearly Ames positive drug to healthy subjects in FIH trial.
- However, according to the ICH M3 (R2) Guidance, it is permissible in Approach 1 and 2 of microdose studies. Five separate administrations of a drug at a dose of 100 µg/day could be possible, if the drug has no structural alerts. There is less concern for a weak Ames-positive that does not possess structural alerts when considering mutagenicity.

Combined response from BfArM and MHRA:

BfArM (Germany)**:** Has not yet dealt with issues with applications for FIH with Ames positive drug candidates outside of "microdosing" scenarios described ICH M3(R2).[7]

Other Regulatory Agencies (continued)

MHRA (UK):

- MHRA would consider a scientific justification as to why a sponsor thought it was acceptable to dose a healthy volunteer with a product that was genotoxic, e.g. positive in the Ames test.

- The sponsor would need to justify why additional studies were not conducted to further evaluate the genotoxic potential of the product and associated clinical relevance (examples where this is possible are given in ICH S2(R1).

- A single dose FIH with a positive Ames may be acceptable, providing adequate justification was provided (e.g. based on TTC/half-life/proposed clinical dose/concentration and strain(s) in which positive result was determined etc.).

- Dosing out to a week would likely not be acceptable without an extensive justification/scientific rationale and without any additional genotoxic data, particularly in healthy subjects.

Presentations

1. **Introduction: How many doses of an Ames-Positive/Mutagenic (DNA Reactive) Drug can be safely administered to Healthy Subjects?** (Dr. Timothy W. Robison)
   a. How do other Regulatory Authorities handle Ames-positive drugs in clinical trials with healthy subjects (US FDA, Health Canada, EMA, Japan)

2. **FDA Requirements for the Protection of Healthy Subjects in Phase 1 Clinical Trials** (Dr. Kevin Prohaska)

3. **Considerations for a Genotoxic API in Clinical Trials: Healthy Subjects or Patients?**(Dr. Bob Dorsam)

Presentations (continued)

4. **4. Literature review for data relevant to administering one or a few doses of a DNA reactive drug to healthy subjects** (short-term exposures to genotoxic/carcinogenic agents and subsequent development of cancer) (Drs. Dayton Petibone and Jennifer Shemansky)

a) An Ames positive test usually results in a carcinogenic effect observed in the 2-year rodent bioassay. Using a database of 709 carcinogens, Cheeseman et al. (1999) found that 45% of carcinogens that tested positive in the Ames test were likely to be potent carcinogens. They also found that mutagenic carcinogens were three times more likely to be potent carcinogens than non-mutagenic carcinogens.

b) By modeling data for both chronic lifetime and stop exposure studies to evaluate cancer risk, Halmes et al. (2000) found that animals exposed to some carcinogens using a stop-exposure experimental approach could potentially have a higher tumor incidence compared to animals which were continuously exposed.

c) A database was compiled with tumor incidences following a single exposure to a suspected agent to estimate risk from less than lifetime exposures, including risk limited to a single dose (Calabrese and Blain 1999).

Presentations (continued)

5. Do the Steps between Genotoxin and Cancer Create Thresholds of Dose or Time?
(Dr. Douglas Brash)

6. Setting Allowable Exposures to Ames-positive Candidate Drugs
(Dr. KS Crump)

a) We will assume that the Ames test is all the toxicological information available upon which to form an answer to this question. We note that the question is vague: How large an increase in cancer risk is deemed "significant"? In other contexts, an answer to this question could perhaps be based on results of a two-year bioassay in which rodents were exposed continuously throughout the two years to various doses of the substance. Even if the results of such a study were available the answer to this question would still be very uncertain owning to several issues, including the uncertainty in applying results obtained in animals quantitatively to humans, the uncertainty in estimating risk from low doses based on experimental results obtained at much higher doses (the low dose extrapolation issue) and the uncertainty in making quantitative estimates of risk from exposures of short duration based upon data collected from much longer dosings. But here we are faced with providing an answer to this question with far less information – results from a short-term bioassay involving bacteria.

Panel Discussion

1. Dr. Alan Boobis
   - Imperial College London

2. Dr. Douglas Brash
   - Yale University

3. Dr. Kenny Crump
   - Louisiana Tech University

4. Dr. Bob Heflich
   - NCTR

5. Dr. Timothy McGovern
   - FDA

6. Dr. Miriam Poirier
   - NCI

7 . Dr. Kevin Prohaska
   - FDA

8. Dr. Errol Zeiger
   - Private Consultant/Formerly from NTP

DNA-Reactive Drugs

- For the today's discussion of DNA reactive drugs, we have chosen to principally focus on Ames-positive drugs as there is a high correlation between chemicals found to be positive in the Ames *in vitro* bacterial reverse mutation assay and positive tumorigenicity findings in the 2-year rodent carcinogenicity bioassay.

- For today's discussion, it should be assumed that the drug has the potential to reactive with DNA (e.g., adduct, strand breaks, intercalation).

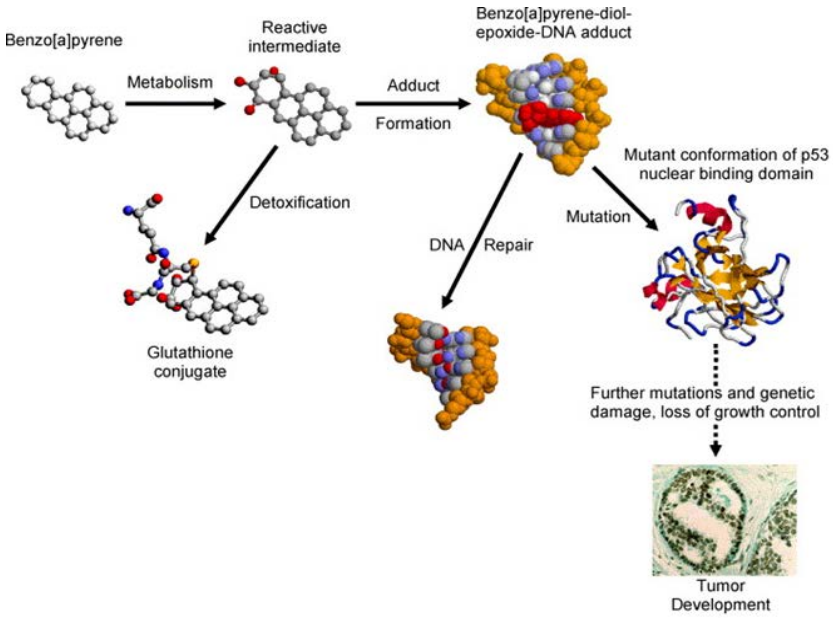
- We note that many genotoxic carcinogens can be positive for both mutation and clastogenicity.

Dr. Dayton Pettibone will provide supportive data in his presentation.

How Mutations may Cause Cancer

DNA damage is an important first step in this carcinogenic process. Chemical carcinogens can cause the formation of

- carcinogen-DNA adducts
- induce other modifications to DNA, such as oxidative damage and alterations to DNA ultrastructure
  - DNA-strand crosslinking
  - DNA-strand breakage
  - Chromosomal rearrangements & deletions



Cells possess mechanisms to repair many types of DNA damage; however, these are not always completely effective.

Mutations:

- Majority of mutations may be largely neutral (e.g., passenger mutations).
- However, mutations in an oncogene, tumor-suppressor gene, or gene that controls the cell cycle can result in a clonal cell population with a proliferative or survival advantage.
  - These mutations are known as "drivers". Driver genes are defined as genes containing driver mutations.

Mutations and Cancer

- Oncogenes are defined as driver genes in which driver mutations are activating or result in new functions. Tumor suppressors are driver genes in which driver mutations are inactivating
    - Oncogenes tend to be affected by focal amplifications or missense mutations at a limited number of codons
    - Tumor suppressors tend to be affected by focal deletions or nonsense, frameshift, and splice-site mutations dispersed across the gene
- The specific type of activating or inactivating mutation can be specific to a driver gene
    - Gene rearrangements almost exclusively activate the *MYC* oncogene in non-Hodgkin lymphoma
    - The V600E base mutation is often the cause of *BRAF* oncogene activation

Mutation and Cancer

- There are always some pre-existing mutations, some of which amplify in the tissue because they are driver mutations
- Mutagens induce additional mutations randomly so that Passenger mutations generally outnumber driver mutations
- Some a driver mutations occur in DNA repair or replication of genes and induce a "mutator phenotype" which results in additional driver and passenger mutations with each cell division.
- Transformation from “normal” to “tumor” usually requires accumulation of 5-8 driver mutations in the same cell, but
  - If most have accumulated already, an “unlucky” mutation induced by a single exposure has a small but measurable chance of initiating the tumor.
  - “Single-mutation” cancers are known: A single rearrangement to form the “Philadelphia chromosome” is probably all that is needed to cause chronic myeloid leukemia.

Mutations and Cancer

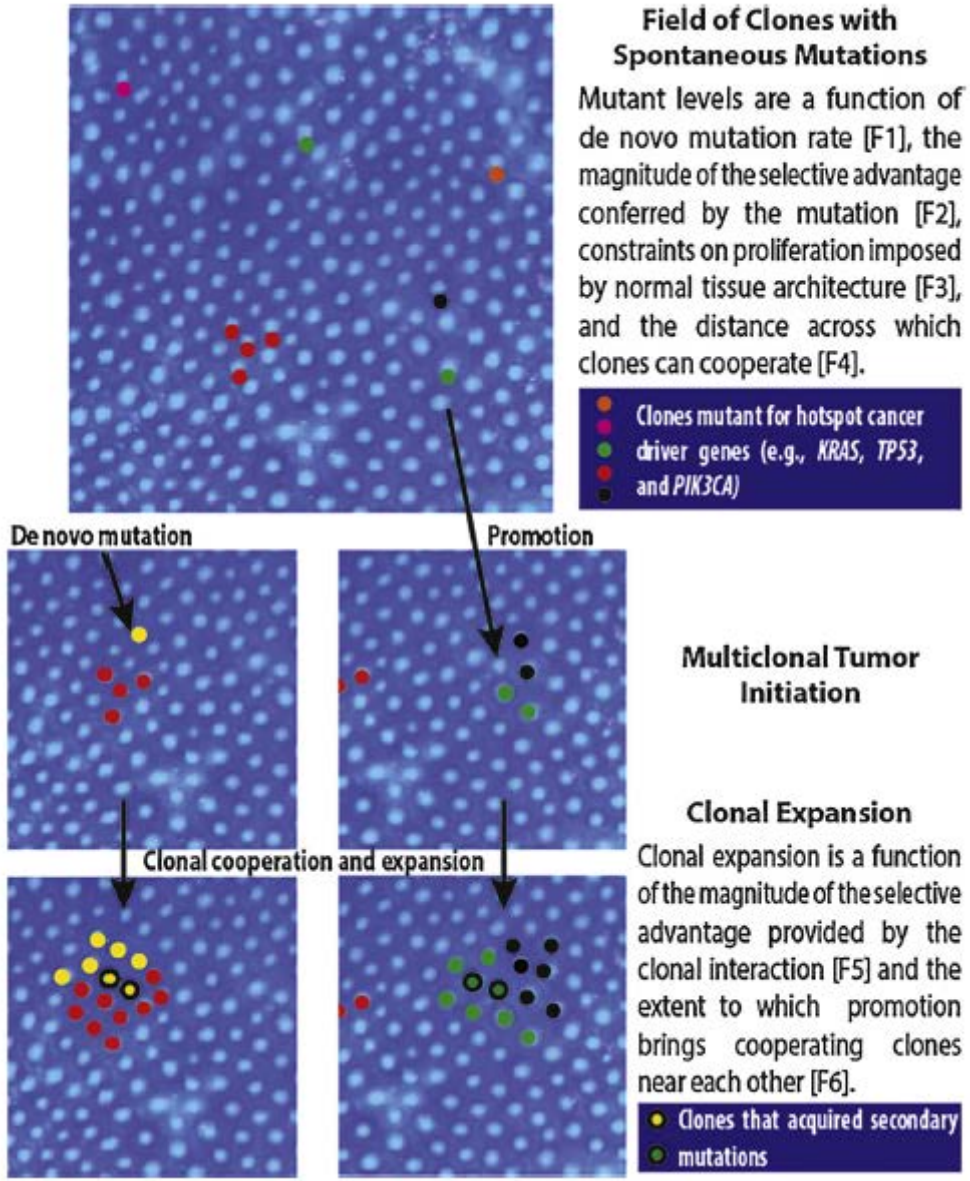
Samples from 11 adult cancer types that had on average 200 somatic point or small insertion/deletion (indel) mutations in exons had only 2 to 6 mutations predicted to be drivers.

At the level of driver genes, common solid tumors had an average of 33 to 66 genes with protein-altering somatic mutations, but only 3 to 6 mutated genes per sample were predicted to be drivers.

- The gold standard of evidence that a mutation is a driver is that
  - the mutation produces a cellular phenotype that contributes a selective advantage to the cells harboring it.
  - Such phenotypes may be related directly or indirectly to survival and proliferation.

- In clonal expansion, mutant daughter cells each create a pair of mutant daughters, exponentially increasing the prevalence of mutant cells .
  - Only one of this clone's cells needs to acquire the next driver.
  - Numerically, this is key to making multiple-genetic-hit cancers.

Mutation, Multiclonal Tumor Initiation, and Clonal Expansion



BL Parsons, Mutation Research-Reviews in Mutation Research 777 (2018): 1-18

Single Exposure to a Carcinogen Can Cause Cancer

- Published studies (thru 1999) have reported that a single dose of 426 agents led to the development of tumors in males and females of numerous animal models in all principal age groups.
  - Diverse range of agents

TABLE 2
Chemical Classes in the Single Exposure Carcinogen Database

Chemical class(es)	# of positive chemicals per chemical class
PAH	67
Inorganic	49
Nitrosamine	35
Ether	17
Amide, fibers/minerals	16
Polymer	15
Halocarbon, phenol	14
Aromatic amine, azo compound, heterocyclic compound	13
Alcohol, carboxylic acid	10
Hydrazine, nitrosourea, triazene	7
Nitro compounds	6
Aldehyde, anthracycline antitumor antibiotic, carbamate, ester, ketone, steroid	5
Alkaloid, epoxide	4
Amine, azoxy compound, mycotoxin, radionuclide, sulfate ester	3
Glutamic acid pyrrolysate, sulfide, sulfonate	2
Coumarin, cyclic sultone, imide, lactone, nitrile, organometal, PBB, pyrrolizidine alkaloid, sulfonic acid, thiol	1
Miscellaneous	18

Calabrese and Blain, Toxicological Sciences 50: 169-185, 1999.

Intermittent Exposure (e.g., 5 half-lives separating each dose)

- In a pharmacokinetic study with a drug administered to healthy subjects, doses are typically separated by a washout period (e.g., up to 5 half-lives allowing >95% of the drug to be cleared).

- Intermittent exposures may have different risks compared to short term exposures.

- Considering the total number of days of treatment, if there are intermittent exposures where there is a washout period (e.g., 5 half-lives) between treatment
  - greater probability that either the pharmacokinetics (i.e., not reaching or maintaining steady state)
  - or mode of action (i.e., recovery time)

  → could decrease the potential risk of a potential adverse health outcome.

- For the same daily exposure at the same dose rate
  - 5 mg/kg/day continuous daily exposure for 10% of a lifetime vs. 5 mg/kg/day intermittent exposure once every 10 days for a lifetime
  - more likely that the intermittent exposure will be associated with a lower risk (Toxicological Sciences 58: 32-42, 2000).

Intermittent Exposure (continued)

- The continuous exposure could saturate DNA repair capacity or other physiological processes,

- Whereas the intermittent exposure would allow time for DNA repair and other adaptive or inducible physiological processes.

- Therefore, one approach for intermittent exposures is to consider the potential for repeated exposures during a lifetime, and to then “combine” these into the equivalent short-term scenario, and finally apply the same approach as one would for that short-term exposure.

Use of genotoxic drug in healthy Subjects - extrapolation from experience with genotoxic impurities [ICH M7 (R1)]

- Use of drugs that are genotoxic, based upon the standard battery of genetic toxicity tests, in healthy subject
  - All risk and no benefit

- Minimize risk to healthy volunteers:
  - A virtually safe dose (VSD) of a genotoxic carcinogen has generally been defined as the dose which after lifelong exposure will result in one additional cancer case in a population of one million

- Linear extrapolation of a VSD to a 1 or 10-day exposure results in daily dose levels of 25,000VSD or 2500VSD, respectively, at which exposures the lifetime cancer risk is likewise considered as acceptable.

- If sensitive subpopulations can be identified an additional dose-rate correction factor of 10 is applied resulting in 10-fold lower daily doses. Up to these dose levels the additional lifetime cancer risk is considered to be negligible since they are set for susceptible sub- populations.

*70-year lifespan = 25,000 day

Exposure to a DNA-Reactive Drug for 1-day or up to 10 days

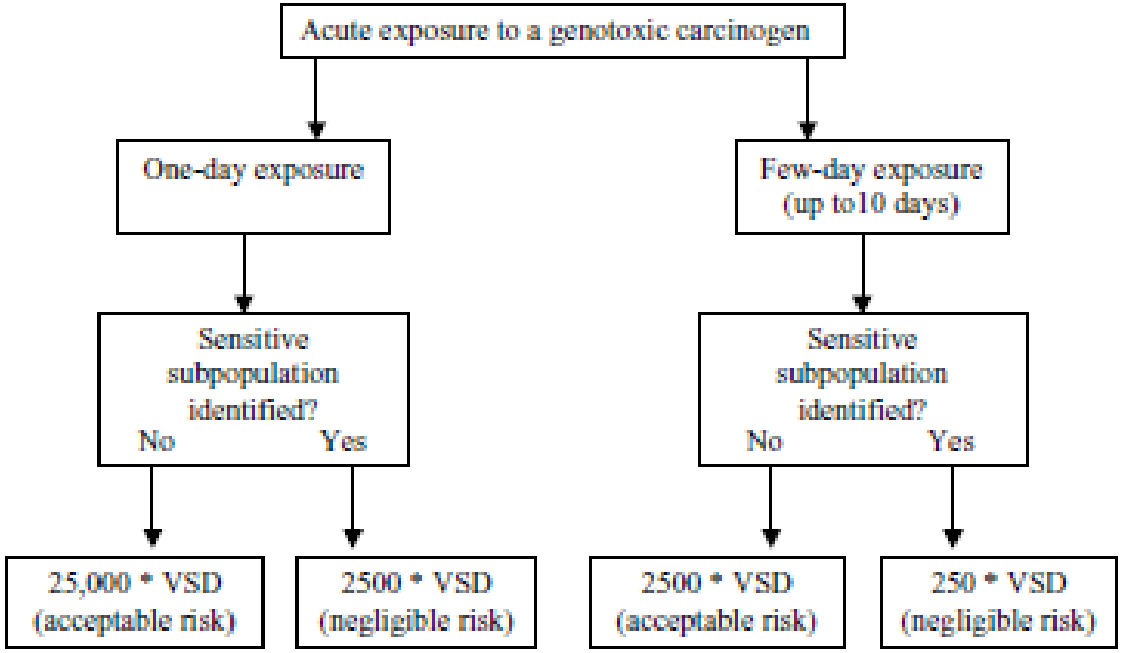


Fig. 1. Pragmatic decision tree for the assessment of a daily dose associated with an additional lifetime carcinogenic risk following acute exposure to a genotoxic carcinogenic substance.


FDA
U.S. FOOD & DRUG
ADMINISTRATION

Definitions

Mutation: the changing of the structure of a gene, resulting in a variant form that may be transmitted to subsequent generations, caused by the alteration of single base units in DNA, or the deletion, insertion, or rearrangement of larger sections of genes or chromosomes.

Clastogen: an agent giving rise to or inducing disruption or breakages of chromosomes, leading to sections of the chromosome being deleted, added, or rearranged. Most cells are killed by the clastogenic effect. If the damage becomes fixed in the chromosome, it can be transmitted to subsequent generations.

From ICH M3 (R2):

IX. GENOTOXICITY STUDIES (9)

An assay for gene mutation is generally considered sufficient to support all single dose clinical development trials. To support multiple dose clinical development trials, an additional assessment capable of detecting chromosomal damage in a mammalian system(s) should be completed (Ref. 10). A complete battery of tests for genotoxicity should be completed before initiation of phase 2 trials (Ref. 10).

If a positive finding occurs, an assessment, and then possibly additional testing (Ref. 10), should be conducted to determine if further administration to humans is still appropriate.

The genotoxicity studies recommended to support Exploratory Clinical Study approaches are discussed in section VII (7).

Pharmacokinetics of [^{14}C]-Benzo[a]pyrene (BaP) in humans: Impact of Co-Administration of smoked salmon and BaP dietary restriction [Food and Chemical Toxicology 115: 136-147, 2018 (IND 117175)]

Benzo[a]pyrene (BaP), a polycyclic aromatic hydrocarbon (PAH), is a known human carcinogen. In non-smoking adults greater than 95% of BaP exposure is through diet. The carcinogenicity of BaP is utilized by the U.S. EPA to assess relative potency of complex PAH mixtures. PAH relative potency factors (RPFs, BaP=1) are determined from high dose animal data. We employed accelerator mass spectrometry (AMS) to determine pharmacokinetics of [^{14}C]-BaP in humans following dosing with 46 ng (an order of magnitude lower than human dietary daily exposure and million-fold lower than animal cancer models). To assess the impact of co-administration of food with a complex PAH mixture, humans were dosed with 46 ng of [^{14}C]-BaP with or without smoked salmon. Subjects were asked to avoid high BaP-containing diets and a 3-day dietary questionnaire given to assess dietary exposure prior to dosing and three days post-dosing with [^{14}C]-BaP. Co-administration of smoked salmon, containing a complex mixture of PAHs with an RPF of 460 ng BaPeq, reduced and delayed absorption. Administration of canned commercial salmon, containing very low amounts of PAHs, showed the impacts on pharmacokinetics were not due to high amounts of PAHs but rather a food matrix effect.

Cigarette Smoke

S.S. Hecht Journal of the National Cancer Institute, Vol. 91, No. 14, July 21, 1999

Table 1, A. Summary of carcinogens in cigarette smoke*

Type	No. of compounds
Polycyclic aromatic hydrocarbons	10
Aza-arenes	3
N-Nitrosamines	7
Aromatic amines	3
Heterocyclic aromatic amines	8
Aldehydes	2
Miscellaneous organic compounds	15
Inorganic compounds	7
Total	55

Table 1, B. Pulmonary carcinogens in cigarette smoke†

Carcinogen class	Compound	Amount in mainstream cigarette smoke, ng/cigarette‡	Sidestream/ mainstream ratio§	Representative lung tumorigenicity in species‖	Reference No.‖
Polycyclic aromatic hydrocarbons	Benzo[*a*]pyrene	20–40	2.5–3.5	Mouse, rat, hamster	*(41,42)*
	Benzo[*b*]fluoranthane	4–22		Rat	*(41–43)*
	Benzo[*j*]fluoranthane	6–21		Rat	*(41–43)*
	Benzo[*k*]fluoranthane	6–12		Rat	*(41–43)*
	Dibenzo[*a,i*]pyrene	1.7–3.2		Hamster	*(41,42,44)*
	Indeno[1,2,3-*cd*]pyrene	4–20		Rat	*(41–43)*
	Dibenz[*a,h*]anthracene	4		Mouse	*(41,42,45)*
	5-Methylchrysene	0.6		Mouse	*(42,45)*
Asz-arenes	Dibenz[*a,h*]acridine	0.1		Rat	*(41,42,46)*
	7H-Dibenzo[*c,g*]carbazole	0.7		Hamster	*(41,42,47)*
N-Nitrosamines	*N*-Nitrosodiethylamine	ND–2.8	<40	Hamster	*(48,49)*
	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	80–770	1–4	Mouse, rat, hamster	*(22,50)*
Miscellaneous organic compounds	1,3-Butadiene	$20–70 \times 10^3$		Mouse	*(51)*
	Ethyl carbamate	20–38		Mouse	*(52)*
Inorganic compounds	Nickel	0–510	13–30	Rat	*(53)*
	Chromium	0.2–500		Rat	*(53)*
	Cadmium	0–6670	7.2	Rat	*(54)*
	Polonium-210	0.03–1.0 pCi	1.0–4.0	Hamster	*(55–58)*
	Arsenic	0–1400		None¶	*(59)*
	Hydrazine	24–43		Mouse	*(60)*

*Adapted from *(19,20)*. Compounds for which there is "sufficient evidence for carcinogenicity" in either laboratory animals or humans, according to evaluations by the International Agency for Research on Cancer.

†Compounds from Table 1, A, for which there is convincing evidence of pulmonary tumorigenicity in at least one species.

‡Data from *(19,37)*; all values in ng/cigarette except polonium-210; ND = not detectable.

§Data from *(61)*.

‖Studies in laboratory animals.

¶Epidemiologic studies indicate that inorganic arsenic compounds are skin or lung carcinogens in humans.

Adjusting cancer risk assessment based on lifetime exposure to short-term exposure

The National Advisory Committee on Acute Exposure Guideline Levels (NAC/AEGL), operating under the auspices of the US EPA, recommends multiplying the results of a cancer risk assessment based on lifetime exposure by a factor of between 2 and 6 to account for the number of stages in the multistage model applicable to the particular chemical and exposure scenario of concern.

In the case of a very short-term exposure, such as that which occurs in most instances of catastrophic accidental releases of chemicals, the AEGL Committee uses a factor of 6—the maximum number of stages in the multistage model—unless there is evidence that the chemical is a late-stage carcinogen or operates by mechanisms different from those assumed in development of the linearized multistage model (NRC, 2001).