FDA

FDA Genetic Toxicology Workshop: Can a mutagenic drug candidate be administered safely to Healthy Subjects?

Literature review for data relevant to administering one or a few doses of a DNA reactive drug to healthy subjects

November 4, 2019

Disclaimer: The views expressed herein do not necessarily reflect the official views of the US Food and Drug Administration

DATA BASES SURVEYED AND SEARCH TERMS QUERIED

Databases or search engines include:

Pubmed, **Google Scholar**, Google, PubChem BioAssay, PubMed Health, Pharmapendium, SciFinder, Embase, Medline, and **IARC Monographs on the Evaluation of Carcinogenic Risks to Humans**

Search Terms:

mutagenicity, mutagen, mutagenic, **DNA reactive**, **genotoxicity**, genotoxicant, genotoxin, **carcinogenicity**, carcinogen, carcinogenic, tumor, **Ames test**, bacterial gene mutation assay, micronucleus assay (in vitro or in vivo), chromosome aberration test, reproductive toxicity, germline mutagen, cytotoxic drugs, antineoplastic drugs, cancer, secondary cancer/tumors, drug/chemical/ingredient, **milligram dose (range)**, clinical trials, occupational exposure, environmental exposure, pollution, accident/unintentional exposure, epidemiological, **animal model studies**, **human subjects**, **healthy adult (subjects/volunteers)**, human biomonitoring, preventive medicine, **threshold of toxicological concern (TTC)**, **threshold of regulation**, **less-than-lifetime exposure**, pharmacologically active dose, active pharmaceutical ingredient (API), **single dose**, **two doses**, **few/several/multiple doses**, **short-term/duration (year/week) exposure**, **(quantitative) risk assessment**, cohort of concern, virtually safe dose, short term drug use/abuse, drug abuse, excessive drug intake/use, iatrogenic disease, chemical/radiation disaster/spill clean-up workers, chemical/radiation countermeasure workers

SEARCH RESULTS

- **SUMMARIZED FINDINGS FROM 100+ RELEVANT AND SUPPORTING DOCUMENTS**
  - **Data modeling and database analyses**
  - **Animal studies with single, few or short duration exposure to DNA reactive agents and tumor endpoints**
  - **Cancer epidemiology of environmental and occupational exposures to DNA reactive agents**
  - **Cancer epidemiology of medical exposures to DNA reactive agents**
- **COMPILED 1,300+ MANUSCRIPTS CONTAINING SINGLE EXPOSURES WITH TEST ARTICLES AND TUMOR ENDPOINTS IN ANIMAL MODELS**

PART I: DATA MODELING AND DATABASE ANALYSES FOR AMES POSITIVE CHEMICALS

PART II: CANCER DATA FROM SINGLE, FEW OR SHORT-DURATION EXPOSURES TO AMES POSITIVE CHEMICALS IN ANIMAL MODELS AND HUMANS

PART I: DATA MODELING AND DATABASE ANALYSES FOR AMES POSITIVE CHEMICALS

- ***An analysis of genetic toxicity, reproductive and developmental toxicity, and carcinogenicity data: I. Identification of carcinogens using surrogate endpoints*** **(Matthews *et al.*, 2006)**

- ***Reevaluating cancer risk estimates for short-term exposure scenarios*** **(Halmes *et al.*, 2000)**

Associations between Ames test and rodent cancer bioassay data

- Database (EPA GENE-TOX) of 3,596 chemicals with gene tox data
- 1,607 (44.7 %) had Ames (*Salmonella*) data and 988 (27%) also had rodent cancer bioassay data

	Carcinogenic	
Mutagenic (Ames)	Positive	Negative
Positive	275 a (true +)	85 c (false +)
Negative	282 b (false -)	346 d (true -)

Overall concordance:	62.9%	a+d/(a+b+c+d)	fraction of matching results
Sensitivity:	49.4%	a/(a+b)	fraction of carcinogens which are mutagens
Positive predictive value:	76.4%	a/(a+c)	fraction of mutagens which are carcinogens
Specificity:	80.3%	d/(c+d)	fraction of non-carcinogens which are not mutagens
False Positives:	19.7%	c/(c+d)	mutagenic non-carcinogens
False Negatives:	51.9%	b/(a+b)	non mutagenic carcinogens
Correlation indicator	78.3%		indicator of a positive finding in the rc bioassay

- The Ames test is a reliable indicator of a positive finding in the rodent cancer bioassay for mutagenic agents

Data derived from Matthews *et al.*, 2006; table compiled by Dan Levy, CFSAN

CHRONIC LIFETIME (2-YEAR) AND STOP EXPOSURE DATA MODELING

- **Cancer risk assessments assume that excess risk increases as a linear function of the cumulative carcinogen dose administered at a given rate (Haber's law)**

- **To test this assumption 11 carcinogens from NTP combined chronic lifetime- and stop-exposure studies were modeled to determine maximum likelihood estimated dose corresponding to a 1% cancer risk (ED_{01})**

Chemical	*Salmonella* mutagenicity assays
1-Amino-2,4-dibromoanthraquinone (ADBAQ)	+
2,2-Bis(bromomethyl)-1,3-propanediol (BBMP)	+/–
* 1,3-Butadiene	+/–
Coumarin	+/–
3,4-Dihydrocoumarin	–
Furan	–
Methyleugenol	–
o-Nitroanisole	+
Oxazepam	–
Pentachlorophenol	–
Salicylazosulfapyridine	–

modified from Halmes *et al.* (2000)

- **Tumor incidence was significantly higher for 6 of 11 chemicals for short-term exposures suggesting more effective tumor producers vs. continuous exposure**

- **Most of the carcinogens in the stop-exposure studies had significantly higher (≥ 2-fold response) carcinogenic potencies (lower ED_{01}) than the chronic lifetime exposures for at least one tumor site**
  - **Example: BBMP, 1,3-butadiene, and *o*-nitroanisole were positive for increased tumors at sites only when the stop exposure data was included**

- **Findings from stop-exposure modeling suggest that short-term exposures could pose cancer risks not identified in continuous exposure studies**
  - **Example: 1,3-butadiene exposures for 13, 26, 40 or 52 weeks produced a much larger tumor response (heart hemangiosarcoma) than compared with continuous lifetime exposure at the same dosing rate**

PART I: SUMMARY

- **The GENE-TOX (EPA) database analyses suggest that the Ames test is a reliable indicator of a positive finding in the rodent carcinogenicity bioassay for a mutagenic agent**

- **Majority (5 of 6) of chemicals in stop exposures that gave a ≥ 2-fold response in cancer potency were positive in at least one *Salmonella* mutagenicity assay**

- **Findings from stop-exposure modeling suggest that short-term exposures could pose cancer risks not identified in continuous exposure studies**

PART II: CANCER DATA FROM SINGLE, FEW OR SHORT-DURATION EXPOSURES TO AMES POSITIVE CHEMICALS

- **ANIMAL CARCINOGENICITY STUDIES:**
  - ***The Single Exposure Carcinogen Database: assessing the circumstances under which a single exposure to a carcinogen can cause cancer*** **(Calabrese and Blain, 1999)**
- **EPIDEMIOLOGICAL DATA: Studies identified for 2 occupational exposures and 3 medical exposures in limited exposures or for short durations (i.e. ≤ 1 year)**

- Beryllium (Be): wide ranging products, production worker cohort
- Aromatic amines of benzidine: cohort exposed through components of dyes, manufacture, use, purification
- Phenacetin: (OTC analgesic) use discontinued in the US, Canada, and UK
- Chloral hydrate: (prescription sleep aid)
- Thorotrast (contrast agent) contains α-particle-emitting thorium 232 (^{232}Th) radionuclide. Used from 1920s to 1950s

SINGLE EXPOSURE CARCINOGEN DATABASE IN ANIMAL MODELS: Assessing the circumstances under which a single exposure to a carcinogen can cause cancer

- **Database of tumor incidences following a single exposure to a suspected agent to estimate risk from less than lifetime exposures**

- **Contains over 5,500+ studies for 800+ chemicals from 2,000+ articles that addressed single-exposure carcinogenesis**

- Criteria for inclusion in single-exposure carcinogen database (SECD):
  - **Agent administered only once**
  - **No additional treatment**
  - **Tumors were examined as the endpoint**

- SECD information compiled:
  - **# Citations**
  - **Chemical details (CAS#, synonyms, chemical class)**
  - **Study design (controls and treatment groups, animal model, age, sex, exposure route, pathology, dose-response relationships, statistics)**

Calabrese and Blain (1999)

SINGLE EXPOSURE CARCINOGEN DATABASE IN ANIMAL MODELS: Assessing the circumstances under which a single exposure to a carcinogen can cause cancer

Single dose administration of 426 chemicals (with bioactivation or direct-acting) in 17 chemical classes

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

	Number of studies (% of total positive; 4271)	Number of studies (% of total negative; 1295)
Sex:		
Males	1285 (30%)	366 (28%)
Females	1796 (42%)	459 (35%)
Both	1189 (28%)	470 (36%)
Histology	3384 (79%)	1084 (84%)
Statistics (hypothesis testing)	2039 (48%)	454 (35%)
Used controls:	2492 (58%)	869 (67%)
Concurrent	2151 (50%)	744 (57%)
Vehicle	1318 (31%)	505 (39%)
Historical	242 (6%)	85 (7%)
Subjects in groups:		
>10	3607 (84%)	1027 (79%)
>30	1696 (40%)	418 (32%)
>50	820 (19%)	177 (14%)
Age:		
Newborn	425 (10%)	106 (8%)
Transplacental	277 (6%)	63 (5%)
Most reported organs:		
Liver	613 (14%)	131 (10%)
Mammary	800 (19%)	81 (6%)
Skin	577 (14%)	139 (11%)
Respiratory	1220 (29%)	175 (14%)
Most examined animal models:		
Rats:	1659 (39%)	364 (28%)
Sprague-Dawley	621 (15%)	85 (7%)
Wistar	188 (4%)	66 (5%)
Fisher 344	124 (3%)	30 (2%)
Mice:	2260 (53%)	785 (61%)
Swiss	151 (4%)	99 (8%)
Strain A	259 (6%)	50 (4%)
C3H	76 (2%)	40 (3%)

modified from Calabrese and Blain (1999)

SINGLE EXPOSURE CARCINOGEN DATABASE IN ANIMAL MODELS: Assessing the circumstances under which a single exposure to a carcinogen can cause cancer

TABLE 3
Species with Positive Results in the Single Exposure Carcinogen Database

Species	Number of strains and/or substrains with positive results
Mice	464
Rats	141
Hamsters	20
Fish	9
Rabbits	9
Guinea pigs	5
Primates	3
Gerbils	2
Birds	5
Dogs	1
Opossums	1

GENERAL FINDINGS:

- **A single dose of many agents produce tumors in both males and females, in numerous animal models (i.e. hamster, gerbil, rabbit, guinea pig, possum, and fish), and in all age groups (fetal, neonate, and adult stages)**

- **Doses causing tumors were generally a low proportion of the LD_{50} dose (between 0.1 and up to LD_{50}) and not acutely life-threating**

- **Tumorigenic responses observed for a single exposure to (DNA reactive) chemicals with wide structural diversity and in all principal animal models imply that humans are also likely to exhibit a qualitatively similar response**

Calabrese and Blain (1999)

SINGLE EXPOSURE CARCINOGEN DATABASE IN ANIMAL MODELS: Assessing the circumstances under which a single exposure to a carcinogen can cause cancer

TABLE 9

Chemicals where Dose Fractionation Was Performed, Sorted by the Results of a Single Dose vs. Dose Fractionation

Chemicals where a single dose caused fewer tumors than that dose fractionated	Chemicals where a single dose caused more tumors than that dose fractionated	Chemicals where the single dose and the dose fractionation produced similar results
DMBA	1,2-Dimethylhydrazine	3-Hydroxyxanthine
Benzo(a)pyrene	Methylnitrosourea	DMBA
Radiation	Procarbazine	Procarbazine
MCA	DMBA	N-OH-2-FAA
3-Hydroxyxanthine	Methyl(acetoxymethyl)nitrosamine	Benzo(a)pyrene
PBB		Ethyl carbamate
Potassium bromate		Methyl-bis(2-Chloroethyl)amine
N-nitrosobis(2-Acetoxypropyl)amine		Hydrochloride
Cadmium chloride		DMN

- **Single dose vs. fractionated lower daily dose for a near lifetime exposure (i.e. cancer bioassay)**
  - **Some mutagenic chemicals produced more tumors as a single dose, as a fractionated dose, or similar results**
  - **Some mutagenic chemicals had mixed results (DMBA, B[a]P, 3-hydroxyxanthine, procarbazine)**
- **Suggests there is chemical-specific carcinogenic response or varied responses to single vs. fractionated doses, and that a single dose can have carcinogenic effects not observed in lifetime exposures**

Calabrese and Blain (1999)

EPIDEMIOLGICAL DATA FOR LIMITED EXPOSURE TO DNA REACTIVE CHEMICALS

- **LIMITED DATA FOR DNA REACTIVE/MUTAGENIC EXPOSURES IN HUMANS**
- **“SHORT DURATION EXPOSURE”: LESS THAN ONE YEAR OR EXPOSURE TO A FEW DOSES**
- **HUMAN SUBJECTS: EXCLUDE CANCER OR TERMINALLY ILL PATIENTS**
- **“TREATMENT”: INCLUDE DRUGS WITH POSITIVE MUTAGENICITY DATA; EXCLUDE ANTI-NEOPLASTICPLASTIC DRUGS**

SHORT-DURATION OCCUPATIONAL EXPOSURES IN HUMANS

		Human and Exp. Models					
	Agent	Mutagenicity	Clastogenicity - Aneugenicity	Cohort	Exposure Level	Exposure Duration (≤ 1 year)	Citation
Occupational Exposures	Beryllium (Be)	(-) Ames test, (+) *HGPRT* mutation	(+)cAbs, (+) SCE	421 white male subjects entered into the beryllium case registry	Beryllium exposure levels not quantified	One year or less of employment as a beryllium exposed worker resulted in a significant excess of lung cancer	Infante *et al.*, 1980; Wagoner *et al.*, 1978
				3,685 white males employed in manufacturing beryllium (control: similarly employed vicose rayon workers)	Beryllium exposure levels not quantified		Mancuso, 1980
	Aromatic amines of benzidine (AABs)	(+) Ames	(+) MN, (+) cAbs, (+) SCE	4,622 males involved in the manufacture (I), use (II) or purification (III) of AABs	Men in manufacturing had highest cancer rates	Overall risk of dying from a bladder tumour is approximately 30X that of the general population if exposed at least 6 months and exposures of ≤1 year carried a significant risk	Case *et al.*, 1954

SHORT-DURATION BERYLLIUM OCCUPATIONAL EXPOSURES IN HUMANS

TABLE 5

LUNG CANCER CASES AMONG WHITE MALES ENROLLED IN THE BCR COHORT

Case	Type of respiratory illness	Type of exposure	Length of exposure (years)	Year of initial exposure	Years of entry into registry	Date of death	Interval since initial exposure (years)
1	Acute chemical bronchitis and pneumonitis	Extraction, smelting	< 1	1947	1952	05-13-66	19
2	Acute chemical bronchitis	Extraction, smelting	< 1	1943	1952	02-23-72	29
3	Chemical bronchitis	Extraction, smelting	< 1	1947	1952	05-18-59	12
4	Acute chemical bronchitis	Extraction, smelting	< 1	1943	1954	07-23-59	15
5	Acute chemical bronchitis	Extraction, smelting	< 1	1947	1954	08-12-74	27
6	Chemical bronchitis	Extraction, smelting	4	1943	1952	04-10-70	26
7	Pulmonary fibrosis	Tube disposal	n.p.d.[a]	1936	1964	10-09-68	32

[a] Not possible to determine.

Infante *et al.*, 1980

*Data on smoking history was not collected

Lung cancer cases in the beryllium exposed cohort*

- **Five cases of lung cancer were observed where Be exposure was less than 1 year: 1 month (3 workers), 2 months (1 worker), and 6 months (1 worker) (Infante *et al.*, 1980)**
- **Two lung cancer deaths (~20 years after last exposure): one employed for 6 months and one employed for 21 months (Mancuso, 1980)**

SHORT-DURATION AAB OCCUPATIONAL EXPOSURES IN HUMANS

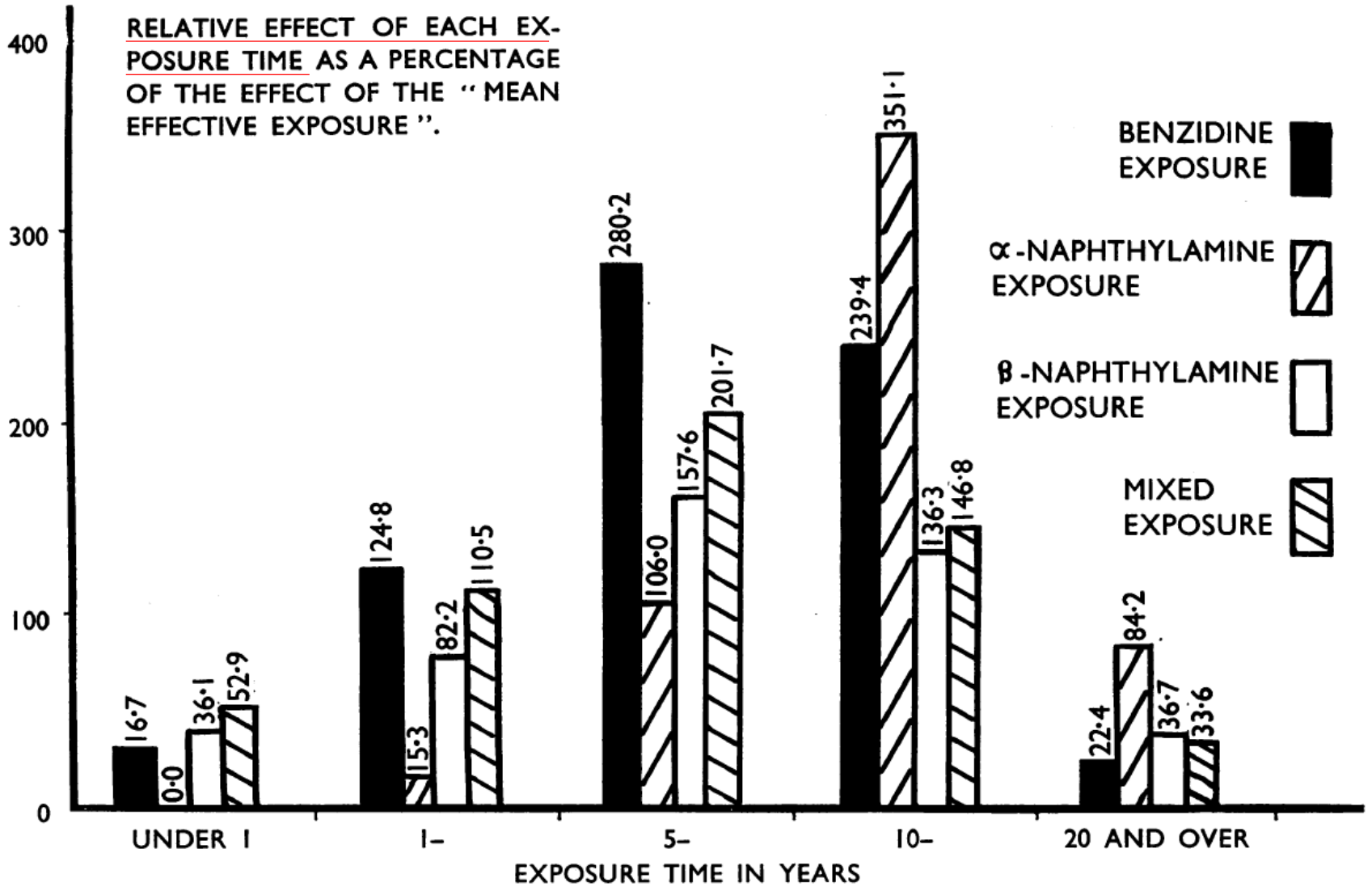


FIG. 7.—The effect of exposure time by exposure class.

Case et al., 1954

- **Mean effective exposure: length of exposure time necessary for a given exposure class to produce average risk**
- **≤ 1 year exposure increased bladder tumor risk for workmen (7 workers w/ < 1 year, 50 workers w/ 1 year exposure)**
- **Increased risk observed with increased duration of exposure**

*Data on smoking history was not collected

SHORT-DURATION MEDICAL EXPOSURES IN HUMANS

		Human and Exp. Models					
	Agent	Mutagenicity	Clastogenicity - Aneugenicity	Cohort	Exposure Level/Exposure	Exposure	Citations
Medical Exposures	Phenacetin (OTC analgesic)	(+/-) Ames test (hamster S9); (+) *LacZ* reporter gene mutation	(+) cAbs, (-) MN	314 subjects between 1978-1982	Phenacetin dosage was 300 mg four to six times per day; daily dose was not to exceed 2 g	Chronic (>30 consecutive d/yr) exposure in phenacetin users, > 30 d/y, < 30 d/y, no use	Ross et al., 1989
	Chloral hydrate (sleep aid)	(+/-) Ames test	(+) MLA, (+) cAbs, (+) MN	2,290 chloral hydrate users (i.e. sleep aid) between 1969 and 1973	500mg dispensings of chloral hydrate	0, 1, 2-3, or 4+ dispensings over a 4 year period	Haselkorn *et al*, 2006
	Thorotrast (radiological imaging agent)	(+) T-cell receptor mutations, (-) glycophorin A, (+/-) *p53*, (+/-) KRAS	(+) cAbs	9,000+ persons injected with Thorotrast during the period between 1929 and 1956	Acute administration of 250 g/L, volumes range from 1ml up to ~100 mLs	Dose dependent increase in tumors observed following Thorotrast injection	Andersson *et al*. 1997; van Kaick et al. 1999

SHORT-DURATION HEAVY-USE OF PHENACETIN

Reference, study location and period	Organ site (ICD code)	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Ross et al. (1989) Los Angeles County USA, 1978–82	Renal pelvis and ureter	187 (127 men, 60 women) selected from the Los Angeles County Cancer Registry, diagnosed under age 75; response rate 80%	187 neighbourhood controls selected at random and matched individually to cases by sex, date of birth (± 5 yr), and race	Structured interview over the telephone	*Phenacetin-containing analgesics* > 30 d/yr > 30 consecutive d/yr	 11 7	 1.1 ($P = 0.83$) 1.4 ($P = 0.56$)	Age, sex, race	Response rate not given for control subjects. Use of analgesic measured up until date of diagnosis or equivalent date for control. Reference category defined as 'no use or use fewer than 30 times in a yr'. Non-prescription compounds in focus of the study

- **Phenacetin is classified as a Group 1 carcinogen: *carcinogenic to humans* (IARC 2012)**
- **Withdrawn from US market in 1983**
- **Long term Phenacetin use causes renal pelvis and ureter tumors in humans**
- **Ross *et al.*, (1989) found a n.s. slight increase risk for renal pelvis and ureter tumors following heavy short-duration phenacetin use: >30 consecutive days/yr vs. > 30 days/year, no < 30 days/yr, or no use**

SHORT-DURATION CHLORAL HYDRATE ADMINISTRATION

Table IV. Distribution of chloral hydrate dispensings by cancer site and case/control status

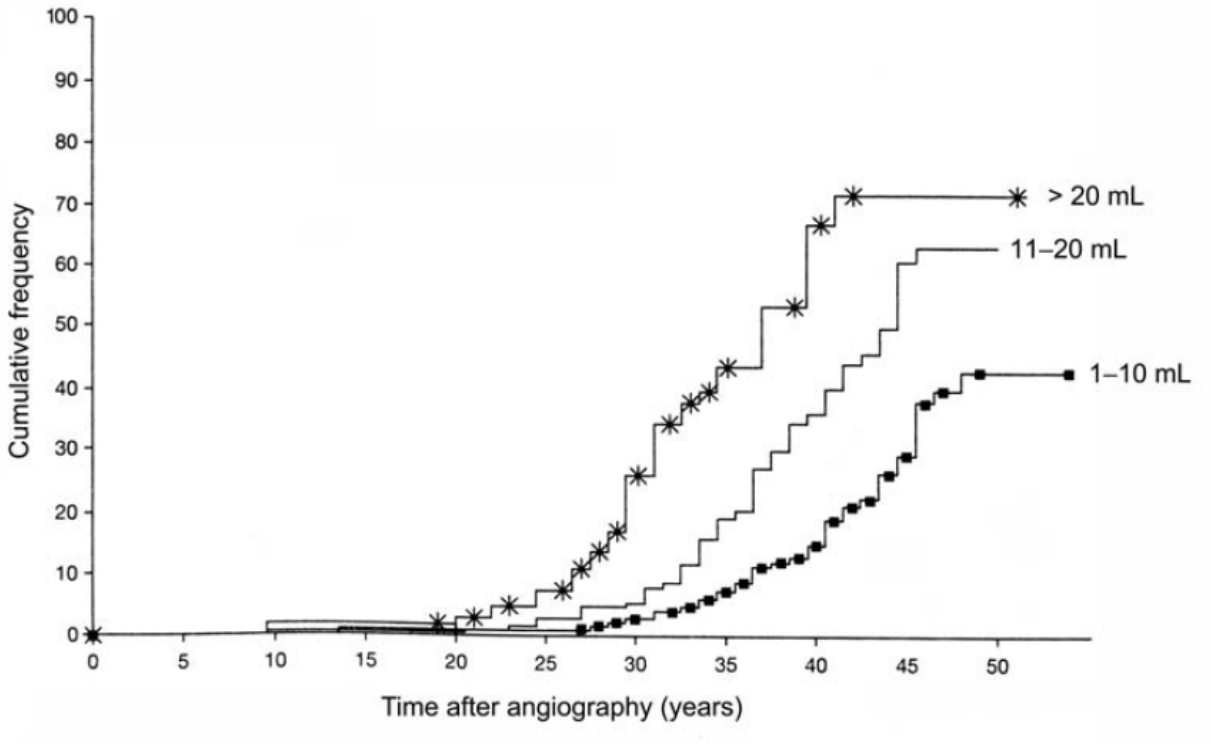
Total number of dispensings by cancer site	Cases [no. (%)]	Other chloral hydrate users [no. (%)]
Overall	**n = 85**	**n = 340**
1	32 (38)	140 (41)
2–3	30 (35)	110 (32)
4+	23 (28)	90 (27)
Mean (SD)	3.3 (3.4)	3.6 (5.4)
Minimum, maximum	1, 21	1, 53
Median	2.0	2.0
Prostate cancer	**n = 14**	**n = 56**
1	3 (21)	20 (36)
2–3	5 (36)	17 (31)
4+	6 (43)	19 (34)
Mean (SD)	4.3 (3.7)	4.4 (7.9)
Minimum, maximum	1, 13	1, 53
Median	2.5	2.0

- **Chloral hydrate was mutagenic in *Salmonella* and carcinogenic in animal studies, and is a major metabolite of trichloroethylene (TCE), a general anesthetic banned in the US in 1977 due to its ability to induce tumors in rodents**
- **Chloral hydrate used in a 500mg capsule form (dispensing). Based on study, most patients received a 500mg dose only a few times for short-term use (e.g. for insomnia)**
- **There was no evidence of a chloral hydrate dose-response for all cancers combined**
- **Suggestion of increased risk (n.s.) of prostate cancer with increased dispensings**

Modified from Haselkorn *et al.*, 2006

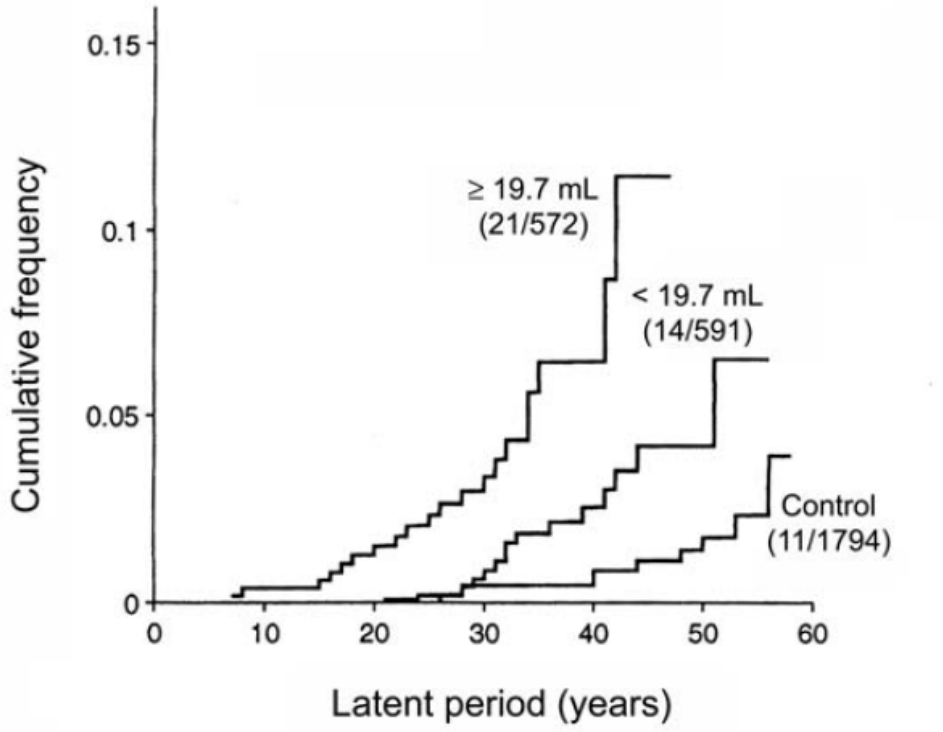
ACUTELY ADMINISTERED THOROTRAST IN HUMANS

Figure 5. Cumulative frequency of liver tumours with time after angiography in relation to the volume of Thorotrast injected (Kaplan-Meier estimates, log rank test: $p < 0.0001$)



From Andersson (1997)

Figure 6. Cumulative frequency of haematopoietic malignancies (myeloid leukaemia and myelodysplastic syndrome) among persons injected with Thorotrast



The numbers in parentheses are the number of malignancies per number of patients. Note that the frequency and the latency are related to the injection volume (i.e. dose rate) (van Kaick *et al.*, 1999).

- **Increasing the level of Thorotrast injected resulted in increased risk for cancer**

PART II SUMMARY

- **Tumorigenic responses observed for a single exposure to carcinogens with wide structural diversity and in all principal animal models imply that humans are also likely to exhibit a qualitatively similar response**

- **Cancer epidemiology studies provide suggestive, but not conclusive, evidence of a causal relationship between short duration exposures to mutagenic compounds and cancer**

- **Animal experiments have limited exposures, other than the doses administered, whereas humans are exposed to additional environmental/lifestyle mutagens**

Questions

A review of the genotoxicity of marketed pharmaceuticals

Genotoxicity and carcinogenicity profiles of marketed pharmaceuticals

- **Reviewed the 1999 Physicians Desk Reference (+ peer-reviewed literature & NTP) list of 352 pharmaceuticals marketed in the US with at least 1 gene-tox assay result**
  - **101/325 (29%) had at least one positive genetox finding**
  - **Excluded from analysis were anti-cancer drugs and nucleosides**
- **201 pharmaceuticals had gene-tox and carcinogenicity results on the drug label**
    - **Marketed pharmaceuticals:**
    - **77/201 (38%) had positive carcinogenicity data**
    - **27/323 (8.3%) had positive findings in the Ames test**

Snyder and Green, 2001

A review of the genotoxicity of marketed pharmaceuticals

- **198 drugs had data for both Ames test and rodent carcinogenicity**

Relationship between Ames test and rodent carcinogenicity

	Bacterial mutation	
	Positive	Negative
Carcinogenicity		
Positive	8	69
Negative	4	117
Total	12	186
P-value of the Fisher's exact test for positive association	0.043	0.137
Sensitivity	0.10(0.05–0.19)[a]	0.30(0.18–0.44)
Specificity	0.97(0.92–0.99)	0.80(0.70–0.89)
Positive predictivity	0.67(0.35–0.90)	0.52(0.33–0.70)
Negative predictivity	0.63(0.56–0.70)	0.62(0.51–0.71)
Concordance	0.63(0.56–0.70)	0.59(0.50–0.68)

[a] 95% confidence interval.

For marketed pharmaceuticals:

- **Determine the association between Ames test and rodent carcinogenicity**
- **8.3% (27/323) of marketed drugs were Ames positive**
- **The Ames assay showed a strong association with rodent carcinogenicity**
- **The bacterial mutation assay had high specificity and a marginally statistically significant P-value for positive association with rodent carcinogenesis**

Snyder and Green, 2001