Swiss Centre for Applied Human Toxicology
Schweizerisches Zentrum für Angewandte Humantoxikologie
Centre Suisse de Toxicologie Humaine Appliquée
Centro Svizzero di Tossicologia Umana Applicata

EUROTOX Advanced Toxicology Course

Risk Assessment – Regulatory Toxicology

Framework and definitions, Hazard evaluation, Dose-response relationships, Exposure assessment

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What is regulatory toxicology?

- **Regulatory toxicology is the process whereby information relevant to assessing the toxicity of agents, which may be biological, chemical or physical in nature, is obtained and evaluated by or on behalf of governmental or international organizations.**
- **The aim is to protect workers, consumers, the public generally and the environment**

Illing HPA & Marrs TC

General and Applied Toxicology, 2009

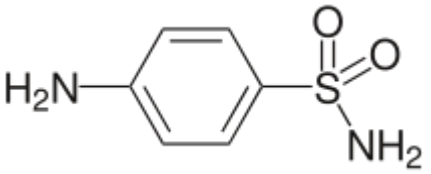
What is regulatory toxicology?

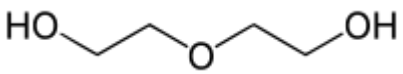
- ***„Regulatory toxicology is to toxicology what military music is to music“***

Sir Colin Berry
Em. Professor of Morbid Anatomy and Histopathology
Queen Mary College
University of London

Sulfanilamide (1937)

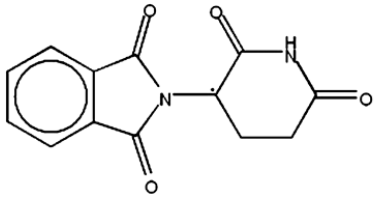
- **Sulfanilamide – antibacterial agent discovered in 1935 and available in tablet and powder form**
- **In 1937, S.E. Massengill company produced ‘elixir sulfanilamide‘ using diethylene glycol as solvent**
- **No toxicity testing had been perfomed, but the product had passed testing for appearance, flavour and fragrance**
- **Within 4 weeks, 353 patients had received treatment, 105 died (incl. 34 children), primarily from renal failure**
- **The incident facilitated the passing of the 1938 Food, Drug and Cosmetic Act which required companies to submit safety testing information to the US FDA**
- **Further mass poisoning incidents occurred in South Africa (1969), India (1986), Nigeria (1990), Bangladesh (1990/92), Haiti (1995/96), China, Panama (2006), Nigeria (2008)**
- **In all cases medicines or personal care products had been prepared with DEG as a substitute of, or contaminant in, other solvents such as glycerine or propylene glycol**



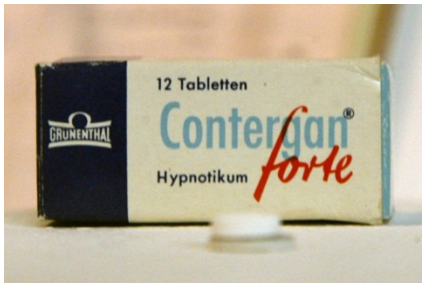


Thalidomide (1958 – 1962)

- Sold as sedative and shown to be particularly effective against morning sickness in pregnant women
- Standard safety tests on non-pregnant rats showed no appreciable toxicity even at high doses
- An estimated 10‘000 – 20‘000 children were born with limb deformities caused by thalidomide
- Extensive investigations in the 1960s showed a marked species difference in teratogenicity with New Zealand White Rabbits being particularly sensitive
- Testing for developmental effects in two species became a regulatory requirement in the USA in 1966



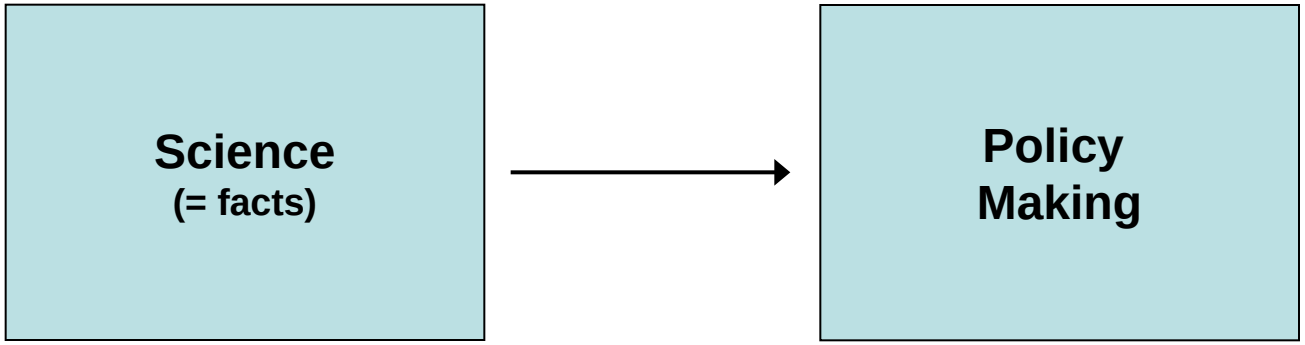
Note: • = asymmetric carbon atom



A paradigm shift

- Historically, public policy makers (such as government ministers) used to claim that policies to regulate safety risks were always and only based on ‘sound science‘

The Technocratic Model

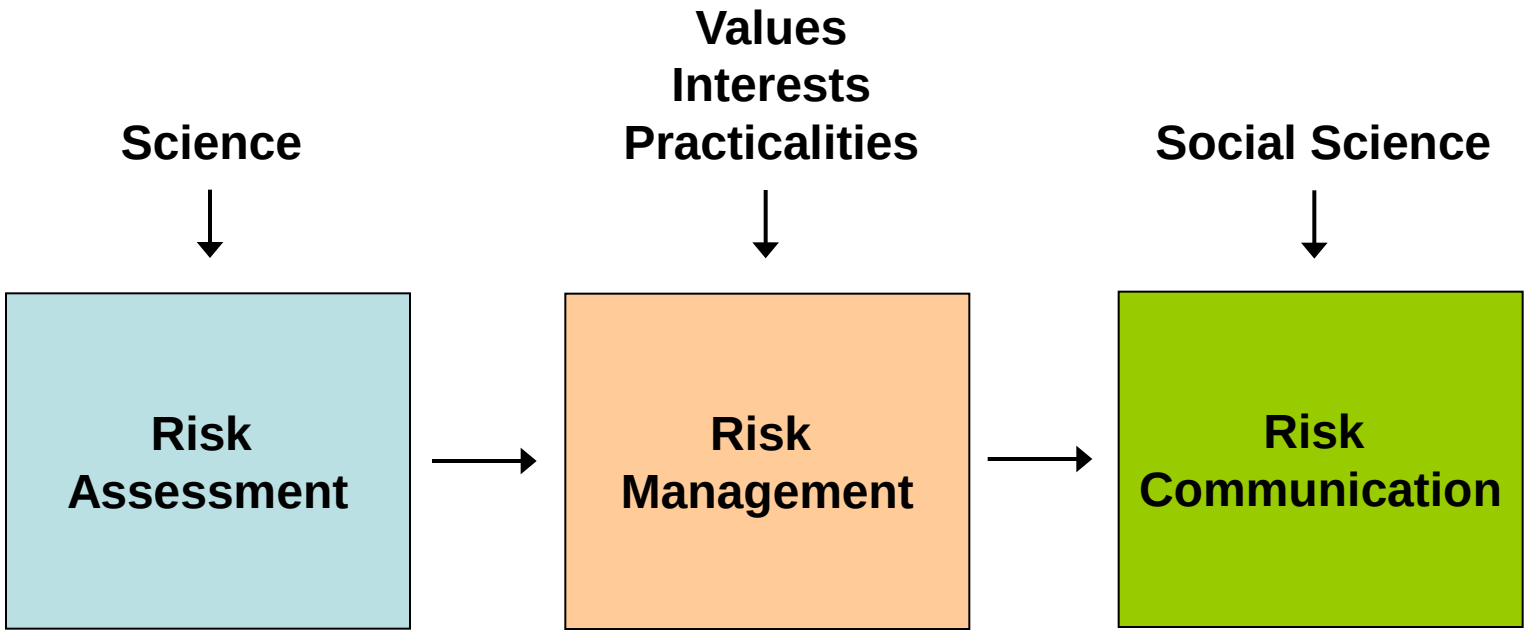


(based on Millstone et al, JRC Report, 2008)

Drivers for change

- **Even if all scientific uncertainties were eliminated, science could still not decide safety policy**
- **Policy judgements are concerned with the *acceptability* of possible risks (and uncertainties) in exchange for anticipated benefits**
- **These are socially variable value judgements – they are policy matters, not scientific issues**

- **The *Red Book* Decisionist Model**



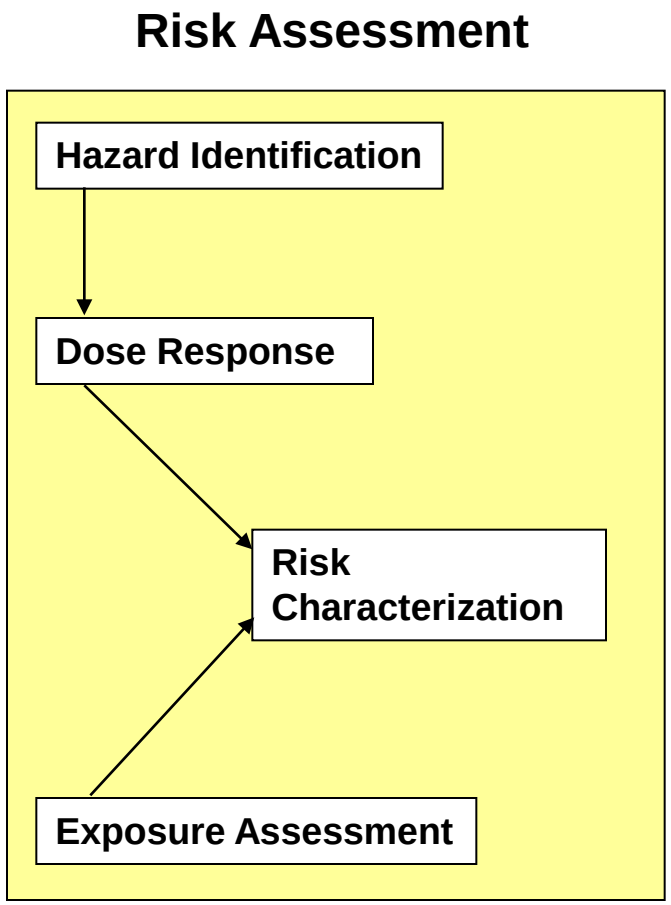
(based on Millstone et al, JRC Report, 2008)

NRC 1983: Risk Assessment in the Federal Government

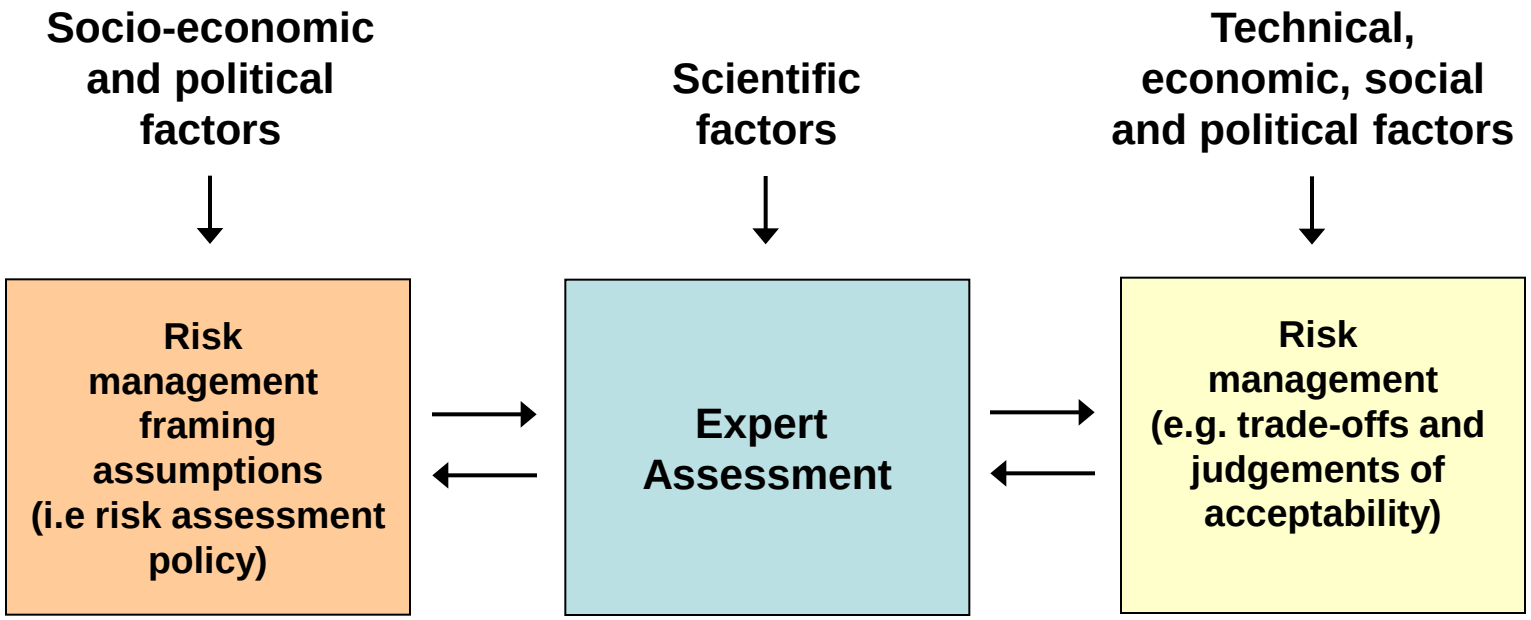
- Introduced the now commonly accepted steps in risk assessment
- Clearly separates risk assessment from risk management and risk communication

But

- Ignores how non-scientific considerations *frame* scientific representations of risk, e.g. choice of ‘target risks‘, ‘target groups‘ and relevant evidence



- **The co-dynamic model: reciprocal links between science and policy**



(based on Millstone et al, JRC Report, 2008)

NRC 2009: Science and Decisions ('Silver Book')

- **Increase the attention to planning and scoping, and problem formulation**
  - Bring risk assessors, risk managers and stakeholders together early in the design of the RA
- **Characterize and communicate uncertainty and variability**
  - Adopt a tiered approach for selecting the level of detail
- **Evaluate background exposures and disease processes, vulnerable populations, and modes of action**
- **Incorporate interactions between chemical and nonchemical stressors in assessments**
- **Increase the role of biomonitoring, epidemiologic, and surveillance data in cumulative risk assessments**
- **Establish a formal process for stakeholder involvement**

Stages of Risk Assessment

Toxicological Hazard Assessment

Does an agent have the inherent property or potential to cause adverse effects when an organism is exposed to that agent?

Hazard identification

- **In silico**
  - **QSAR, structure alerts, selective binding to specific receptors**
- **In vitro**
  - **prokaryotic/eukaryotic, cell/organ culture**
- **In vivo**
  - **Single dose toxicity (also skin and eye irritation)**
  - **Repeat dose toxicity (oral, dermal etc)**
  - **Genotoxicity (in vitro, in vivo)**
  - **Carcinogenicity (rodent lifetime assay)**
  - **Reproduction (fertility, developmental tox)**
  - **Other; sensitization, immunotoxicity, neurotoxicity**

Hazard identification

in silico - OECD QSAR toolbox

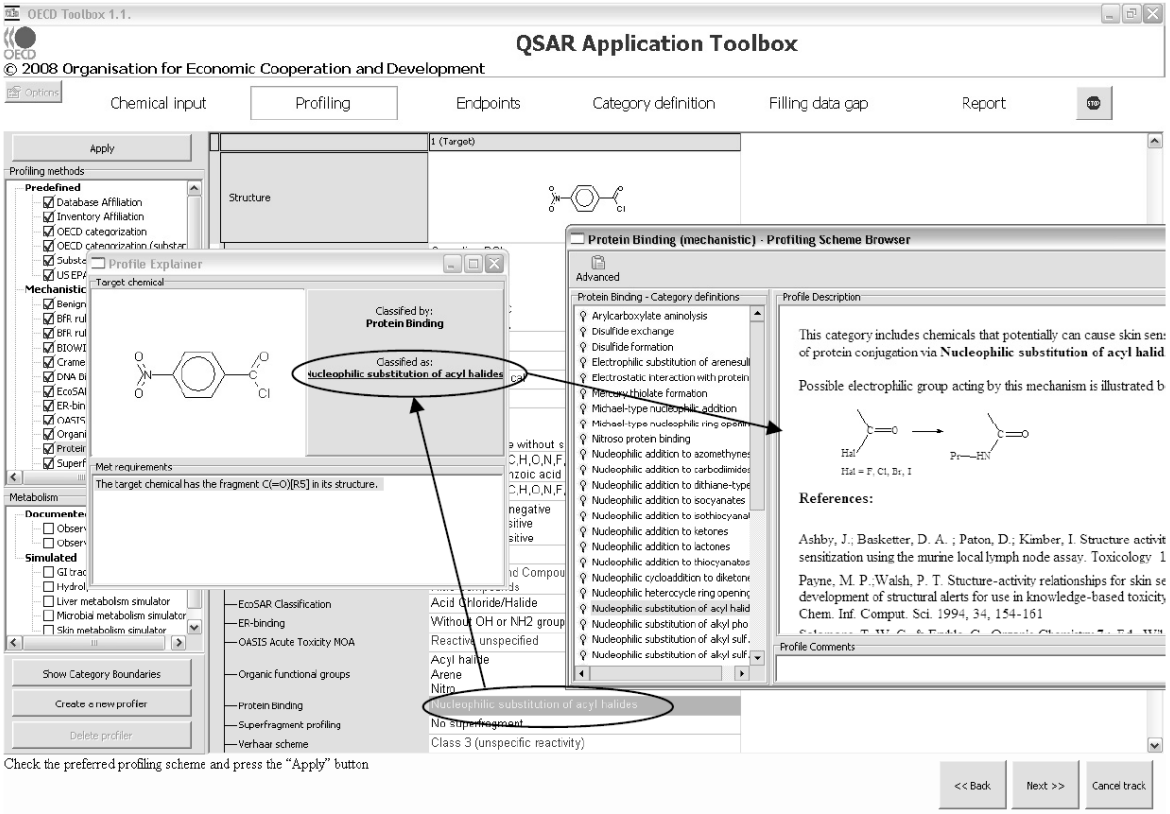
http://www.oecd.org

(Q)SAR APPLICATION TOOLBOX

VERSION 1.1

GETTING STARTED

DECEMBER 2008



Hazard identification

in vitro - ECVAM

- **ECVAM European Centre for the Validation of Alternative Methods develops methods which reduce/replace animals in testing toxicity of chemicals/cosmetics:**
  - Single dose toxicity: New (since 2000) OECD methods reduce the number of animals required from 45 to 3-6 per chemical.
  - Skin sensitisation: Modified test OECD 429 (reduced Local Lymph Node Assay; rLLNA) halves the number of mice per substance.
  - Toxicokinetics: OECD 2004 guideline for in vitro skin penetration.
  - Mutagenicity and carcinogenicity: in vitro micronucleus test validated 2006, became part of REACH legislation.
  - Reproductive toxicity: 3 embryotoxicity tests validated 2002
  - Endocrine disrupters: Validation studies with US ongoing (part of ReProTect).
  - Skin/eye corrosion: OECD accepted tests in 2004.
  - Phototoxicity: OECD accepted tests in 2004.
  - Skin and eye irritation validation studies completed.

(http://ecvam.jrc.ec.europa.eu/)

Hazard identification

in vivo

In vivo ("animal bioassays"):

- **Single dose toxicity (also skin and eye irritation)**
- **Repeat dose general toxicity**
- **Genotoxicity**
- **Carcinogenicity**
- **Reproduction (fertility, developmental tox)**
- **Other; sensitization, immunotox, neurotox…**

GHS/CLP Acute Toxicity Hazard Categories

Exposure route	Category 1	Category 2	Category 3	Category 4	Category 5
					
Oral (mg/kg bw)	5	50	300	2000	5000
Dermal (mg/kg bw)	50	200	1000	2000	
Gases (ppmV)	100	500	2500	20000	
Vapours (mg/l)	0.5	2.0	10	20	
Dusts and Mists (mg/l)	0.05	0.5	1.0	5	

Values are expressed as (approximate) LD_{50} (oral, dermal) or LC_{50} (inhalation values) or as acute toxicity estimates (ATE)

Hazard identification

– acute lethal dose tests

Method	Description	Comment
ALD50 (approximate median lethal dose) (OECD 401)	At least 3 doses, at least 5 animals per dose. Endpoint is death.	Precise estimate with 95% confidence interval. No longer permitted in many countries; superceded by OECD 420, 423, 425.
Fixed dose method (OECD 420)	Sequential testing starting at 1 of 5 fixed doses; 5 animals per dose. Endpoint is signs of toxicity not death.	Designed specifically for classification according to Globally Harmonized Classification System (UN, OECD, EU CLP; does not provide a point estimate of LD50.
Acute toxic class method (OECD 423)	Same 5 doses as in fixed dose method, but tests 3 or animals at each dose. Endpoint is death.	(same as fixed dose method)
Up and down procedure (OECD 425)	Tests individual animals sequentially, with the dose for each animal adjusted up or down, depending on outcome in previous animal. Endpoint is death.	Provides a point estimate of LD50, but does not take advantage of the information available on the sequence of events; uses only the final results.

Hazard identification

– skin, eye irritation

- **used to be done in rabbit (Draize test)**

- **Being replaced by in vitro methods**

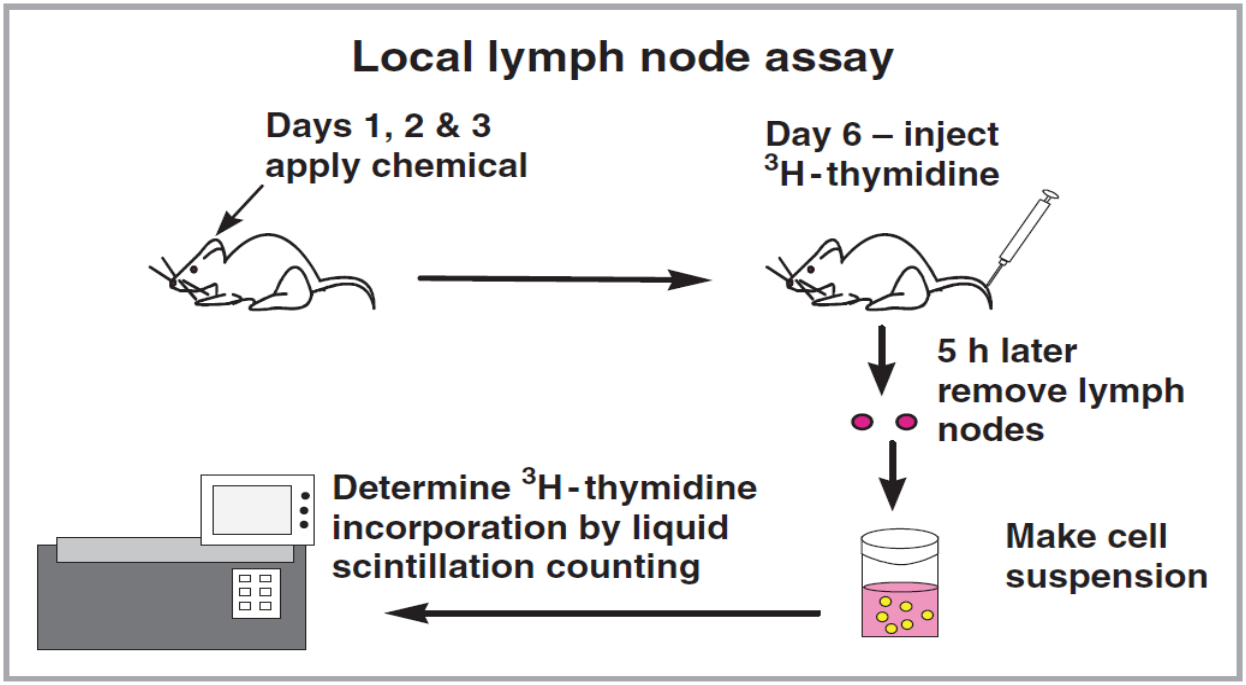
Hazard identification

Skin sensitization

- **The OECD 406 Skin Sensitisation test uses guinea pigs, specifically in the guinea pig maximisation test and the Buehler test.**
- **The mouse Local Lymph Node Assay has been validated (OECD 429) and both it and the two non-radioactive modifications, LLNA: BrdU-ELISA (OECD 442B) and LLNA: DA (OECD 442A), all provide an advantage over the guinea pig tests in OECD 406 in terms of reduction and refinement of animal use.**

Hazard identification

Skin sensitization; LLNA



Basketter DA. Skin sensitization: strategies for the assessment and management of risk. Br J Dermatol. 2008 Aug;159(2):267-73.

Hazard identification

– repeat dose toxicology study; example

Arch Toxicol (2007) 81:361–370
DOI 10.1007/s00204-006-0154-5

ORGAN TOXICITY AND MECHANISMS

Ethylbenzene: 4- and 13-week rat oral toxicity

Werner Mellert · Klaus Deckardt · Wolfgang Kaufmann · Bennard van Ravenzwaay

Hazard identification

Repeat dose toxicology example (OECD 407)

Table 1 Treatment effects in 4-week study

Group	Control	Low	Mid	High	Control	Low	Mid	High
Sex (*N*)	M (5)	M (5)	M (5)	M (5)	F (5)	F (5)	F (5)	F (5)
Dose (mg ethylbenzene/kg/day)	0	75	250	750	0	75	250	750
Body weight (g), day 0 (mean ± SD)	163 ± 5	163 ± 10	165 ± 8	162 ± 8	128 ± 8	128 ± 7	131 ± 10	128 ± 10
Body weight (g), day 28[a]	290	−2%	+1%	−5%	185	−2%	+5%	0%
Clinical signs[b]								
Salivation	0	0	5 (5)	5 (4)	0	0	0	1 (2)
Urine-smeared anogenital region	0	0	0	0	0	0	0	2 (17)
Urinalysis								
Volume (ml)[a]	2.7 ± 0.8	2.9 ± 0.9	3.1 ± 1.4	2.9 ± 0.4	1.8 ± 0.4	−1.2 ± 0.5	2.1 ± 0.7	2.9 ± 1.4
Transitional epithelial cells[c]	0	1	3	5**	0	0	0	0
Granular and epithelial cell casts[c]	0	1	5**	5**	0	0	0	0
Clinical pathology[a]								
Alanine aminotransferase (μkat/l)	0.70 ± 0.09	0.59 ± 0.05	0.67 ± 0.10	0.94 ± 0.17*	0.53 ± 0.10	0.57 ± 0.10	0.67 ± 0.11	0.90 ± 0.19**
Total bilirubin (μmol/l)	1.98 ± 0.51	1.87 ± 0.39	2.26 ± 0.63	3.07 ± 1.16	2.51 ± 0.55	2.00 ± 0.39	2.40 ± 0.49	3.36 ± 0.70*
Cholesterol (mmol/l)	1.81 ± 0.33	1.80 ± 0.20	2.12 ± 0.24	2.57 ± 0.30*	1.50 ± 0.15	1.49 ± 0.23	1.90 ± 0.38	2.09 ± 0.31**
Serum urea (mmol/l)	4.34 ± 0.45	4.42 ± 0.82	4.15 ± 0.73	5.65 ± 0.70*	6.65 ± 1.89	5.28 ± 0.70	5.01 ± 1.57	6.85 ± 1.29
Sodium (mmol/l)	146.2 ± 1.3	146.1 ± 1.0	146.2 ± 1.1	145.0 ± 1.0	145.3 ± 0.8	146.2 ± 1.5	146.1 ± 1.4	142.8 ± 1.6*
Organ weights								
Liver (g)	7.97 ± 0.51	7.90 ± 0.81	9.25 ± 0.74*	9.96 ± 0.30**	5.59 ± 0.43	5.23 ± 0.45	5.91 ± 0.52	6.83 ± 0.56**
Liver (%, related to terminal body weights)	3.00 ± 0.09	3.04 ± 0.09	3.47 ± 0.13**	4.02 ± 0.23**	3.32 ± 0.21	3.20 ± 0.12	3.39 ± 0.07	4.04 ± 0.15**
Kidneys (g)	1.97 ± 0.15	2.04 ± 0.22	2.28 ± 0.11	2.21 ± 0.12	1.34 ± 0.14	1.35 ± 0.11	1.40 ± 0.10	1.49 ± 0.11
Kidneys (%, related to terminal body weights)	0.74 ± 0.03	0.78 ± 0.04	0.86 ± 0.03**	0.89 ± 0.03**	0.80 ± 0.05	0.83 ± 0.05	0.81 ± 0.05	0.89 ± 0.09
Histopathology[d]								
Hepatocyte centrilobular hypertrophy	0	0	5 (1.6)	5 (3.0)	0	0	0	5 (2.4)
Hyaline droplet nephropathy	1 (2.0)	3 (1.0)	5 (2.8)	5 (2.8)	0	0	0	0

*$P \leq 0.05$, **$P \leq 0.01$ versus control by Wilcoxon test or Dunnett's test (body weights) or Fisher's exact test (urinalysis except volume)

[a] For controls, group means are shown, for treated groups, % differences from controls. Statistical significance is based on actual data (not on % differences)

[b] Clinical signs data are number of affected animals, with day of onset in brackets

[c] Data are number of affected animals

[d] Pathology data are animal incidences with mean histopathology grade in brackets (1 = minimal, 2 = mild/slight, 3 = moderate, 4 = marked)

Genotoxicity - history

1973: Paper by Bruce Ames “carcinogens are mutagens”

1980: US test guidelines
- bacterial mutagenicity (Ames)
- in vitro cytogenetics
- in vitro mammalian mutation
- in vivo mammalian cytogenetics (micronucleus test)

(adopted by OECD 1983-1986)

1987: US NTP National Toxicology Program describes “non-genotoxic carcinogen” concept…

2009: WHO/IPCS Harmonized Scheme, mutagenicity testing update (Eastmond DA et al. Mutagenicity testing for chemical risk assessment: update of the WHO/IPCS Harmonized Scheme. Mutagenesis. 2009 Jul;24(4):341-9)

Hazard identification

Genotoxicity – Ames test (OECD 471)

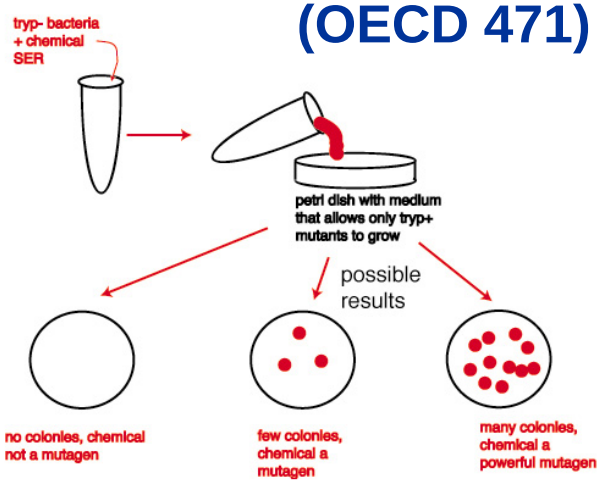
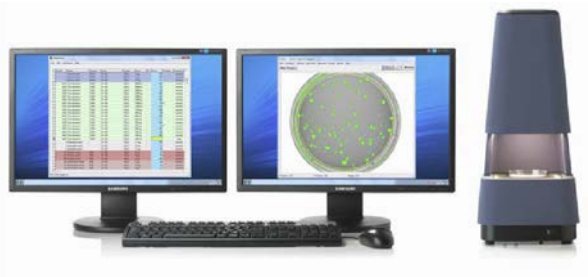


Table 2 - Data obtained in the evaluation of the mutagenicity of root extract (RE) from *C. mollis* with TA98 and TA100 *Salmonella typhimurium* strains concurrent with or without metabolic activation (S9).

Dose (µg/plate)	TA98- S9	Dose (µg/plate)	TA 98+ S9	Dose (µg/plate)	TA100 - S9	TA 100 + S9
0	24.33 ± 4.51	0	33.33 ± 6.11	0	139 ± 3.00	102.33 ± 2.89
4NQO	520 ± 105.83*	2AA	700 ± 100.00*	4NQO (TA100 - S9) or 2AA (TA100+ S9)	1030 ± 62.44*	890 ± 85.44
5	19 ± 2.65	0.005	T	5	139.33 ± 4.51	108 ± 5.57
50	17.67 ± 2.08	0.05	T	50	142 ± 4.36	116.67 ± 7.09
500	21.67 ± 2.52	0.1	T	100	144 ± 6.0	117 ± 7.0
3000	19.67 ± 2.08	0.25	T	200	140.33 ± 4.51	116 ± 9.85
4000	21.33 ± 1.53	0.5	T	300	151 ± 9.54	114 ± 8.89

T = toxic.
*Significant at 5%.
4NQO (0.5 µg/plate) = positive control in tests without S9.
2AA (2.5 µg/plate) = positive control in tests with S9.

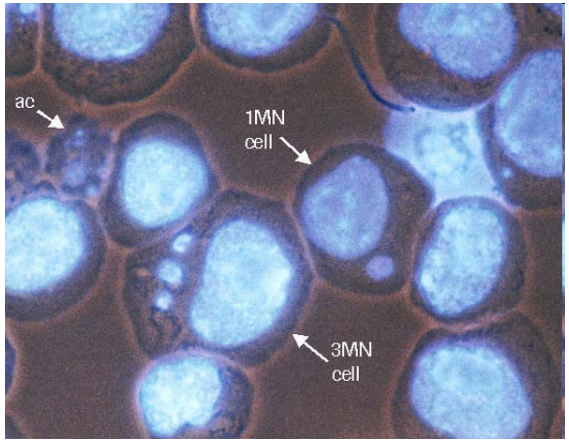


- Ames test = a revertant mutation assay using various strains (TA97, TA98, TA100, TA102, TA1535) of the bacterium *Salmonella typhimurium*. Named for its developer, Bruce Ames.
- Defective gene prevents synthesis of histidine (His-); only DNA “back mutation” revertants grow on histidine-free medium
- Strains show differential sensitivity to various mutagens
  - TA1535 and TA100 to base pair substitution mutagens.
  - TA97 and TA98 to frameshift mutagens
  - TA102 to oxidizing & some cross-linking agents (this is only strain with excision repair)
- To simulate animal metabolism, culture dishes include liver fraction (S9 mix) from rats treated with liver CYP450 enzyme inducers (phenobarbital, benzoflavone etc).

Hazard identification

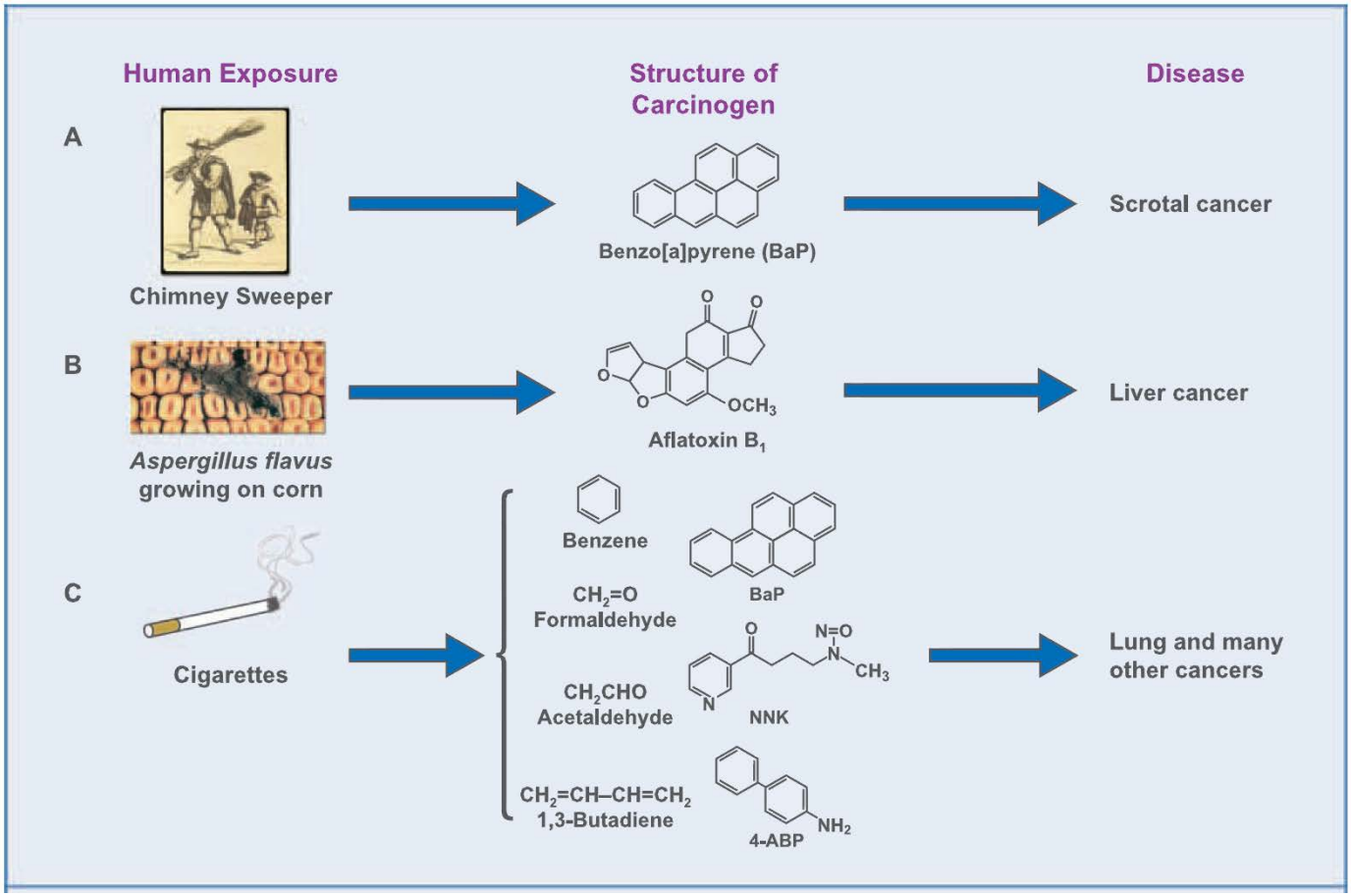
Genotoxicity; Micronucleus test (OECD 487)

Micronuclei may originate from acentric chromosome fragments (i.e. lacking a centromere), or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division. The assay detects the activity of clastogenic and aneugenic chemicals in cells that have undergone cell division during or after exposure to the test substance.



Hazard identification

Carcinogenicity



Loeb LA, Harris CC. Advances in chemical carcinogenesis: a historical review and prospective. Cancer Res. 2008 Sep 1;68(17):6863-72.

Hazard identification

Carcinogenicity assay

- **Two species (mouse, rat), both sexes, at least 50 animals per dose per sex, at least 3 doses + control = at least 800 animals (often run with 2 control groups)**
- **“Sufficiently characterised strains” required**
- **Duration 18-24 months (“majority of lifespan”) plus histopathology + reporting = 3+ years for study**
- **Often combined with Chronic Toxicity study (= interim sacrifice at 12 months)**
- **Special emphasis on time of onset and incidence of non-neoplastic and neoplastic histopathological findings**

Hazard identification

Rodent tumor responses which are not predictive of cancer risk for humans

Renal tumors in male rats	Chemical binds to a2u-globulin. Accumulation in target kidney cells. Increased necrosis, increased regenerative hyperplasia. Renal tubular calcification, neoplasia.	a2u-globulin is a male rat specific low-molecular weight protein not found in female rats, mice, monkeys or humans	Unleaded gasoline: 4-Dichlorobenzene, D-limonene Isophorons, Dimethylmethylphosphonate, Perchloroethylene, Pentachloroethane, Hexachloroethane
Urinary bladder	Chemical precipitates out at high concentration, induces cytotoxicity and reactive hyperplasia.	Rodent exposure levels exceed solubility, not relevant for human exposure	Saccharin, melamine, nitrilotriacetic acid, fosetyl-A2
Forestomach	Direct irritation of stomach by gavage application. Local cytotoxicity. Hyperplasia.	Rodent gavage treatment, exposure conditions not relevant for human exposure	BHA, propionic acid, ethyl acrylate
Thyroid gland tumors	Substance alters thyroid homeostasis. Decreased thyroid hormone production. Sustained increase in thyroid stimulating hormone (TSH). Thyroid tumors.	Lack of thyroid-binding protein in rodents versus humans. Decreased t1/2 for T4; increased TSH levels in rodents	Ethylene bisdithio-carbamate, fungicides, amitrole, goitrogens, sulfamethazine
Rat lung	Substance overwhelms clearance mechanisms	High dose effects seen with rodent models	Various particles, titanium dioxide

Casarett & Doull 2008, p.113

Hazard identification

Number of animals required by test guidelines

Type	OECD TG No.	Animals/ dose	Animals/ test
Acute toxicity	420, 423, 425	2–5	8–15
Acute dermal toxicity	402	5	25–30
Acute inhalation toxicity	403	5	40–50
Repeated dose toxicity (28 d)	407, 410, 412	5	40
Repeated dose toxicity (90 d)	408, 411, 413	10	80
Carcinogenicity study	451, 453	50	400
Reproductive toxicity (screening)	421	10	560[a]
Reproductive toxicity (repeated dose)	422	10	412[a]
Reproductive toxicity (prenatal development)	414	20	784[a]
Reproductive toxicity (prenatal development)	414 + 2nd species	20	560[a]
Reproductive toxicity (2-generations)	416	20	3200[a]
Reproductive toxicity (2-generations)	416 + 2nd species	20	2100[a]
Reproductive toxicity (developmental neurotoxicity)	426	20	1400[a]

[a] Includes offspring.

Oberg M. Benchmark dose approaches in chemical health risk assessment in relation to number and distress of laboratory animals. Regul Toxicol Pharmacol. 2010 Aug 25.

Hazard identification

Use of animals by test system…

Animal use in EU regulatatory toxicology studies 1999 by study type:

- **Single dose toxicity (35%)**
- **Repeat dose toxicity (27%)**
- **Reproduction toxicology (13%)**
- **Mutagenicity and carcinogenicity (8%)**
- **Skin sensitisation (5%)**
- **Skin/eye corrosion (3%)**
- **Phototoxicity (3%)**
- **Toxicokinetics (2%)**
- **Endocrine disrupters (2%)**
- **Skin irritation (1%)**
- **Eye irritation (1%)**

(http://ec.europa.eu/dgs/jrc/downloads/jrc_press_animal_ecvam_overview.pdf)

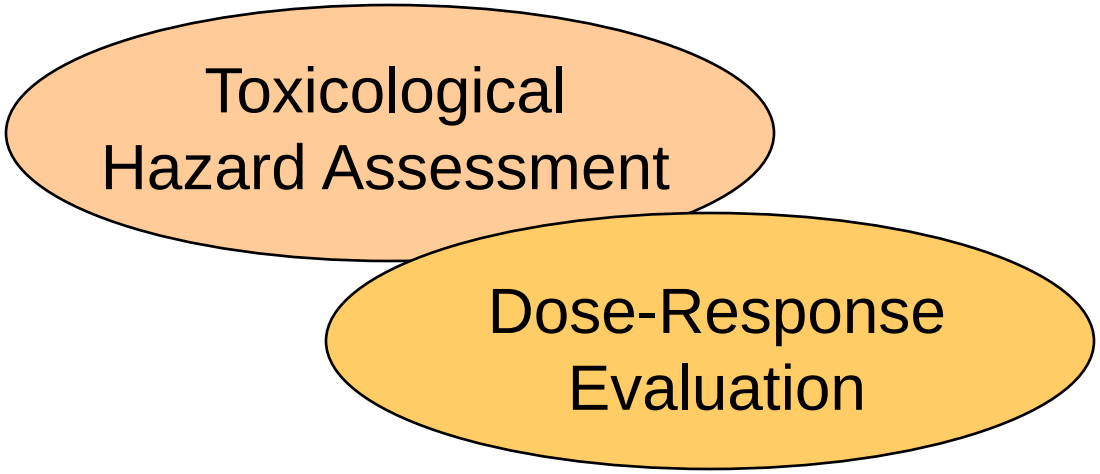
Hazard identification

Conclusion

Hazard identification

- **is a highly structured process regulated by guidelines**
- **requires many animals but as few as possible**
- **Is the basis for human risk assessment**
- **will change as new scientific methods develop**

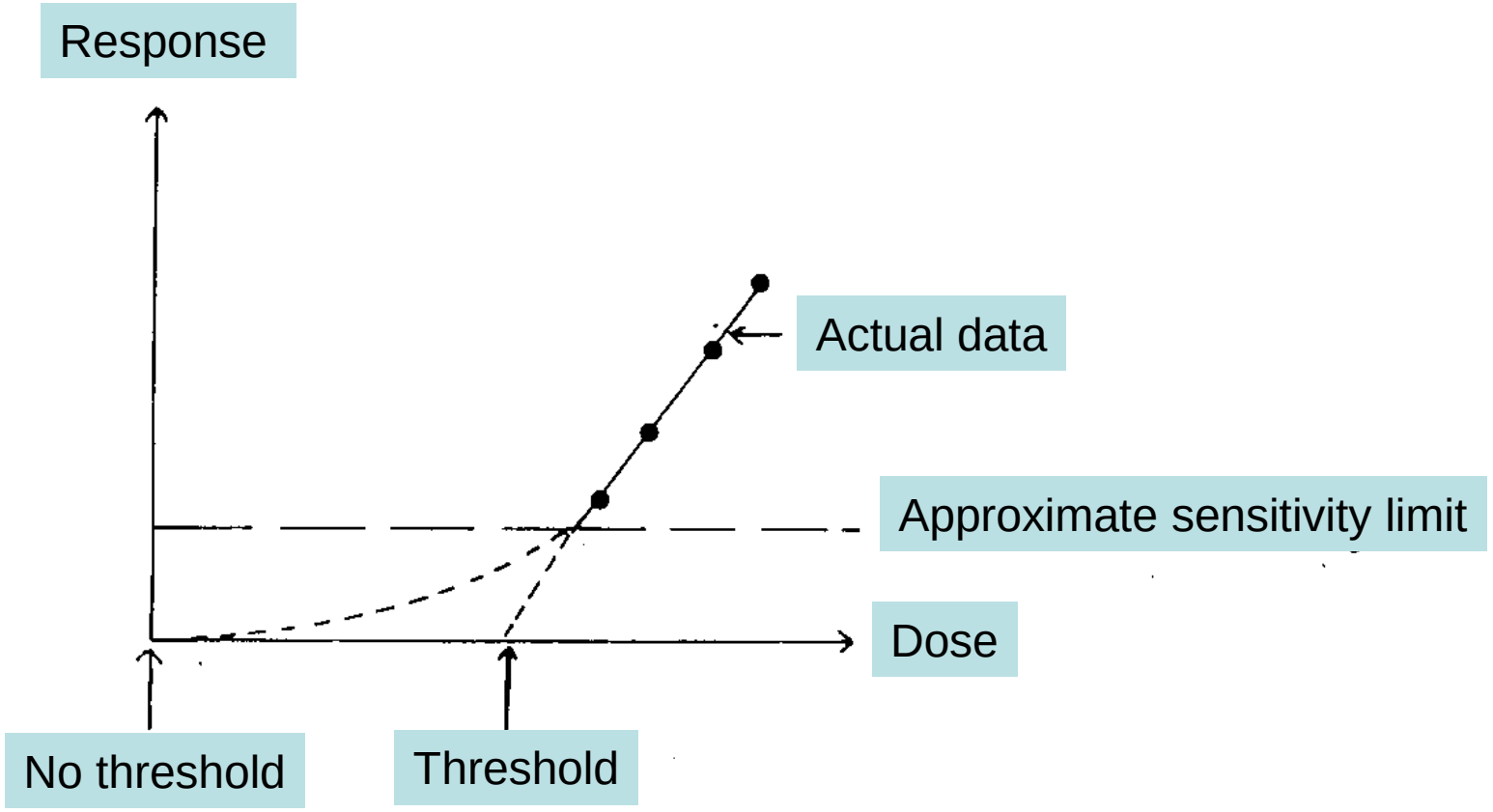
Stages of Risk Assessment



Analysis of the relationship between the amount of an agent taken up by an organism and the changes developed in that organism in reaction to that agent.

Dose-response relationships

The dose-response curve…

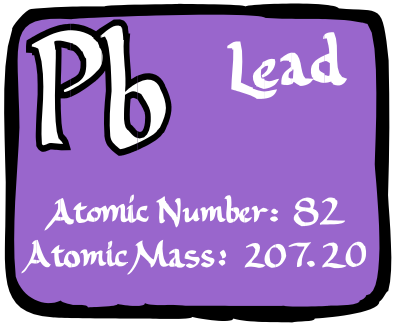


Dose-response relationships

Dose-response threshold...

- **Report of the U.S. Surgeon General 2006**
  - "The scientific evidence indicates that there is no risk free level of exposure to second-hand smoke."

- **CDC 2005**
  - "…data demonstrating that no "safe" threshold for blood lead levels in young children has been identified…"



Dose-response relationships

Dose-response curve

A, B, C, D: Options for low-dose extrapolation (below observed response level)

E: Point of departure (POD)

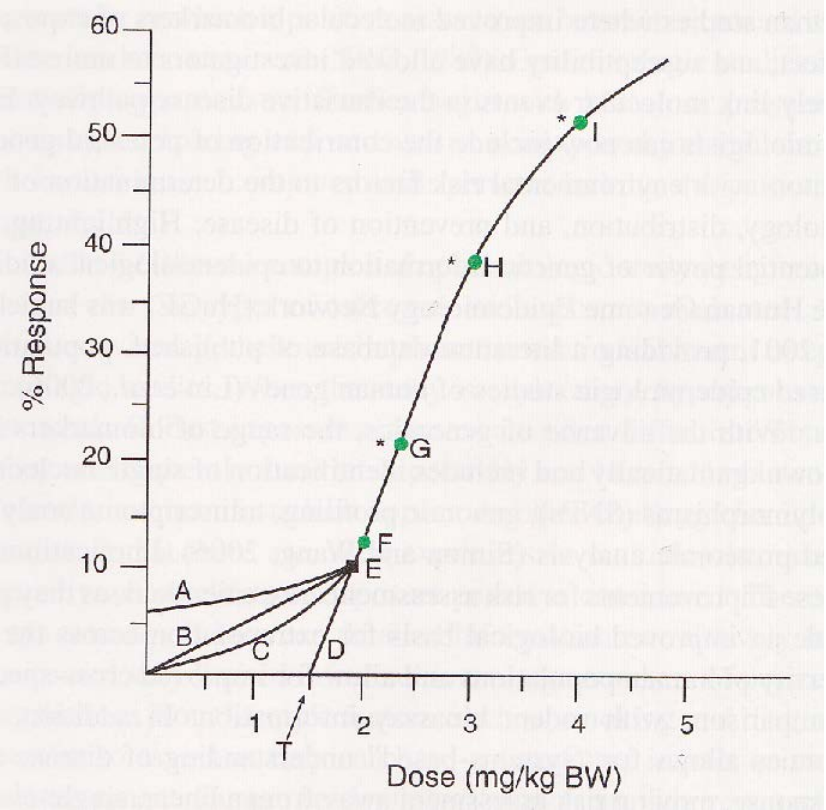
F: No observed adverse effect level (NOAEL)

G: Lowest observed adverse effect level (LOAEL)

H, I: Mid and high dose response

T: Threshold dose

ED10 (EC10)
ED50 (EC50)
etc...



Casarett & Doull 2008

Dose-response relationships

- effect of plotting log dose

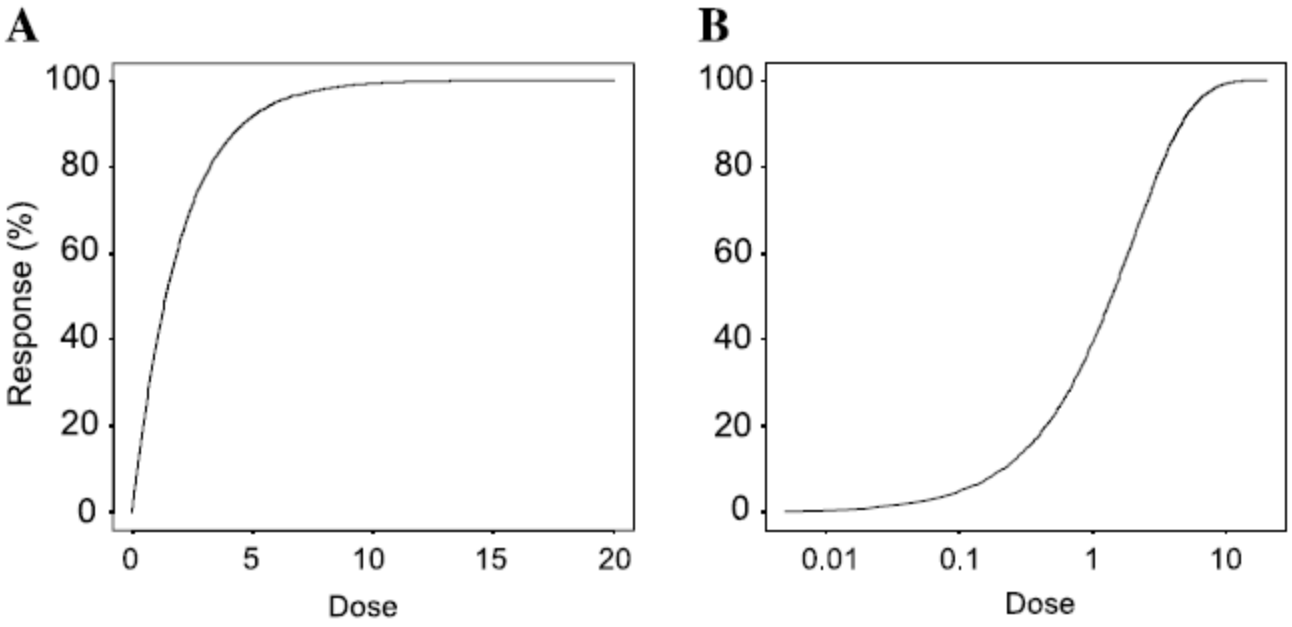
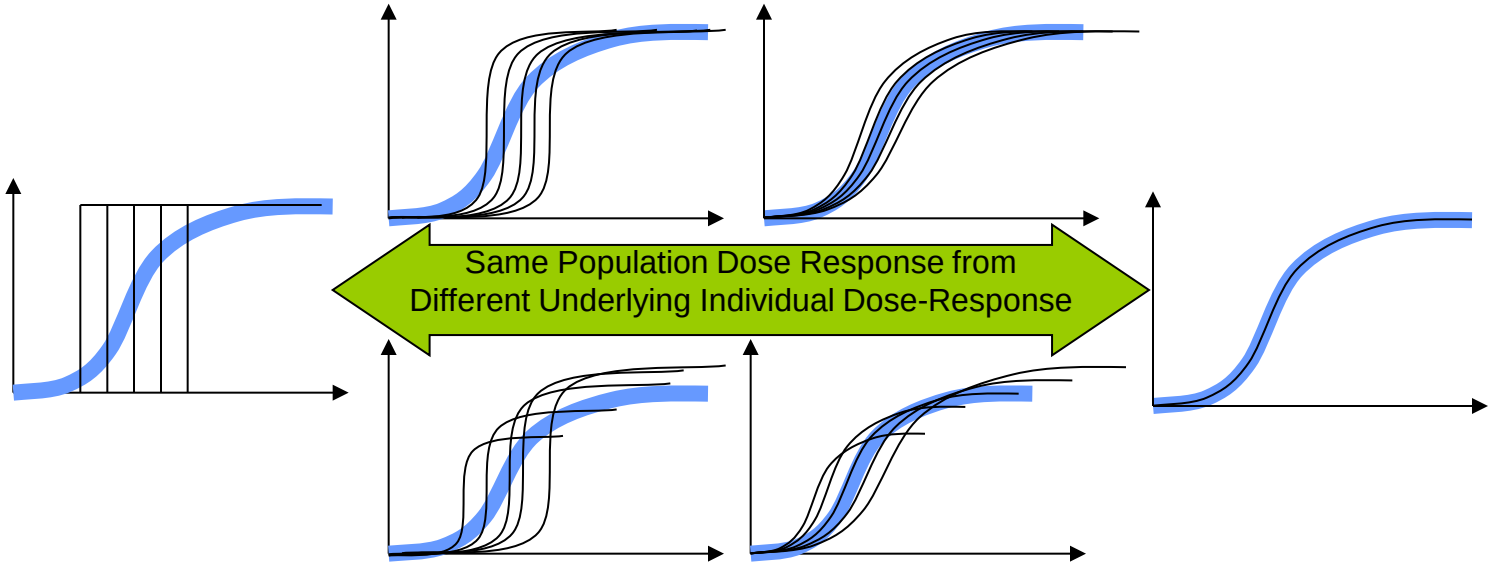


Fig. 1. Dose–response relationship in a receptor-mediated reaction. (A) Arithmetically scaled plot; (B) same data but *x*-axis is now logarithmically scaled (log 10 (*x*)); (Graphics generated by C. Ittrich).

Schwarz & Appel (2005) Reg Tox Pharmacol 43 19

Dose-response relationships

average responses...



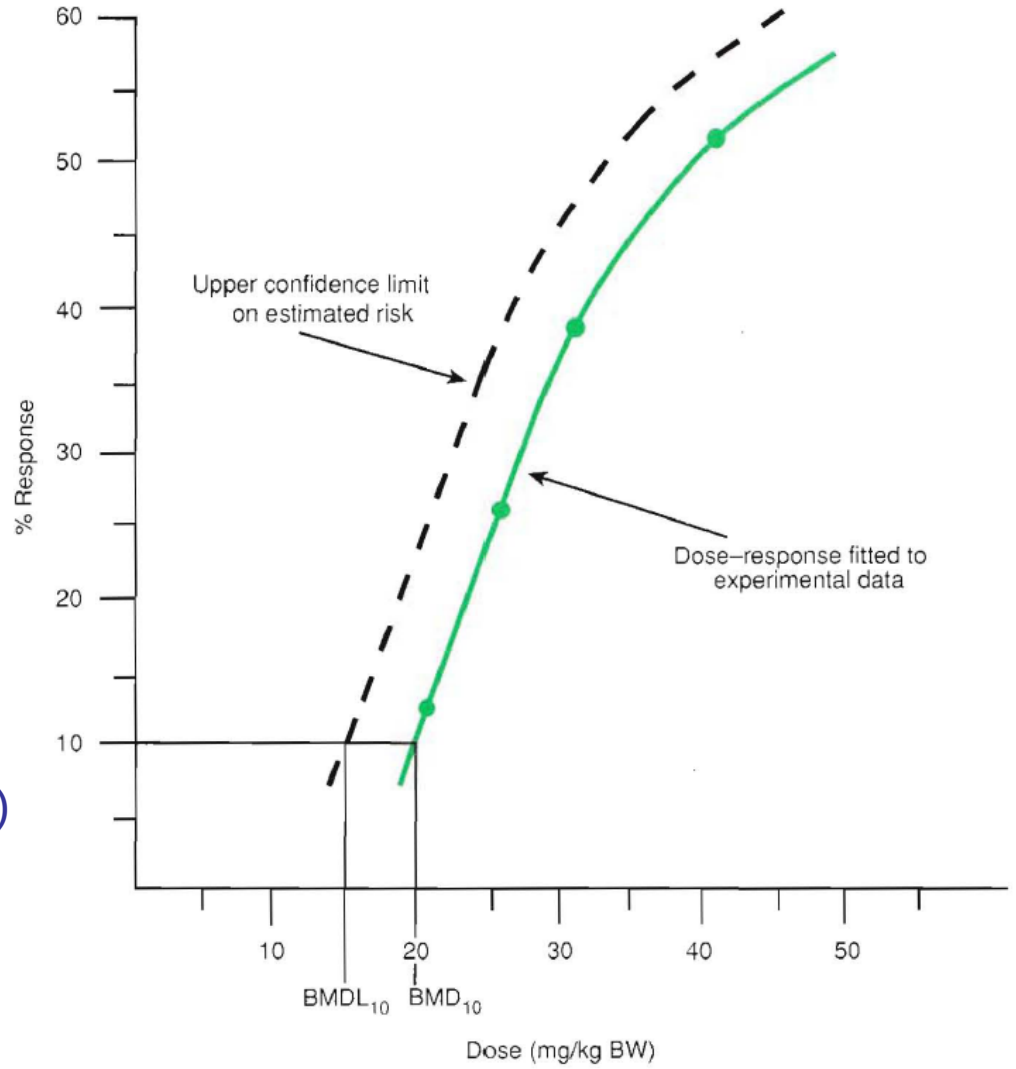
Dose-response relationships
- benchmark dose

Benchmark Dose (BMD)

BMD10 = 10% increase versus control

BMDL10 = lower one-sided 95% confidence interval for BMD10

(EPA software link: http://www.epa.gov/ncea/bmds/)



Casarett & Doull 2008

Dose-response relationships
- benchmark dose

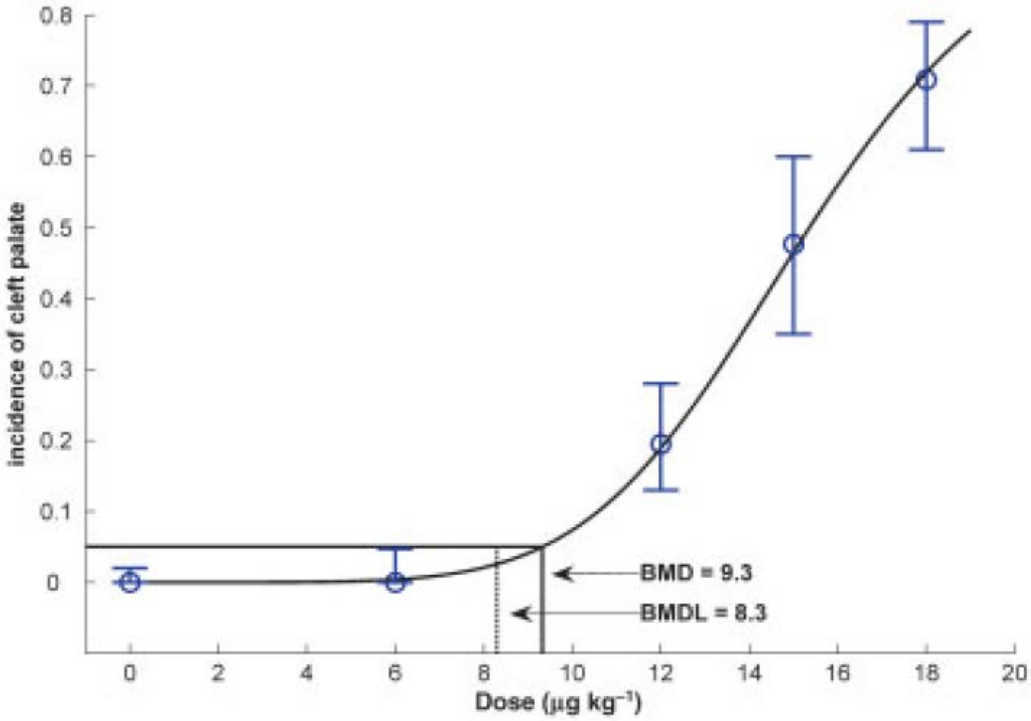
Benchmark Dose (BMD)

Data are cleft palate incidences in mouse fetuses following in utero exposure to 2,3,7,8-tetracholoridibenzo -*p*-dioxin (TCDD) (NOAEL=6, LOAEL=12 µg/kg/day).

Curve fitted by log-logistic function.

BMD = 5% inceased incidence versus controls.

BMDL = lower one-sided 95% confidence interval for BMD



Sand S, Victorin K, Filipsson AF. The current state of knowledge on the use of the benchmark dose concept in risk assessment. J Appl Toxicol. 2008 May;28(4):405-21.

Dose-response relationships

- benchmark dose

BMD advantages

- **includes information about dose–response relationship**
- **can be calculated from experiments lacking NOAEL**
- **confidence interval can be calculated**
- **can be combined with probabilistic exposure analyses**
- **can be used to develop relative potency values used for risk assessment of mixtures**
- **used in risk assessment of genotoxic carcinogens by EPA**

BMD disadvantages

- **there should be at least 5 dose groups for a robust curve fit, but current guidelines specify only 4 (control, low, mid, high)**
- **increasing number of animals and dose groups will produce narrower confidence interval and thus a higher POD compared with the NOAEL, so encouraging use of more animals**
- **animal numbers can be reduced by using less in high dose groups, but this would (will) require a change in the guidelines…**

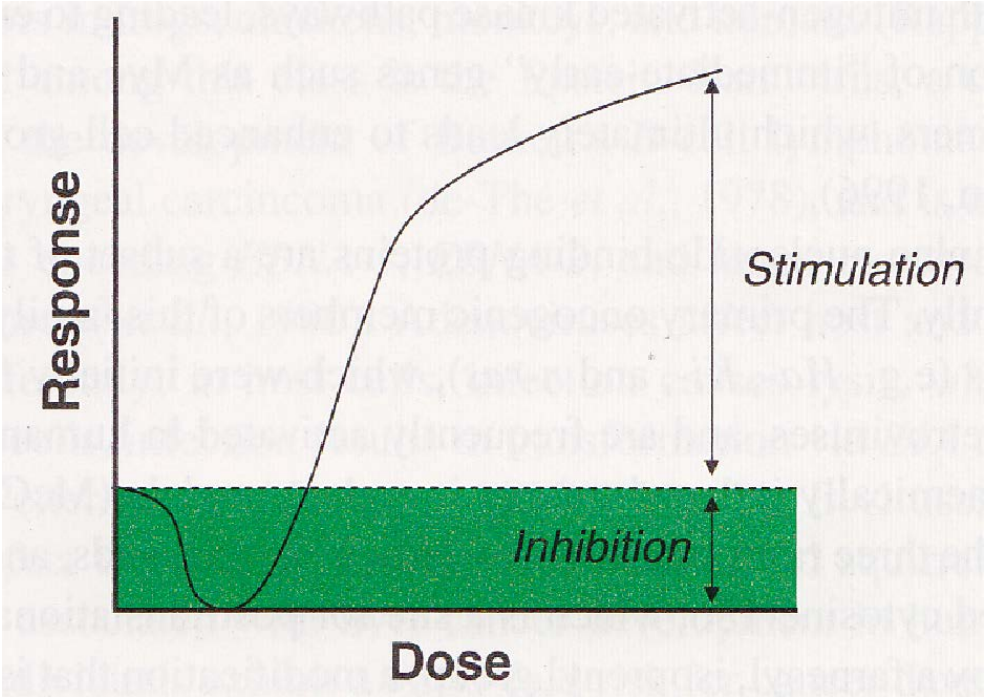
(Öberg M. Benchmark dose approaches in chemical health risk assessment in relation to number and distress of laboratory animals. Regul Toxicol Pharmacol. 2010 Aug 25)

Hormesis

Hormesis = opposite effects at low versus high dose, resulting in either a J-shaped or an inverted U-shaped dose response

Adaptive responses to maintain homeostasis (e.g. enzyme induction) are proposed as mechanisms of action in some cases.

Hormesis is not (yet) included in regulatory toxicology and risk assessment.



Hoffmann GR. A perspective on the scientific, philosophical, and policy dimensions of hormesis. Dose Response. 2009;7(1):1-51.

Dose-response relationships

No-threshold…

No-threshold substances: <u>any</u> exposure is presumed to represent a hazard,

e.g. US EPA (Safe Drinking Water Act) Maximum Contaminant Level Goal "MCLG" is set to zero for

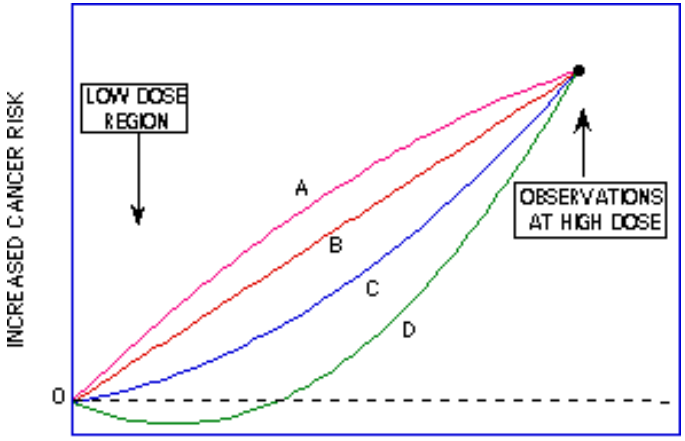
- microbial contaminants that may present public health risk, "because ingesting one protozoa, virus, or bacterium may cause adverse health effects"

- chemical carcinogens without a no-effect-level (implying one molecule may cause cancer).

Dose-response relationships

No-threshold…

- **The Linear No-Threshold model (LNT) assumes damage caused by ionizing radiation is directly proportional to the dose at all dose levels, i.e. no threshold. Sometimes applied to other cancer hazards such as carcinogenic polychlorinated biphenyls in drinking water (EPA)**
  - Alternative 1:
    - below a certain level, radiation exposure is harmless - in other words that there is a threshold for radiation damage.
  - Alternative 2:
    - The radiation hormesis model asserts that radiation is beneficial in very low doses, while still recognizing that it is harmful at high doses.

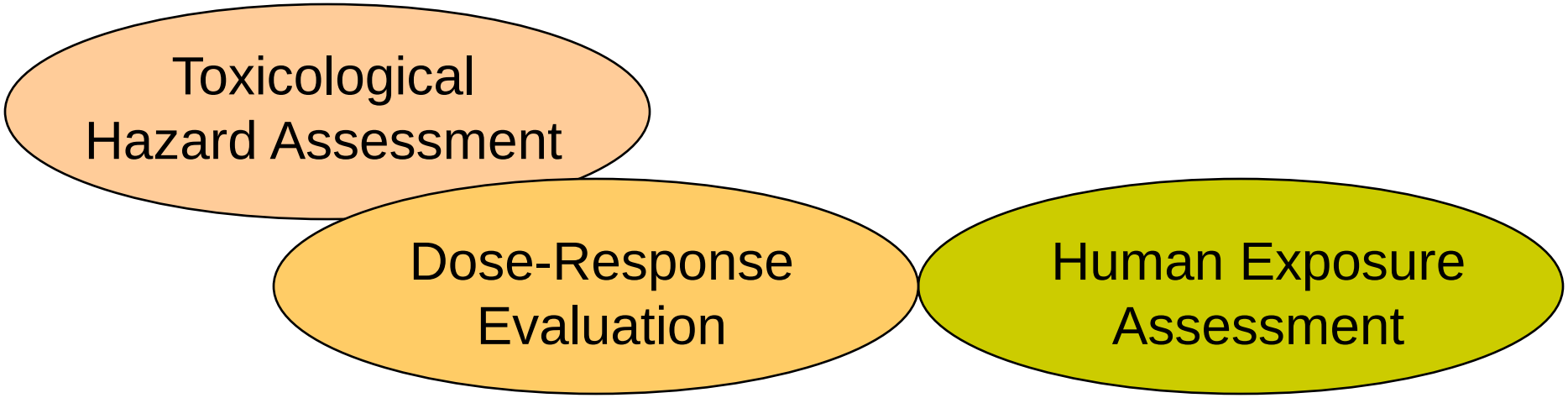


Dose-response relationships

Conclusions…

- **BMD is superior to NOEL for regulatory purposes and is rapidly replacing it in regulatory hazard and risk assessment**
- **Linear/non-linear, threshold/non-threshold, hormesis/low-dose effects all present challenges to be solved in hazard assessment prior to risk characterization**

Stages of Risk Assessment



Assessment of intensity, frequency, duration and routes of exposure for the purpose of quantification of internal dose

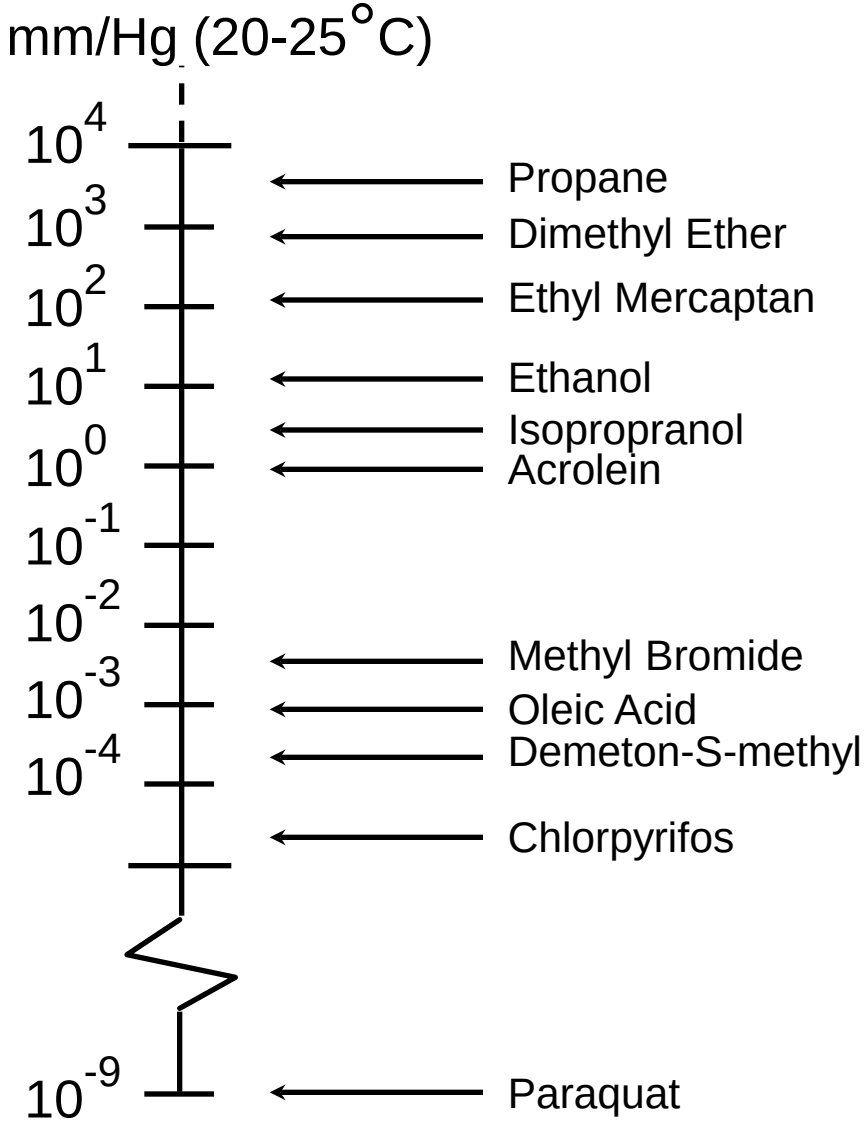
Exposure – Scope

- **Main types of exposure**
  - Occupational
  - Environmental
  - Dietary
- **Main routes of exposure**
  - Inhalation
  - Dermal
  - Oral
- **Methods for exposure assessment**
  - Ambient monitoring
  - Personal monitoring (incl. Biomonitoring)
  - Modelling

Inhalation

- **Volatility**
- **Particle size**

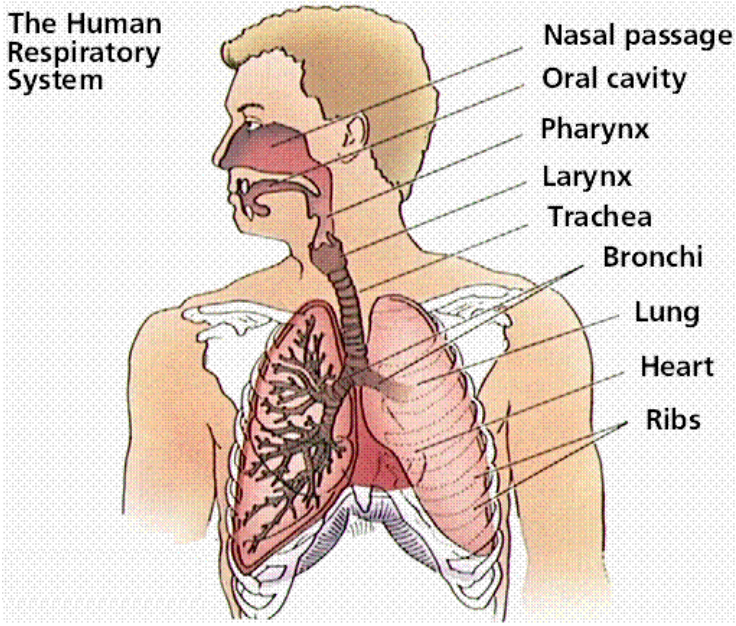
Vapour Pressure of some chemicals and pesticides



Inhalation Exposure

Effect of particle size

Particle Size (μ)	Respiratory Penetration
> 7	Trapped in the mouth/nose & throat, may be ingested 'inhalable'
2 - 7	Trapped in the trachea/bronchi may be ingested after expulsion by lung defence mechanisms
< 2	Penetration to the alveoli may be exhaled or absorbed into blood/lymph systems 'respirable'



Industrial vs. agricultural exposure

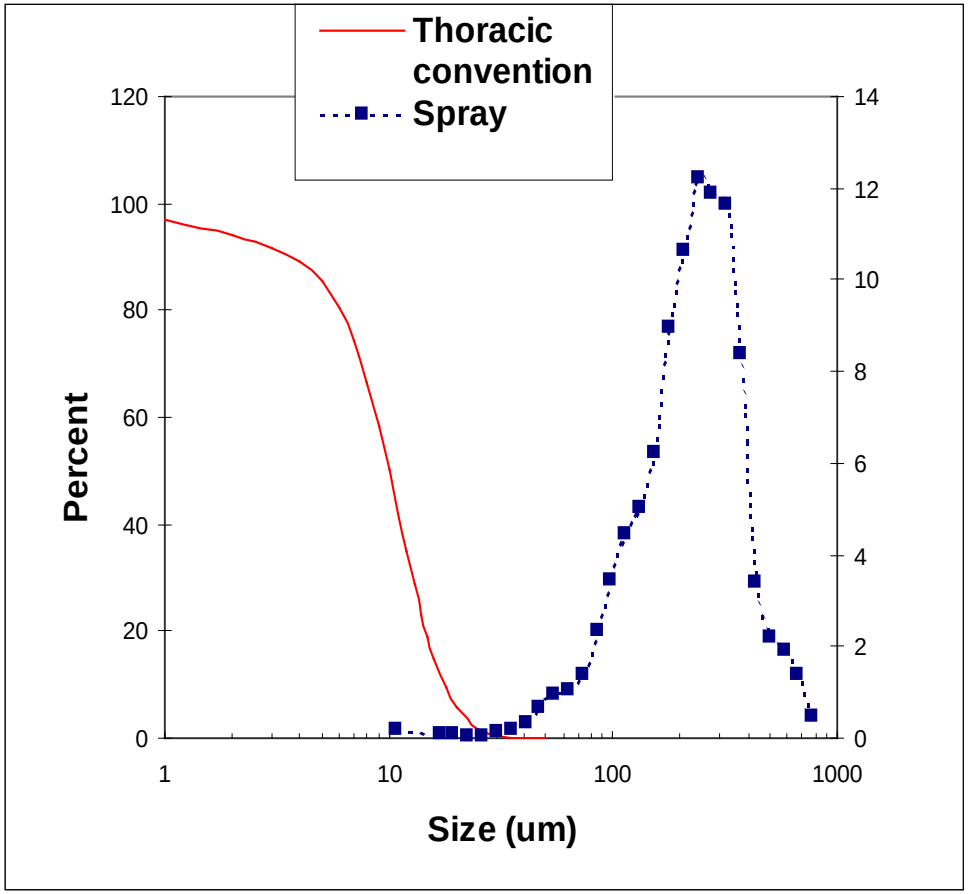
Workplace exposure in factories is primarily through inhalation

Skin exposure is important in specific situations (e.g. cleaning)

Agricultural exposure is primarily via the skin (mainly hands)

Inhalation exposure is important in specific situations (e.g. seed treatment)

Particle size distribution of a typical knapsack spray application compared to respirable particle size in humans



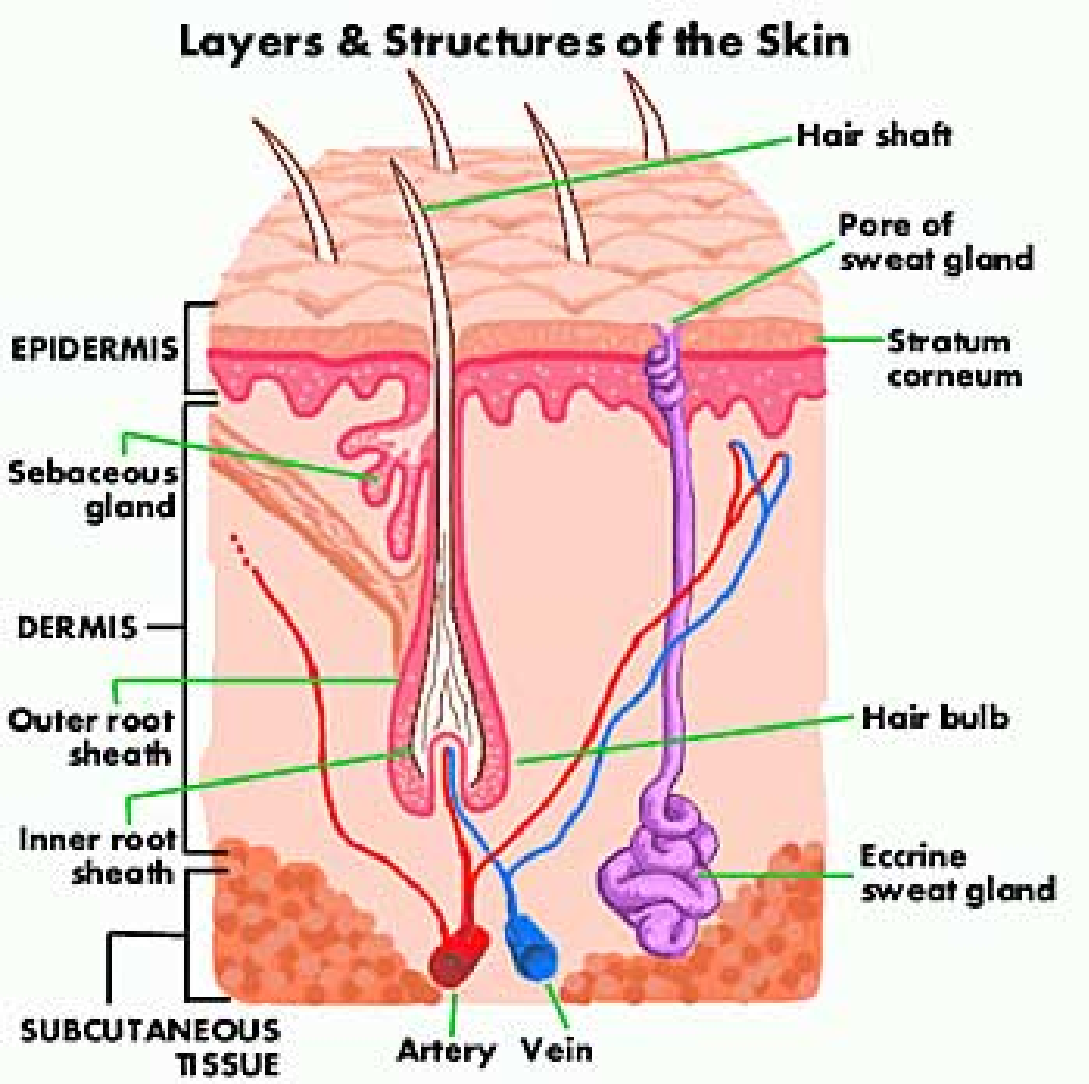
There is practically no overlap, the toxicologically significant fraction is < 0,2%

Skin Contact

The skin is the most important route of exposure for most pesticide applications.

Rate of diffusion depends on:

- Chemical properties
- Amount on skin
- Contact time

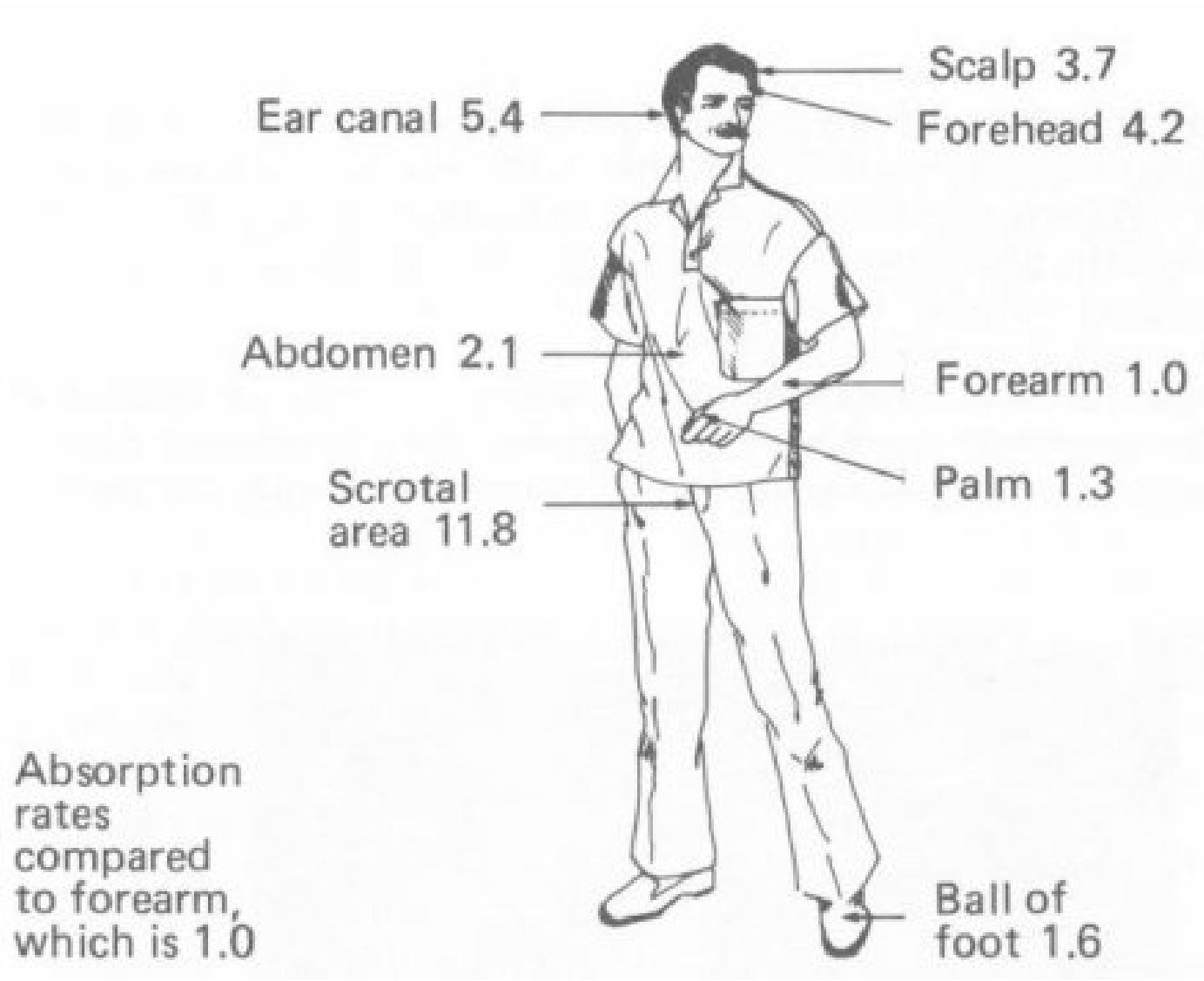


Skin absorption of some pesticides

Compound	% Dose	Compound	% Dose
Carbaryl	73.9	Aldrin	7.8
Propoxur	19.6	Dieldrin	7.7
Azinphos-methyl	15.9	2,4-D	5.8
Monocrotophos	14.7	Ethion	3.3
Parathion	9.7	Diquat	0.4
Lindane	9.3	Paraquat	0.3
Malathion	8.2		

Feldmann and Maibach, 1974
Wester et al., 1984

Absorption through intact skin



Environmental exposure

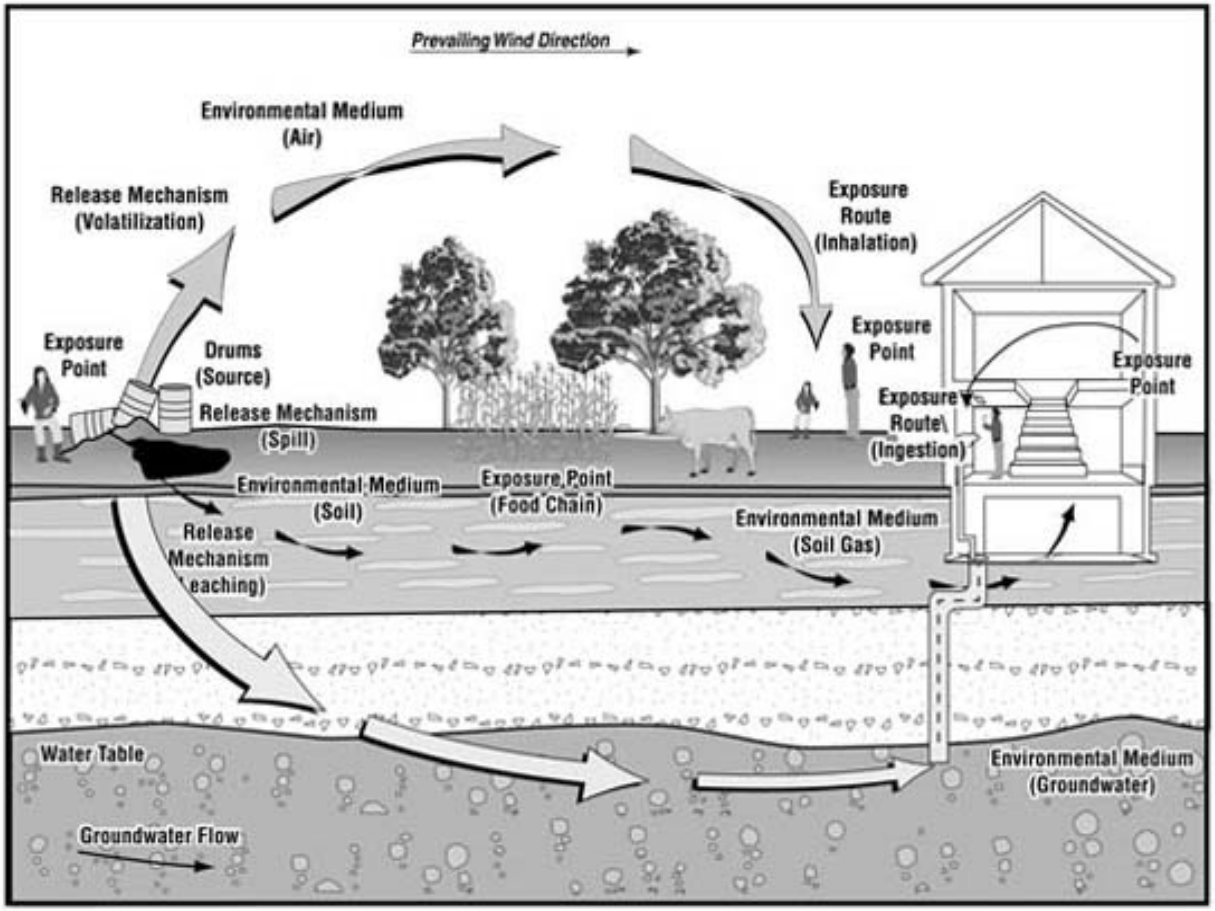


Figure 6-2. Site Conceptual Model—Exposure Pathway Schematic

Occupational vs. environmental exposure

- **Occupational**
  - Small number of chemicals in a single workplace
  - Potentially high exposure
  - Exposure during working week
  - Inhalation is major route of absorption
  - Hierarchy of control measures for individual protection
  - Adult, generally healthy population
- **Environmental**
  - Large number of chemicals
  - Generally low level of exposure
  - Exposure at various times
  - Various routes of exposure depending on source
  - Control measures at population level
  - Large variation in age, health and socioeconomic status

National Report on Human Exposure to Environmental Chemicals

The *Fourth National Report on Human Exposure to Environmental Chemicals* is the most comprehensive assessment to date of the exposure of the U.S. population to chemicals in our environment. CDC has measured 212 chemicals in people's blood or urine—75 of which have never before been measured in the U.S. population. What's new in the *Fourth Report*

The blood and urine samples were collected from participants in CDC's National Health and Nutrition Examination Survey, which is an ongoing survey that samples the U.S. population every two years. Each two year sample consists of about 2,400 persons. The *Fourth Report* includes findings from national samples for 1999–2000, 2001–2002, and 2003–2004. The data are analyzed separately by age, sex and race/ethnicity groups.

The *Updated Tables, July 2010* provides additional data from the 2005-2006 survey period for 51 of the chemicals previously reported through 2004 in the *Fourth Report* and the new addition of four parabens and two phthalate metabolites in 2005-2006.

Text

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Cont

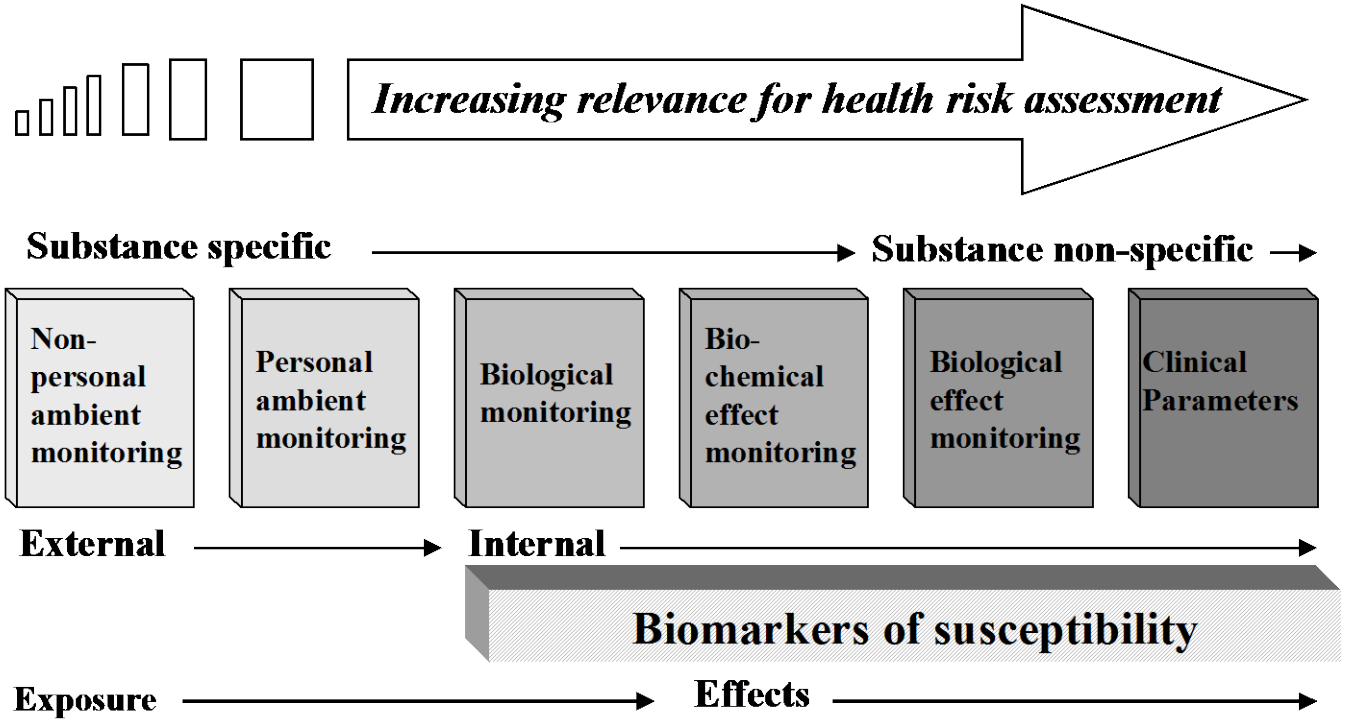
Fourth National Report

National Biomonitoring Program

Exposure Assessment

- **Ambient Monitoring**
  - Concentration in work environment
  - Residue levels in food or water
- **Personal Monitoring**
  - Inhalation exposure
  - Skin exposure
  - Biological monitoring
- **Modelling**
  - Deterministic
  - Probabilistic

Monitoring techniques as part of the exposure-disease continuum



Personal Monitoring Exposure Study

- **Field part (Passive Dosimetry Studie)**
  - Dressing the operators in dosimeters, including fixing of air sampler

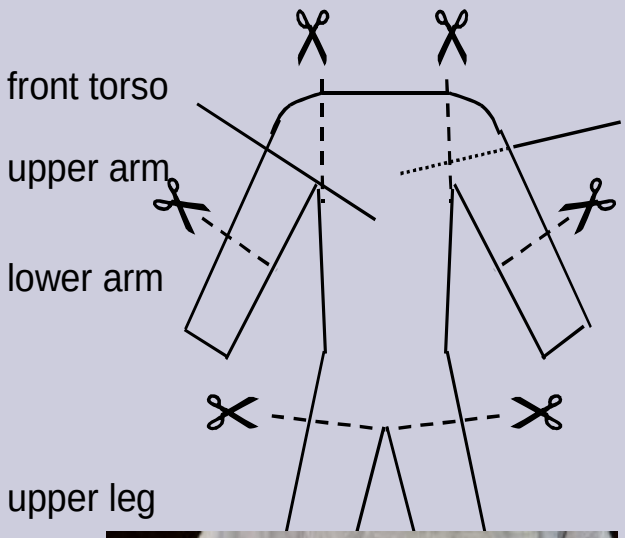
... Application

- Observation and documentation of application process.
- Monitoring during a typical working day

... After Work: Collection of Dosimeters

- Collection of samples after completion of field work

... Preparation of Dosimeters in Field Lab



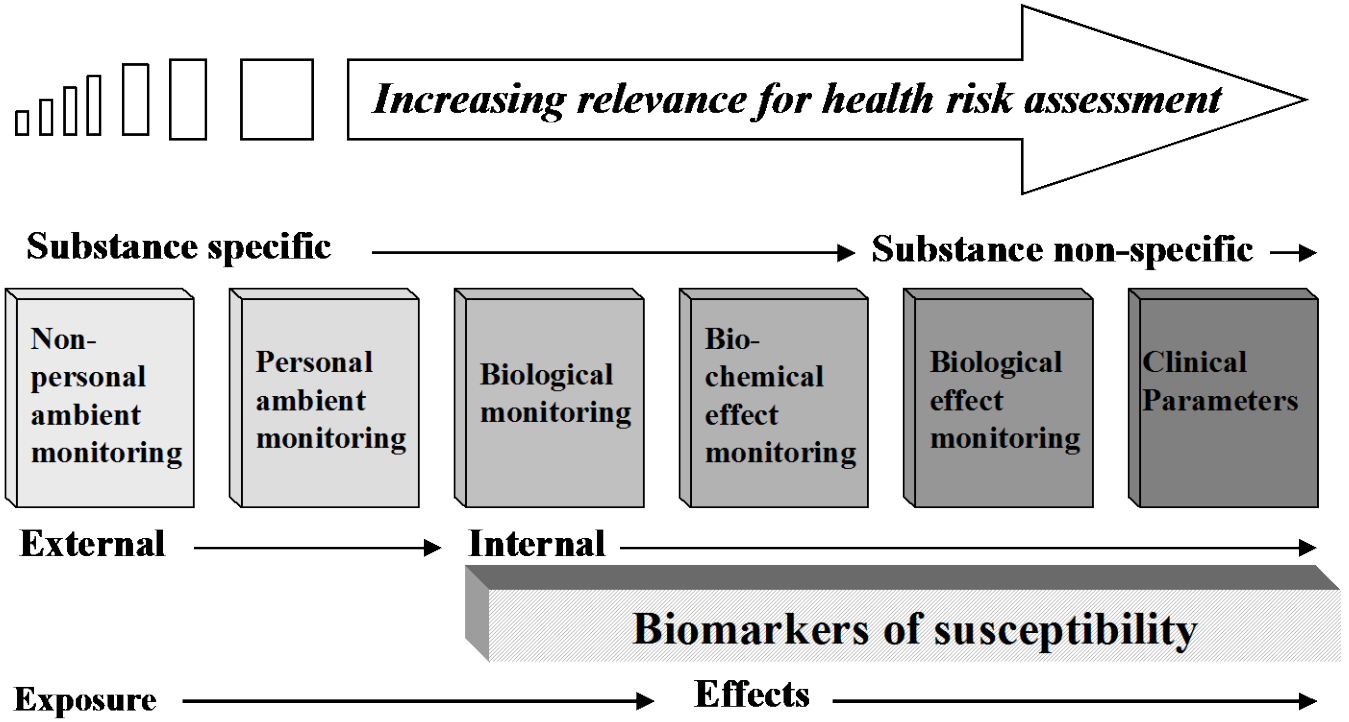
– Sectioning, wrapping up, labelling, shipment to laboratory.

Calculation of Exposure Results

- **Potential Exposure:**
  - Sum of all residues found on inner and outer dosimeter, hand and face wash + respiratory exposure (residues on air samplers corrected by standard breathing volume).
- **Actual exposure:**
  - Sum of all residues found on inner dosimeters, hand and face wash + respiratory exposure .
- **Calculated systemic exposure:**

E = Dermal Exp x Dermal Abs + Inhalation Exp

Monitoring techniques as part of the exposure-disease continuum



Biological Monitoring

- **Measuring of <u>real exposure</u> by monitoring of body fluids (blood or urine)**

Biological markers of exposure

- **Indicative of an action of the chemical at the cellular or molecular level leading to measurable alteration of biochemistry or molecular interaction which may lead to cell death or cell repair.**
  - Inhibition of a marker enzyme (e.g. plasma buturyl (pseudo)cholinesterase, RBC acetyl ChE)
  - Macromolecule adducts (haemoglobin, DNA)

Biological markers of effect/disease

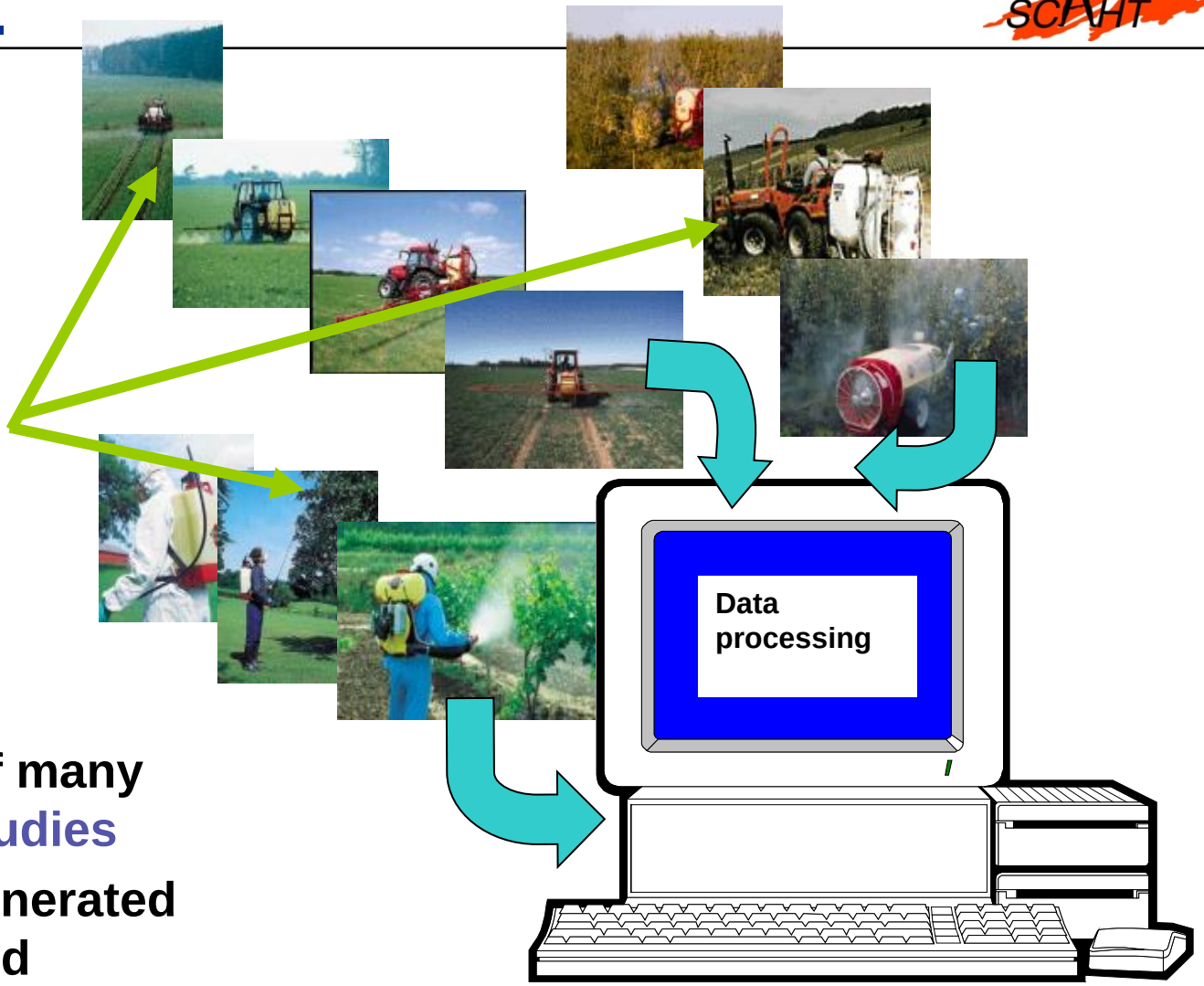
- **These are the consequences of functional change or structural change as a result of organ or system pathology occuring.**
  - Cytogenetic methods (e.g. chromosomal aberration, SCE, micronuclei, mutations in proto-oncogens)
  - Organ toxicity (e.g. renal: urinary proteins and enzymes; nervous system: RBC acetyl ChE, lymphocyte NTE)

- **Identify individuals or (sub)populations who biotransform absorbed chemicals into effective doses at lower exposure levels than the rest of the population, or are less capable of xenobiotic detoxification**
  - Polymorphic drug metabolising enzymes (e.g. epoxide hydrolase, cytochrome P450, glutathion-S-transferases)
  - Paraoxonase (PON1)
  - May be affected by gene/environment interactions

Modelling of exposure

- **Deterministic**
  - Predictive Operator Exposure Models (POEM)
- **Probabilistic**
  - Stochastic Human Exposure and Dose Simulation Multimedia Model (SHEDS)
- **Physiologically Based Pharmacokinetic (PBPK) models**

POEMs Are...



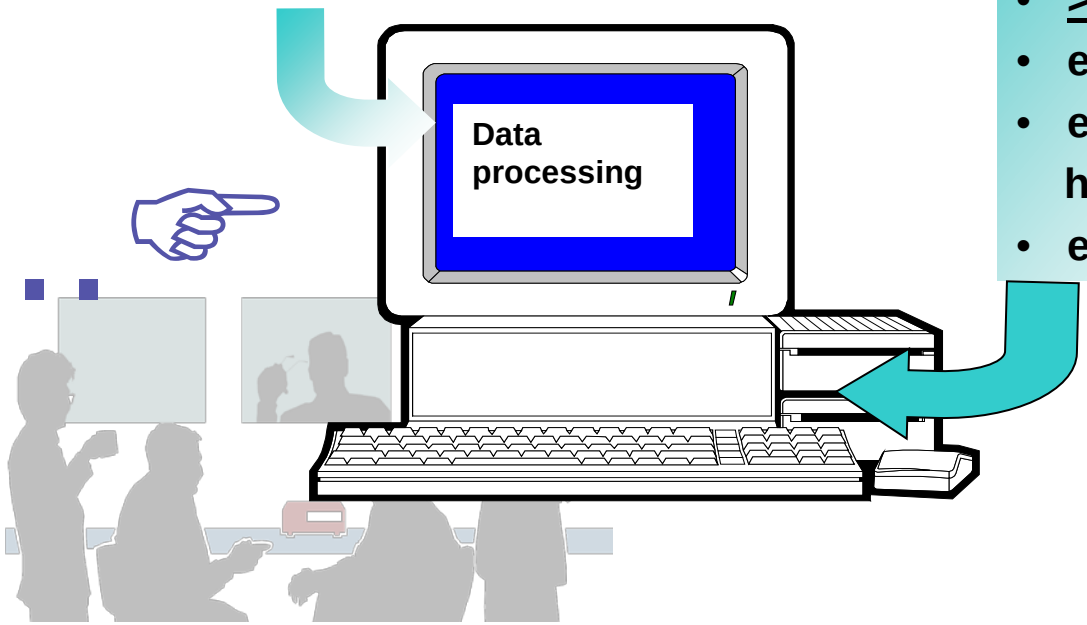
...the sum of many exposure studies

that were generated and compiled

...+

Standard assumptions

- operator body weight
- daily work rate
- dermal absorption



Decision on statistics

- geomean
- 75th percentile
- 90th percentile
- ≥ maximum value
- exposure per hour
- exposure per kg a.i. handled
- exposure per activity

… then they are standardised, and statistical rules are added

EPA
United States
Environmental Protection
Agency

Introduction to and Overview of ORD's Stochastic Human Exposure and Dose Simulation model for multimedia, multiroute/pathway chemicals:

SHEDS-Multimedia

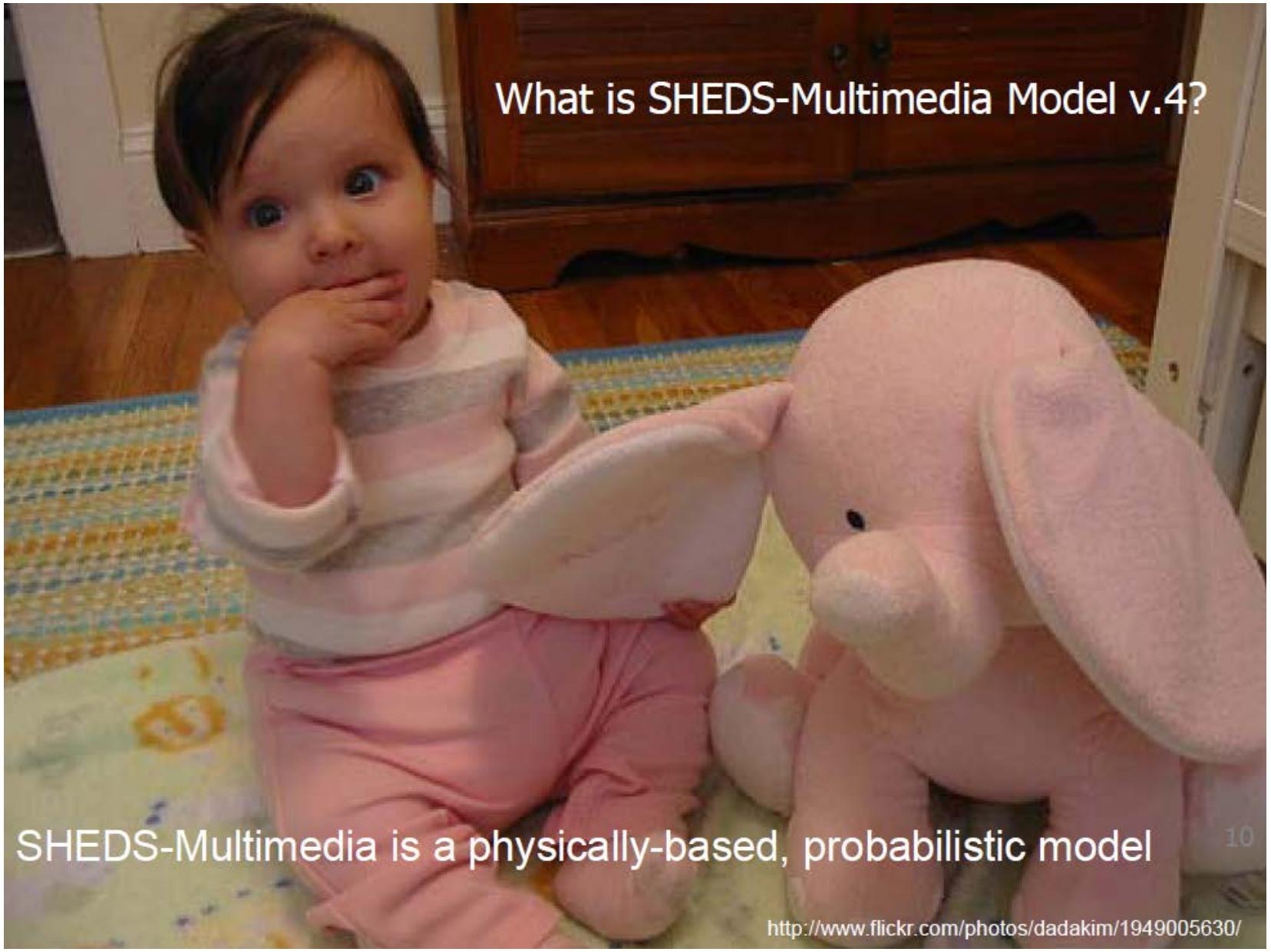
Andrew M. Geller, PhD

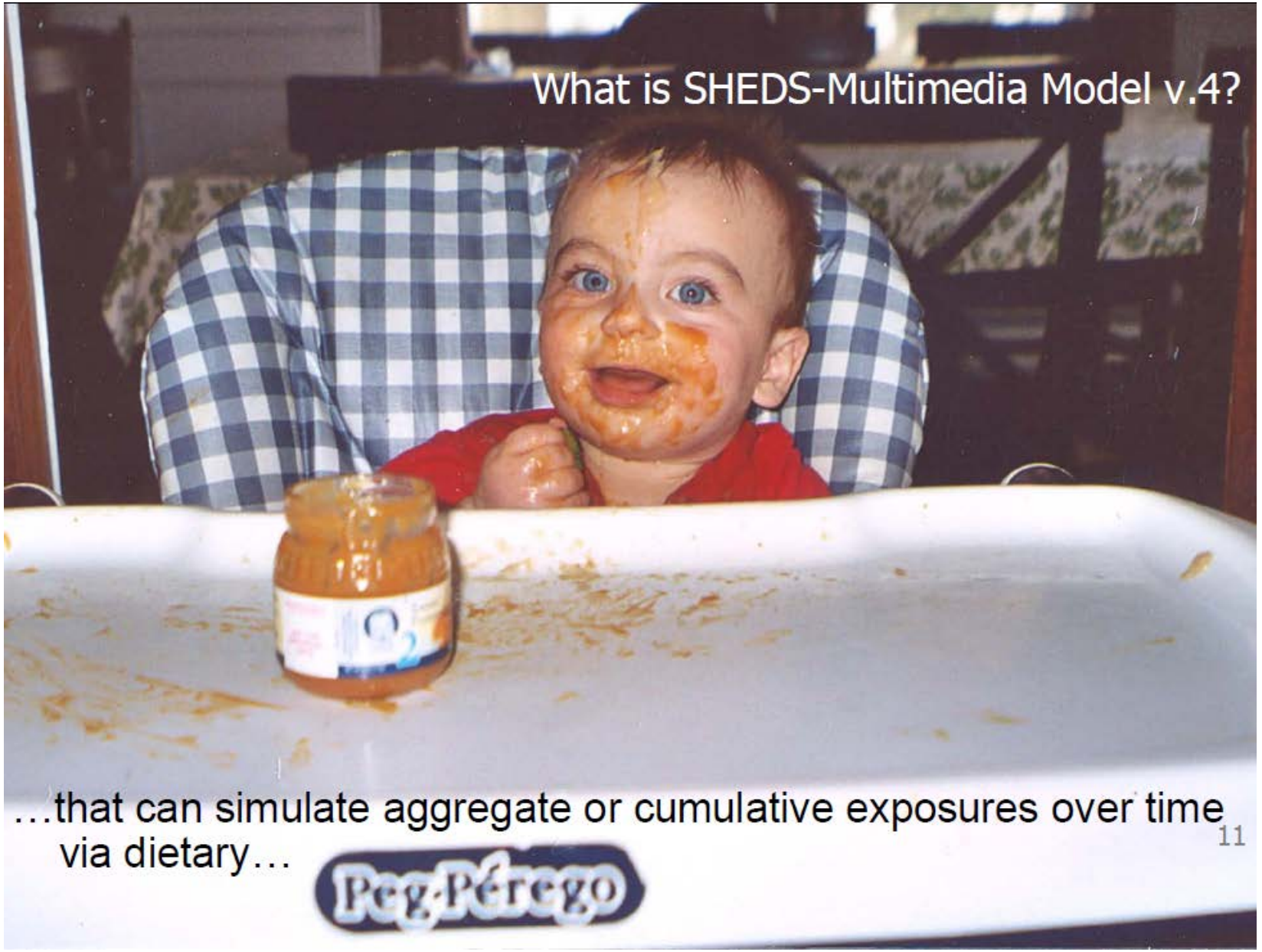
U.S. Environmental Protection Agency
Office of Research and Development

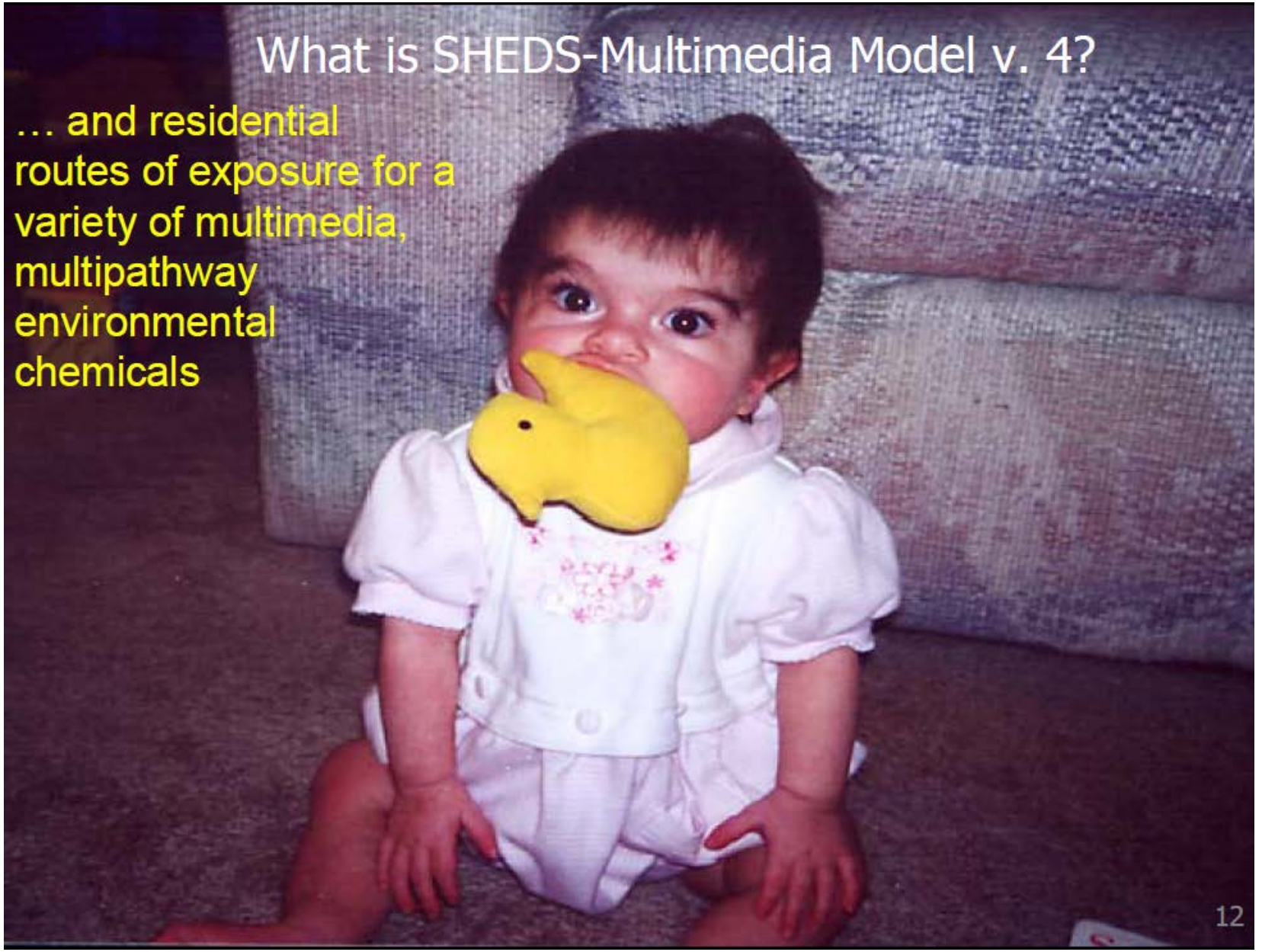
USEPA FIFRA SAP Meeting

July 20-22, 2010

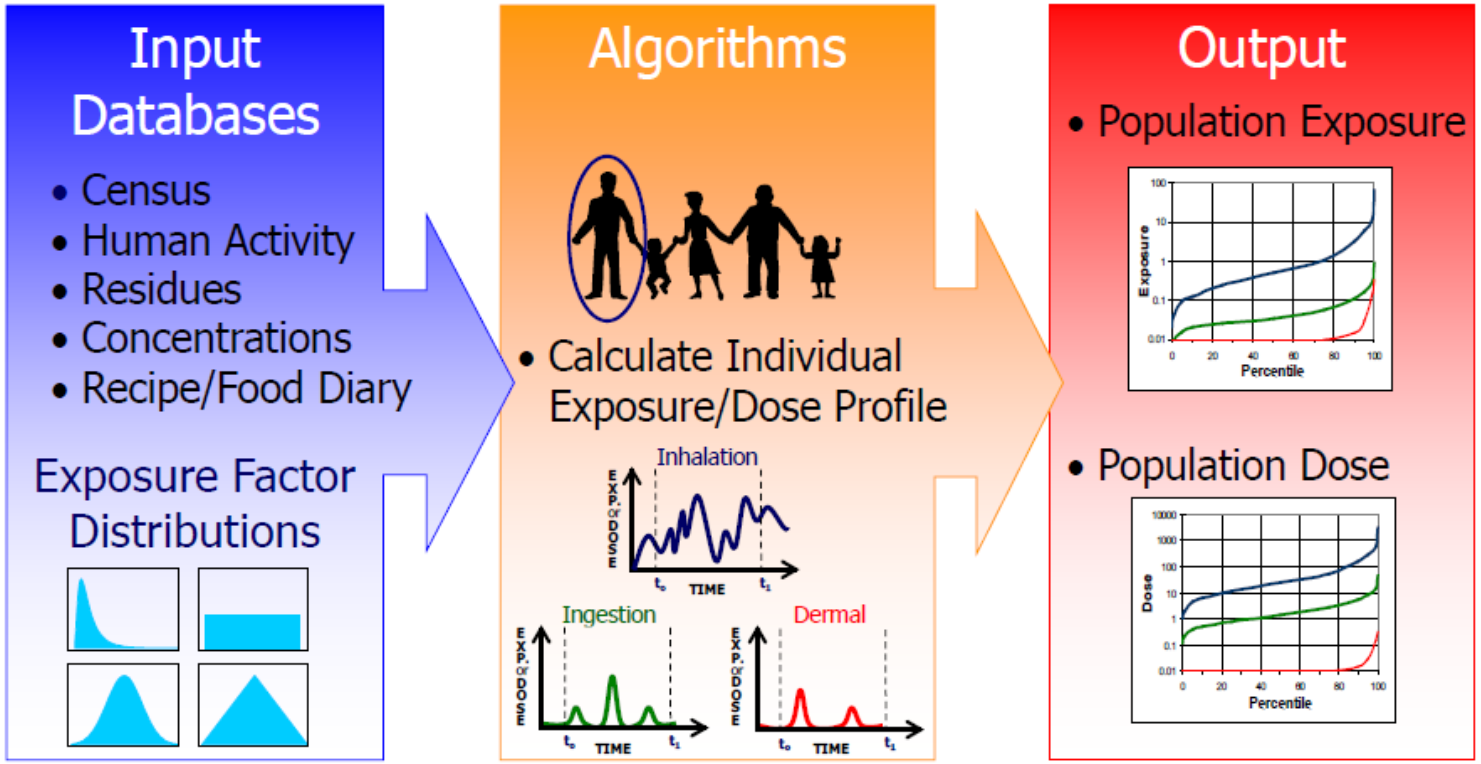
Crystal City, VA


What is SHEDS-Multimedia Model v.4?
SHEDS-Multimedia is a physically-based, probabilistic model
10
http://www.flickr.com/photos/dadakim/1949005630/


What is SHEDS-Multimedia Model v.4?
…that can simulate aggregate or cumulative exposures over time via dietary…
Peg-Pérego


What is SHEDS-Multimedia Model v. 4?
… and residential routes of exposure for a variety of multimedia, multipathway environmental chemicals
12

General SHEDS Model Structure



PBPK models in exposure assessment

- **Used in conjunction with an exposure assessment to improve the quantitative characterization of the dose-response relationship**
- **Identify and evaluate the relationship between an applied dose and biomonitoring or biomarker data, or between an applied dose, biomarker level, and internal target tissue dose**
- **Establish biological exposure indices (e.g., blood or breath concentrations) to protect workers from harmful exposures to solvents**
- **Reconstruct human exposures over time in epidemiology studies**
- **Provide estimates of an internal tissue dose from multiroute (oral, inhalation, dermal) or multichemical exposures**

Sample PBPK Model Structure

A model for simulating diffusion-limited tissue uptake and multi-route exposures.

Dotted lines represent the separation of cellular matrix and tissue blood components.

From: EPA/600/R-05/043F August 2006