Biomarkers of exposure and effect for environmental carcinogens, and their applicability to human molecular epidemiological studies

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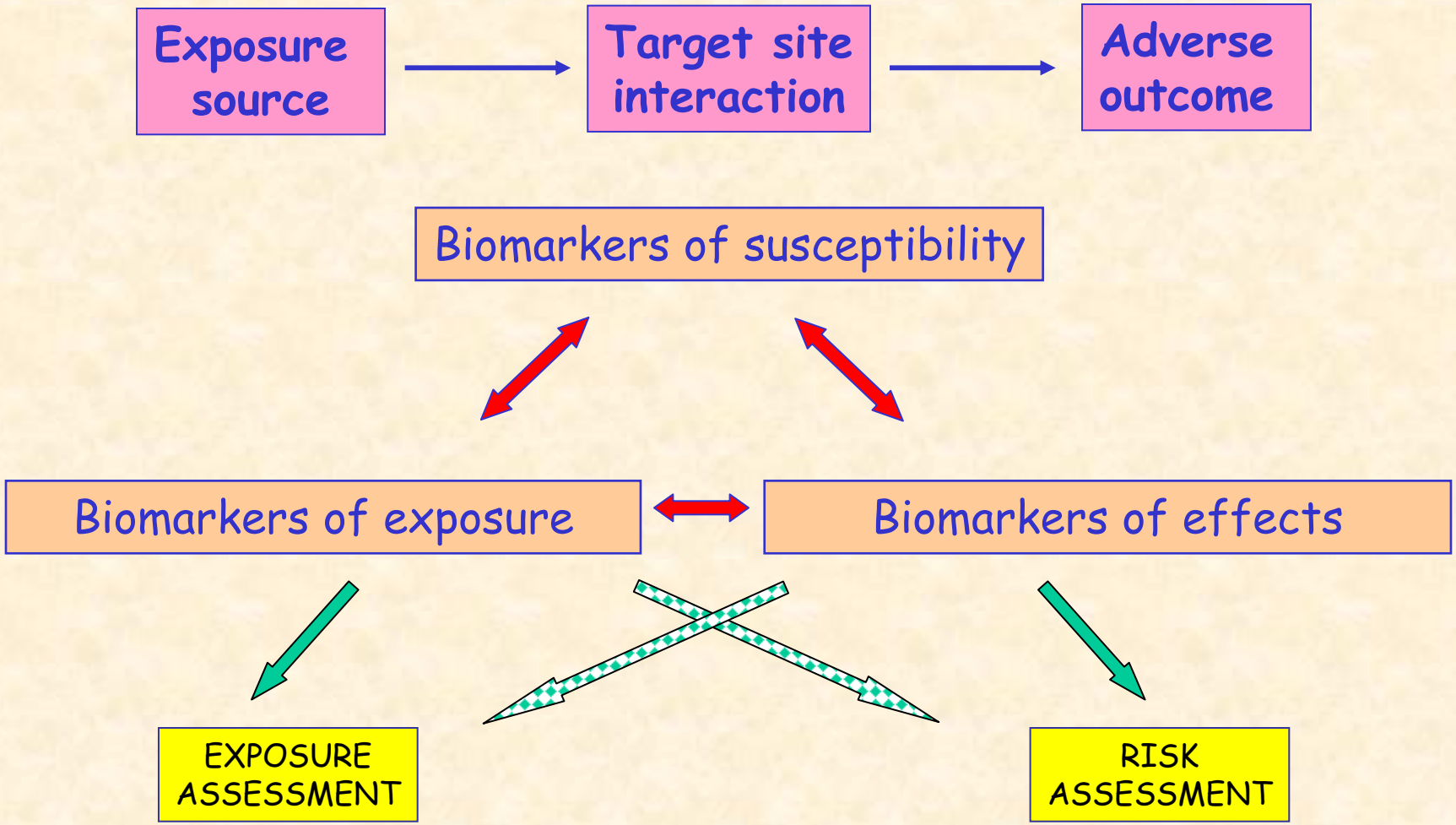
Biocentre, University of Leicester, UK

Exposure to an environmental carcinogen



EU 6th Framework Programme

Environmental cancer risk, nutrition and individual susceptibility

Coordinator: Konrad Rydzynski, Nofer Institute of Occupational Medicine, Lodz, Poland
Duration: 2005-2010
Budget: 11.0 million euro
Network: 25 partners, 13 countries

- *The main focus of this virtual research centre of excellence will be on the use of biomarkers of exposure and bioindicators of disease in molecular epidemiology of cancer*

ECNIS: 25 participants (including SMEs) working in fields related to carcinogenesis such as: diet, environment, occupation, lifestyle, exposure assessment

NIOM (Poland)
VUB (Belgium)
UCL (Belgium)
UC (Denmark)
FIOH (Finland)
DKFZ (Germany)
UM (Germany)
BIU (Germany)
NHRF (Greece)
FJOKK (Hungary)
ISI (Italy)
IRCCS (Italy)
Collegium Med. (Poland)
ICO (Spain)
KI (Sweden)
ULUND (Sweden)
UNIMAS (The Netherlands)
IRAS-UU (The Netherlands)
ULEIC (UK)
ICR (UK)
UNIVDUN (UK)
IARC (France)
NETIX (Poland)
Leocordia AB (Sweden)
ICL (UK)

The overall objectives of ECNIS

1. To overcome the fragmented nature of research in areas related to carcinogenesis caused by the environment, diet, occupation, or lifestyle, within Europe
2. To integrate joint training and mobility programs in area of environmental cancer molecular epidemiology
3. To develop and validate novel biomarkers of exposure, effect and susceptibility for environmental and occupational cancer risk assessment
4. To identify factors that modulate the environmental and occupational cancer risk resulting from nutrition and lifestyle factors
5. To develop hazard and risk assessment strategies based on mechanism of action of carcinogens
6. To disseminate of acquired knowledge to the scientific community and to external stakeholders

14 workpackages

- *Integrating Activities*:

  Co-ordinated research planning, personnel mobility and sharing infrastructures and data

- *Joint Research Activities*:

  Multidisciplinary investigations in the fields of molecular cancer epidemiology, environmental carcinogenesis and its modulation by nutrition and genetics

- *Spreading of Excellence Activities*:

  Training and mobility programmes and sharing of new scientific knowledge with researchers, the general public, regulators, health care specialists, industry, etc.

2005-2007

Inventory of available resources

Reviews/reports

- biomarkers of carcinogen exposure and early effects
- state of validation of biomarkers of carcinogen exposure and early effects and their applicability to molecular epidemiology
- epidemiological concepts of validation of biomarkers for the identification/quantification of environmental carcinogen exposures

Research projects mostly on validation including: urinary DNA oxidation products; acetaldehyde DNA adducts; Comet assay for DNA damage, ^{32}P-postlabeling; genotype methodology; long lived adducts

Major aspects in interpretation of biomarker data: ECETOC

The analytical integrity of data

- *Is the answer right?*

The data's ability to describe exposure

- *Is the answer specific and selective?*

The relationship between the biomarker and effects

- *What is the biological relevance of the answer?*

An overall evaluation and weight of evidence

- *What is the risk assessment and what do we tell the subject?*

Guidance for the interpretation of biomonitoring data. Document No 44, 2005

http://www.ecetoc.org

Carcinogen biomarkers

The analytical integrity of data.
Is the answer right?

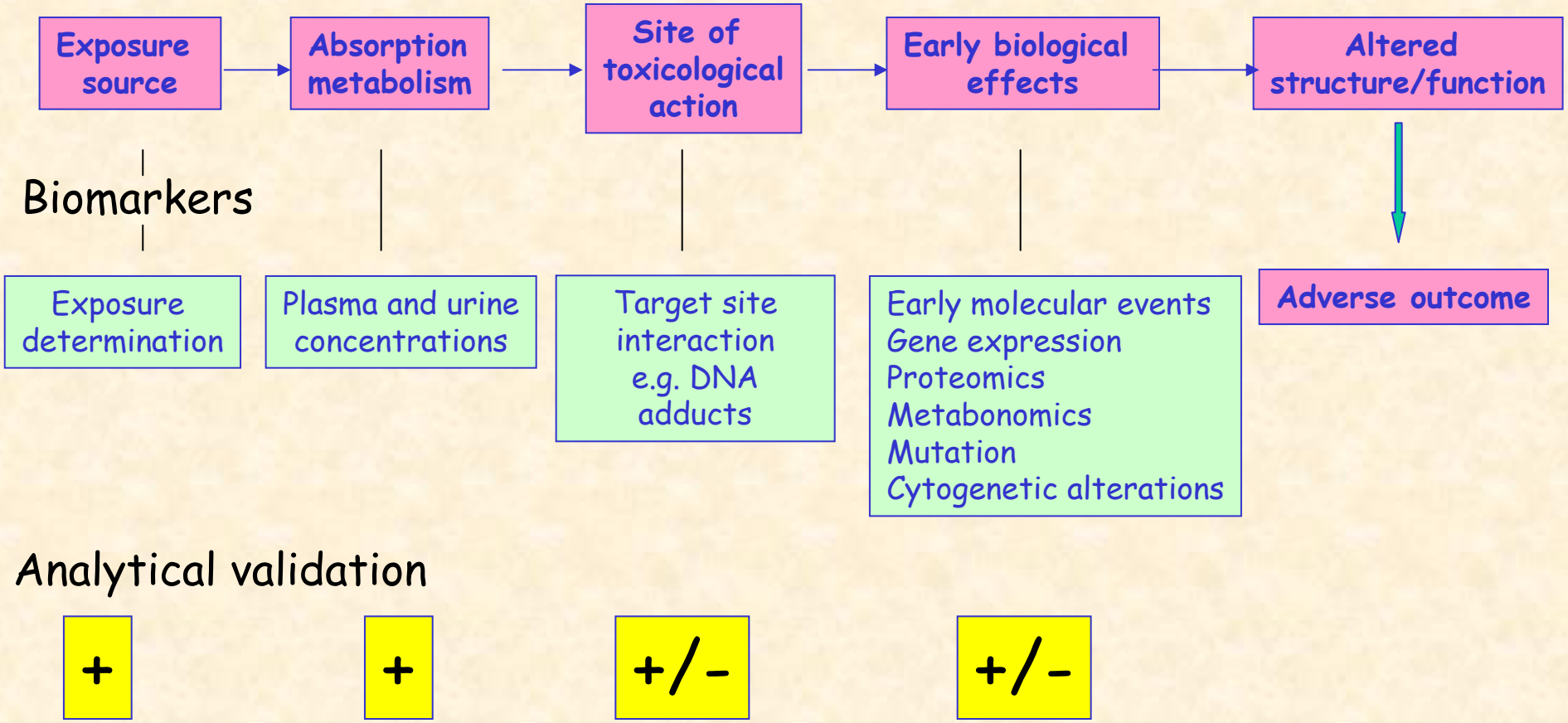
Analytical validation

- standard operating procedures for
  - pre-analytical (sample collection, storage)
  - analytical (procedures, quality controls)
  - post-analytical (statistics, reporting)

- recoveries, reproducibilities and accuracy, limit of detection/quantitation, etc

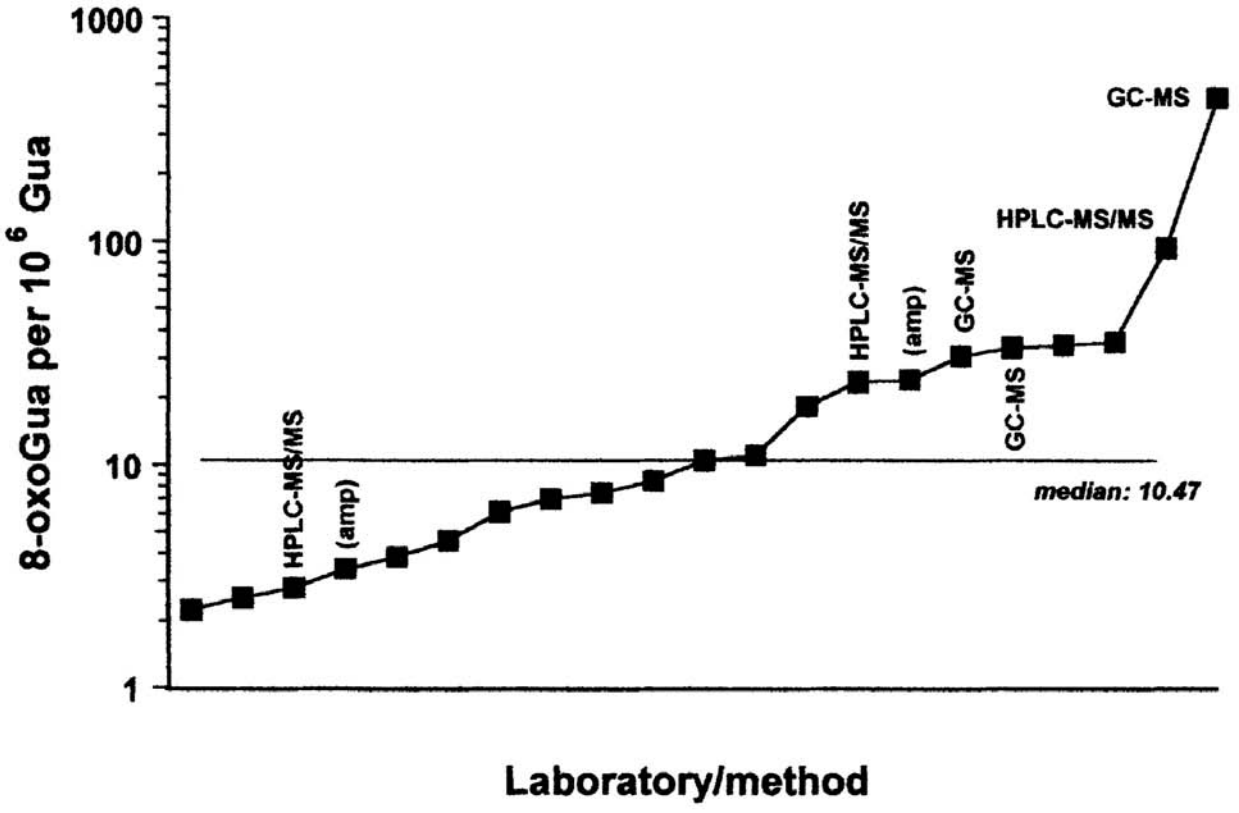
- interlaboratory comparison/ comparison of equipment/platforms

Exposure to an environmental carcinogen: analytical integrity of data



Analytical Validation: interlaboratory, comparison of methods

Comparative analysis of baseline 8-oxo-7,8-dihydroguanine in mammalian cell DNA, by different methods in different laboratories: an approach to consensus ESCODD (European Standards Committee On Oxidative DNA Damage)



Carcinogenesis 2002 23:2129-2133; doi:10.1093/carcin/23.12.2129

Copyright restrictions may apply.

Carcinogenesis

Analytical Validation: Interlaboratory, comparison of methods

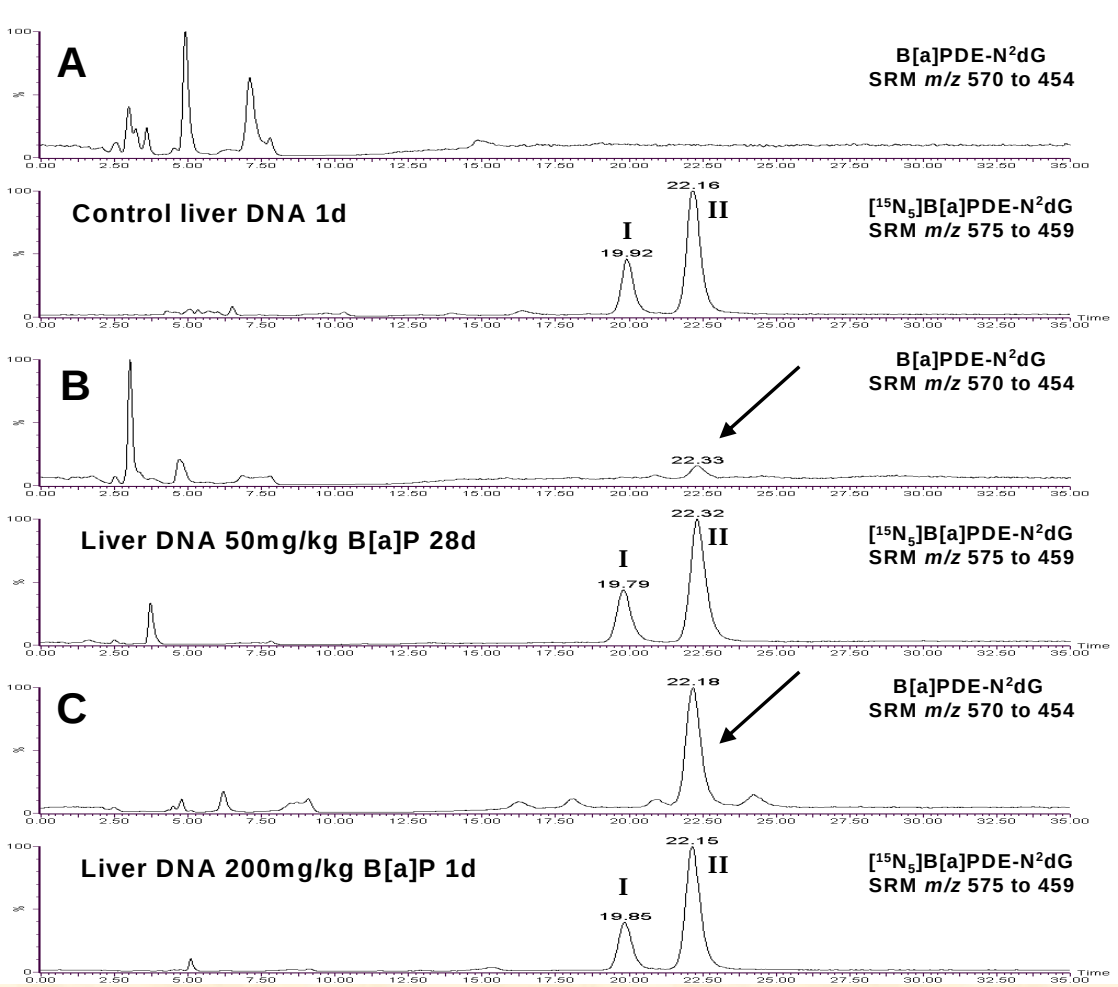
Comparison of the DNA adduct levels obtained by different methods for the samples of the second interlaboratory trial

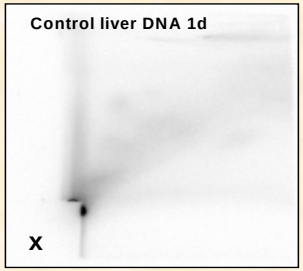
Sample	Method of analysis of DNA adducts		
	^{3}H incorporation	Mass spectrometry	^{32}P-postlabelling
High BaP	137.6	20.5	22.2 ± 9.4 (n=33)
Low BaP	86.4	8.5	11.4 ± 4.1 (n=33)

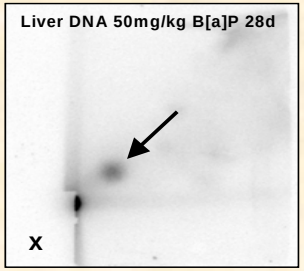
Phillips et al, Mutagenesis, 1999, 14, 301-315

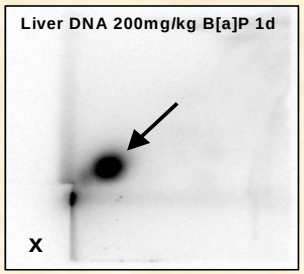
Analytical Validation: Comparison of methods

Benzo(a)pyrene-N^2-dG adducts – LC-MS/MS – mouse liver



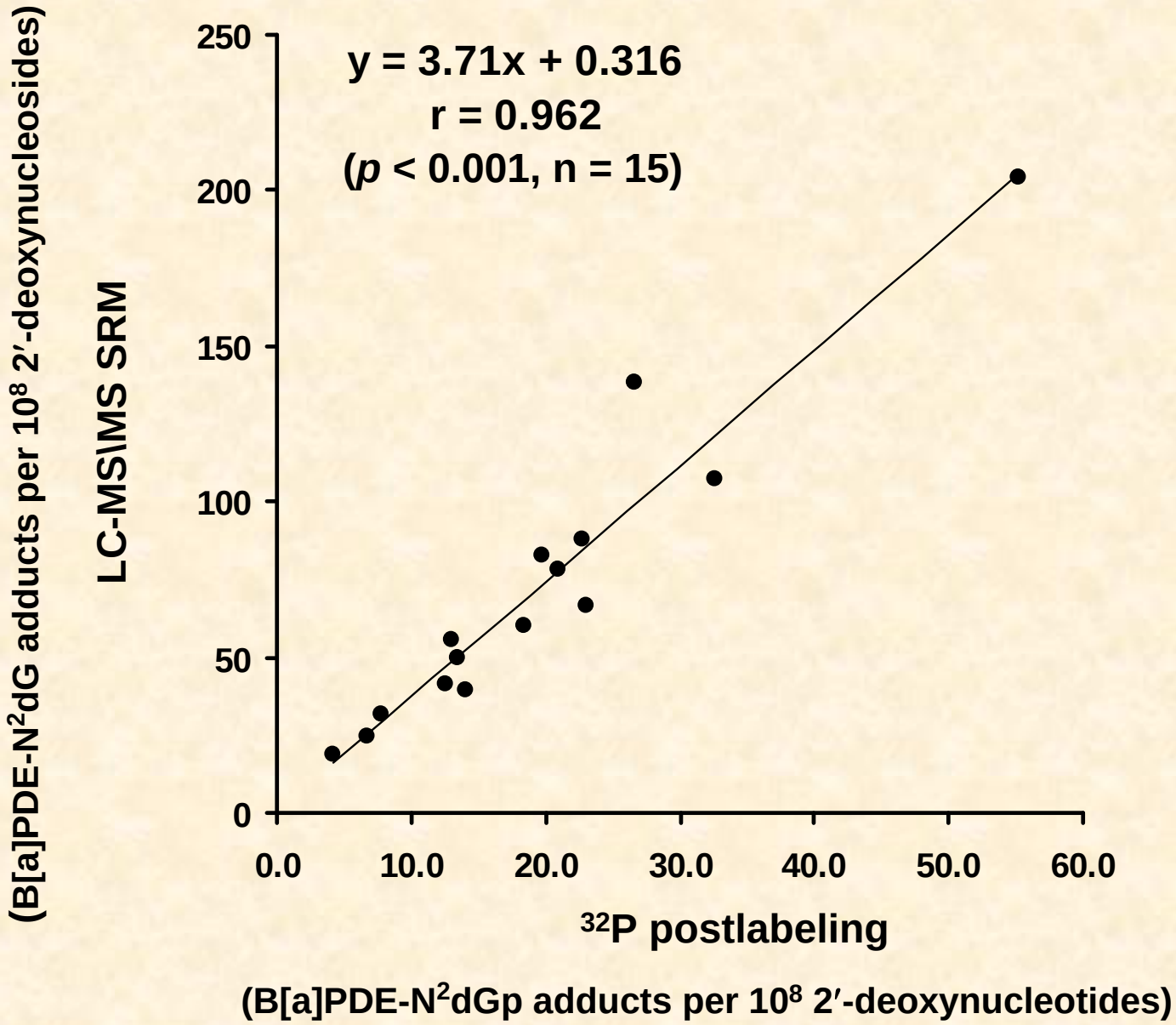






Acknowledgements: S Kyrtopoulos

Singh et al, Chem Res Toxicol, 2006, 19, 868-878



3.7 fold difference between methods

Singh et al, Chem Res Toxicol, 2006, 19, 868-878

Carcinogen biomarkers

The data's ability to describe exposure.
Is the answer specific and selective?

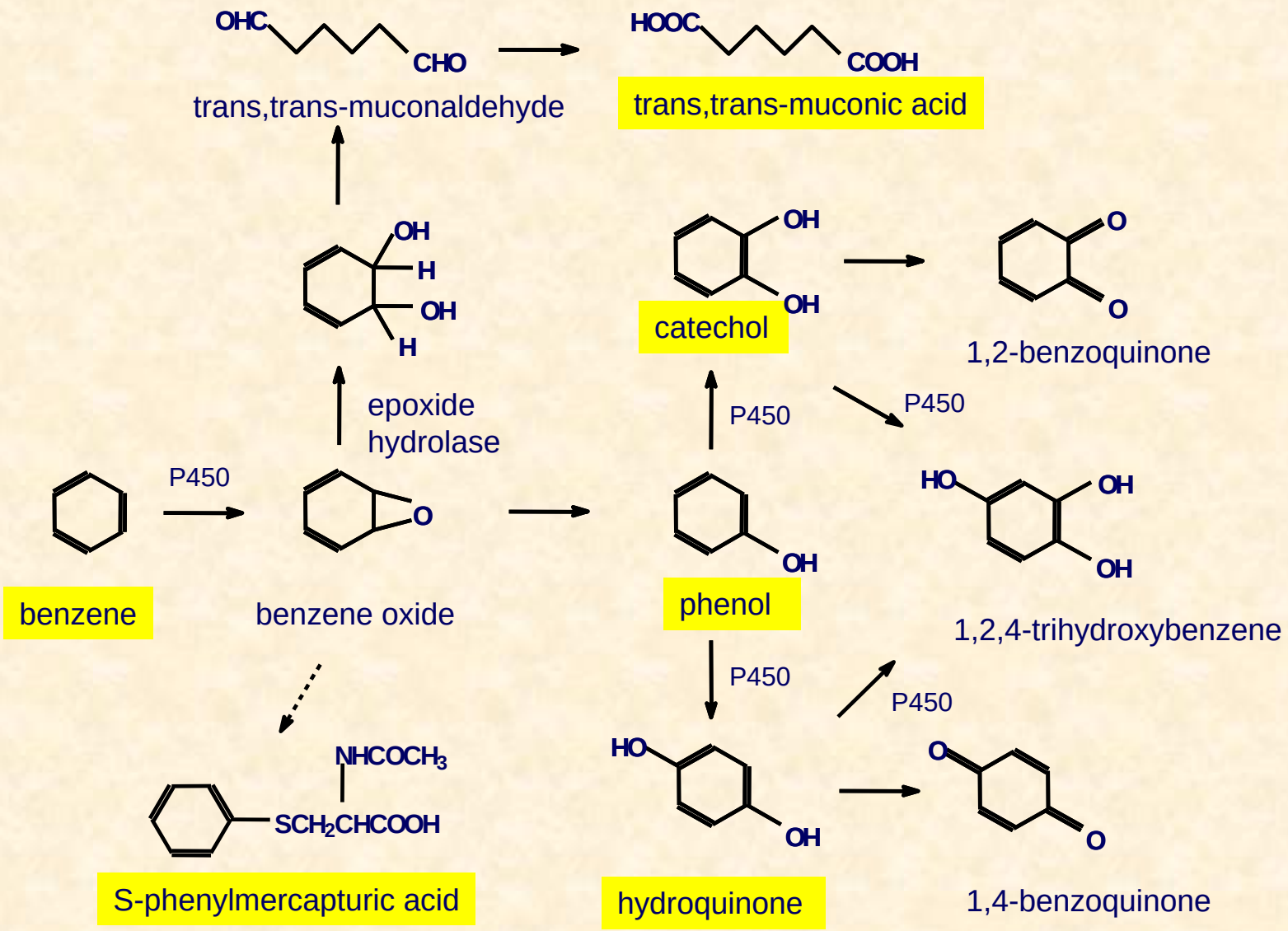
Interpretation may be affected by

- lack of pharmacokinetic data/models

- alternative sources of the biomarker
  e.g. benzene, acrylamide

- endogenous production of the biomarker
  e.g. formaldehyde, ethylene oxide

Benzene metabolism



Using urinary biomarkers to elucidate dose-related patterns of human benzene metabolism

S.Kim, R. Vermeulen, S, Waidyanatha, B.A. Johnson, Q. Lan, N. Rothman, M.T. Smith, L. Zhang, G. Li, M. Shen, S. Yu, S.M. Rappaport

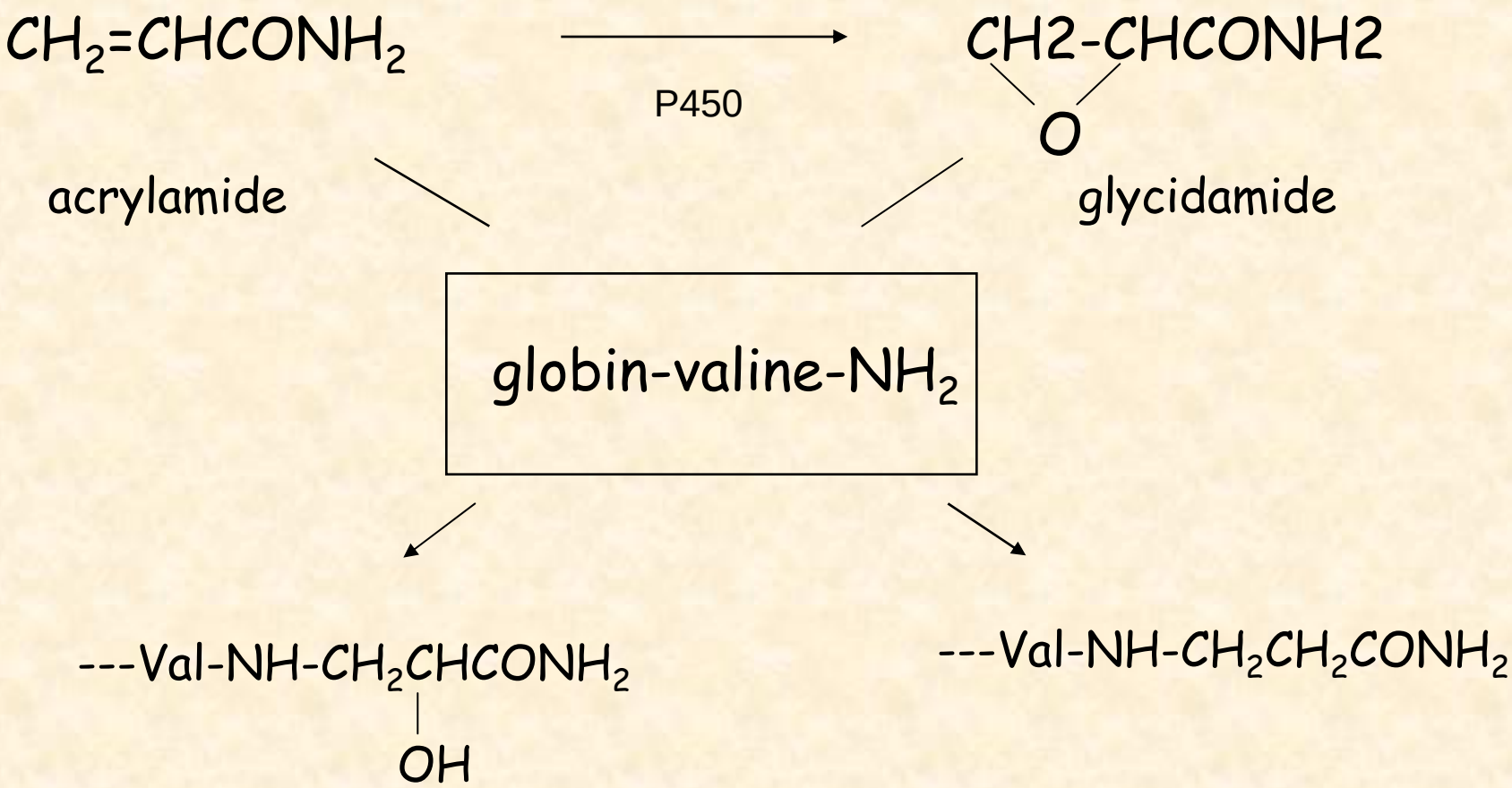
Carcinogenesis, 27, 771-781, 2006.

250 benzene-exposed workers (median 1.2ppm)
139 controls (median 0.004ppm)

Median concentration of each metabolite was elevated when the group's benzene exposure was at or above:
Catechol 2.0ppm
Phenol 0.5ppm
Hydroquinone 0.5ppm
t,t-muconic acid 0.2ppm
S-phenylmercapturic acid 0.2ppm

Sources of background benzene metabolite levels:
Smoke, gasoline, diet, gut flora, medicines (phenol), sorbic acid (t,t-MA)

Acrylamide: globin adducts as biomarker of exposure



Analysed by GC-MS after modified Edman degradation

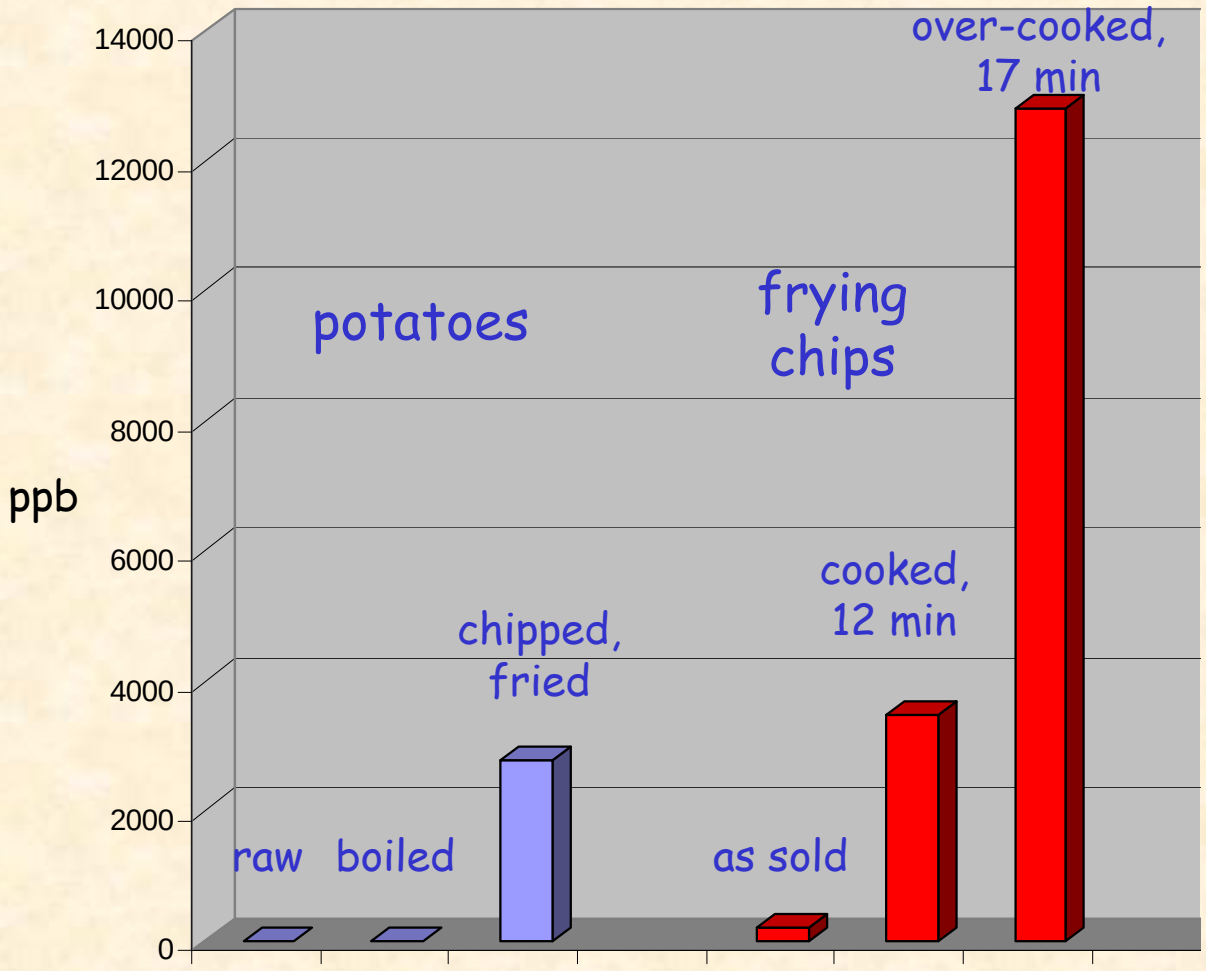
Tornqvist et al, Anal. Biochem. 154 (1986) 255-266

Background adduct levels from acrylamide to N-terminal valine in hemoglobin measured in non-smokers without occupational exposure to acrylamide.

Dybing et al, 2005

Adduct level (nmol/g)	n	Reference
Mean: 0.031; Range: 0.024 - 0.049	8	Bergmark, 1997
Mean: 0.033; Range: 0.020 – 0.047	6	Kjuus et al., 2003
Mean: ~ 0.04 ; Range: 0.02 – 0.07	18	Hagmar et al., 2001
Median: 0.021; Range: 0.012 – 0.050	25	Schettgen et al., 2003
Mean: 0.027 (SD: ± 0.006)	5	Paulsson et al., 2003a

Acrylamide is produced by cooking food - Tareke et al, 2000, 2002



Acrylamide in foods (Ahn et al, 2002)

Source of acrylamide adduct background levels: baked and fried carbohydrate-rich foods

Formaldehyde

Causes nasopharyngeal cancer in humans
IARC: "strong but not sufficient evidence for a causal association between leukaemia and occupational exposure to formaldehyde".
Direct acting *in vitro* mutagen

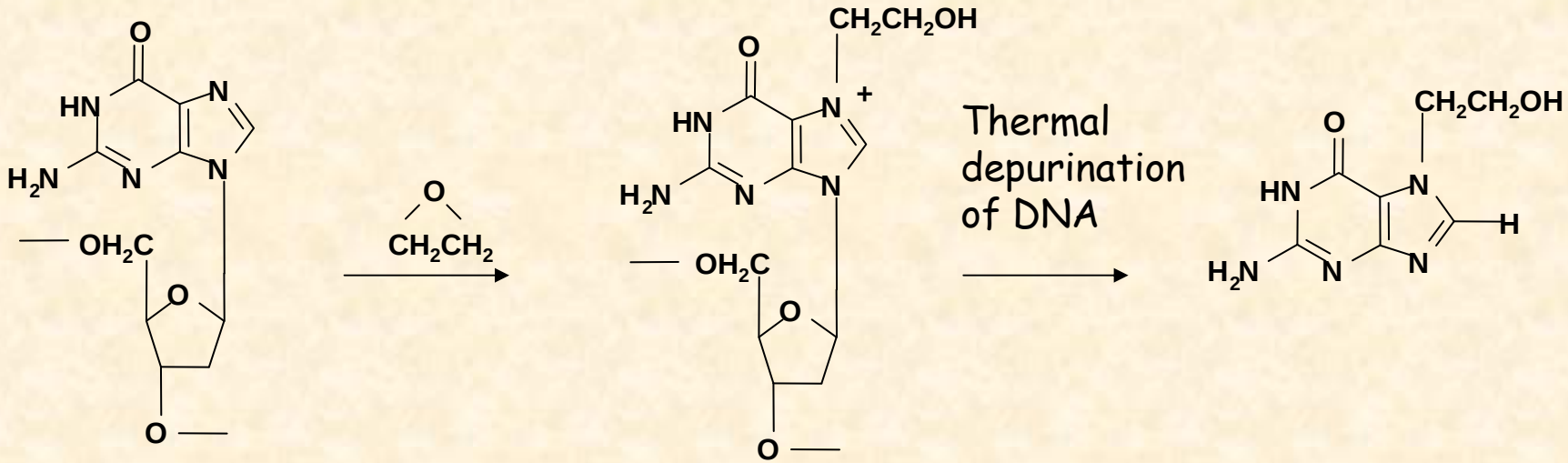
Normal intermediary metabolite in humans.
Endogenous blood concentrations estimated as ca 0.1mM
Exposure of animals to 6ppm formaldehyde did not increase blood levels
Modelling indicates that human exposure at 2ppm (OES) would yield $\leq$0.1% of endogenous levels, i.e. negligible increase

Unlikely to be a direct systemic effect.

Sources of formaldehyde background: normal endogenous metabolism

Ethylene oxide: DNA adducts as biomarkers of exposure

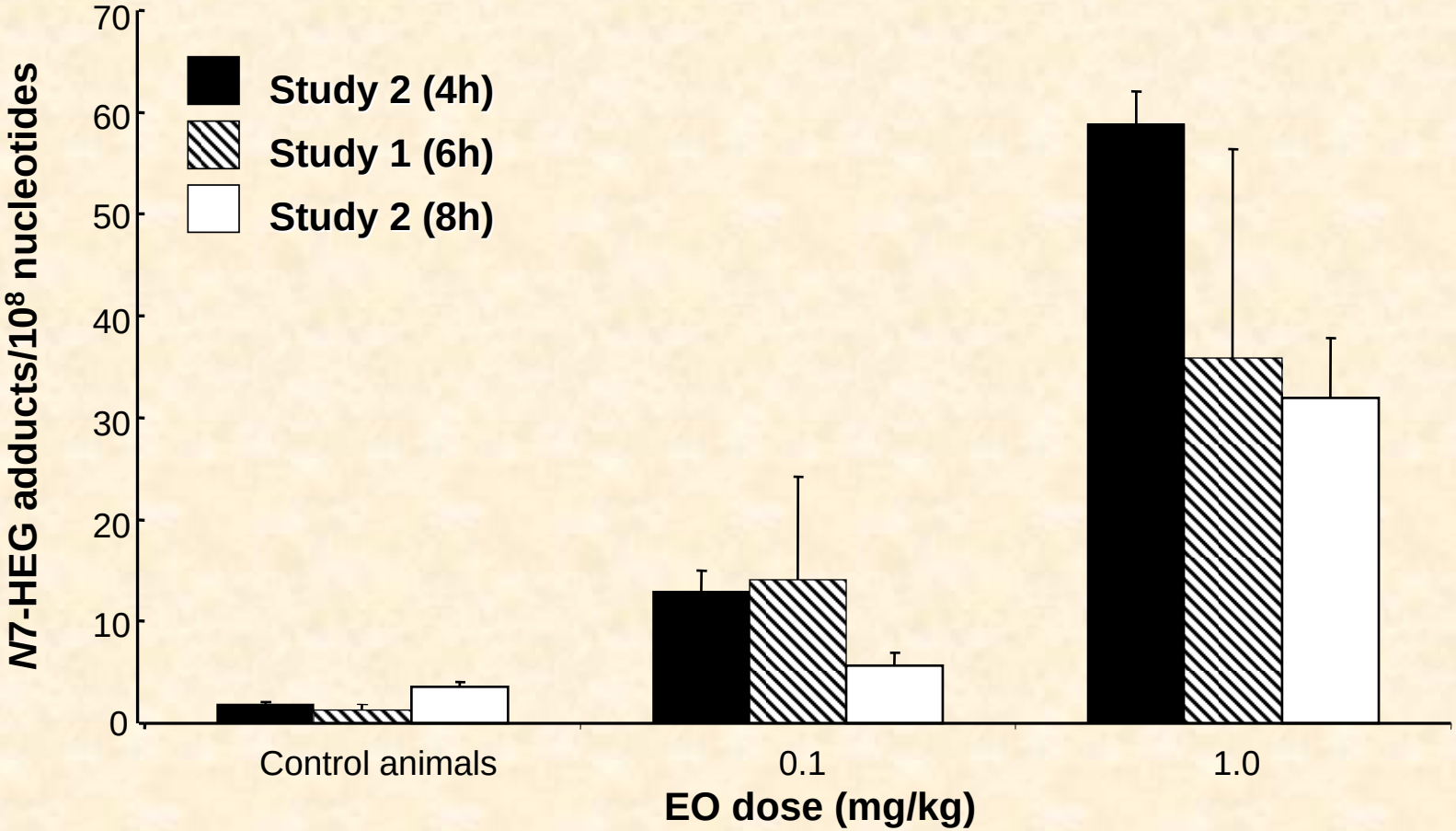
N-7-(2-hydroxyethyl) guanine



ESI LC-MS/MS, LOD 6 adducts/10^9 nucleotides

Marsden et al, Chem Res Toxicol, 20 (2007) 290-299

Dose-response relationship – rat liver (i.p.)



Similar levels of N7-HEG in control heart, colon, lung, kidney, spleen, stomach

Exposure to a single ip dose of 0.01 mg/kg did not increase liver N7-HEG levels over control

Source of EO adducts: endogenous formation of ethylene oxide from ? lipid peroxidation, methionine oxidation, intestinal bacteria

Summary

- Endogenous/background levels of some carcinogens and/or metabolites and many DNA adducts and oxidative DNA damage products (total at least $1/10^6$ nucleotides) have been detected

- May hinder detection of exposure from low doses of exogenous compounds

- Also may result in a lack of observable effect at low dose, (i.e. <u>practical</u> thresholds) for some exogenous compounds

The relationship between the biomarker and effects

What is the biological relevance of the answer?

Interpretation may be affected by

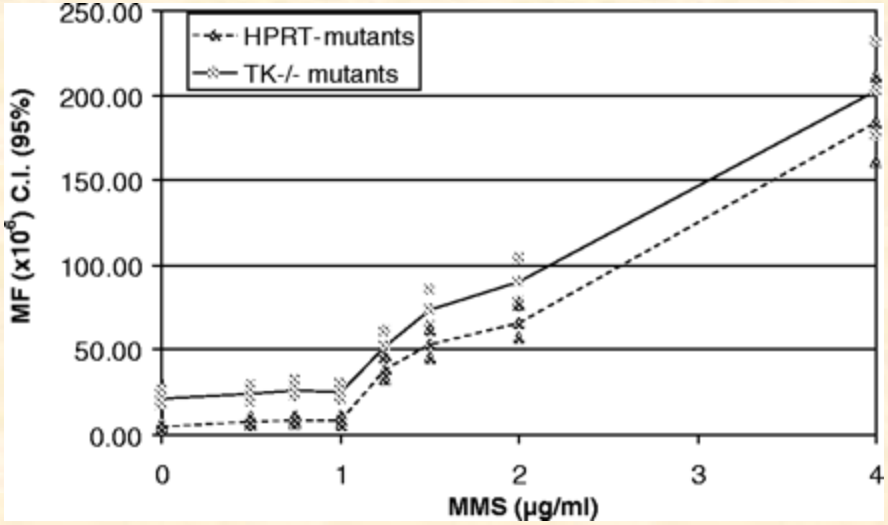
- the mechanism (e.g. DNA reactive or non-DNA reactive)
- the dose response relationship,

  (e.g. is there a <u>biological</u> or <u>practical</u> threshold or a saturable effect)
- background levels
- mixture effects (e.g. synergies, antagonisms)

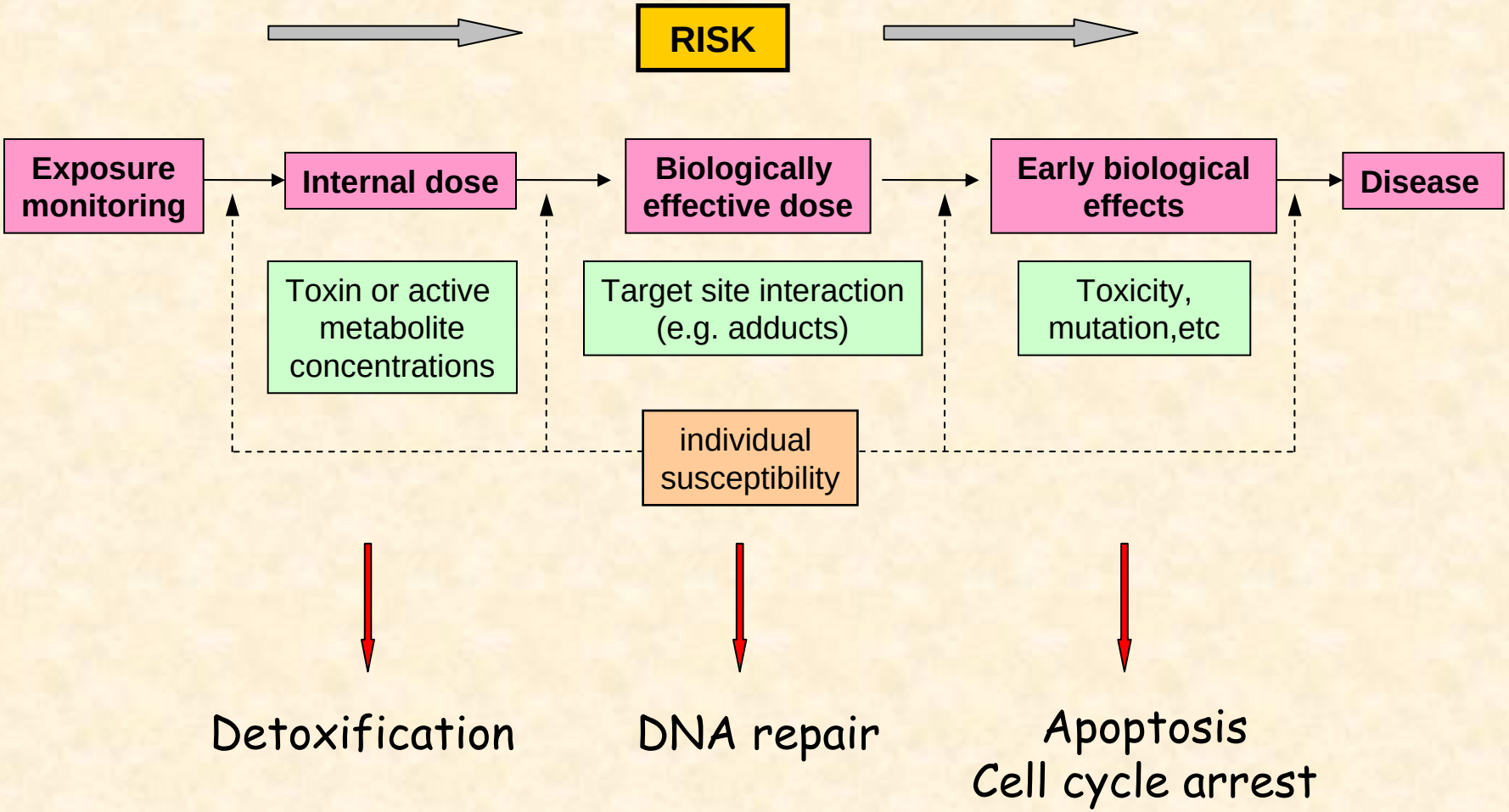
Examples of thresholds

- It is well established that non-DNA reactive carcinogens may show thresholds
- However thresholds for effects are now being shown for some genotoxic compounds

e.g. MMS Jenkins et al, Mutagenesis, 2005, 20, 389) (micronuclei, mutation)
MMS/EMS Doak et al Cancer Res., 2007, 67, 3904 (micronuclei, mutation)



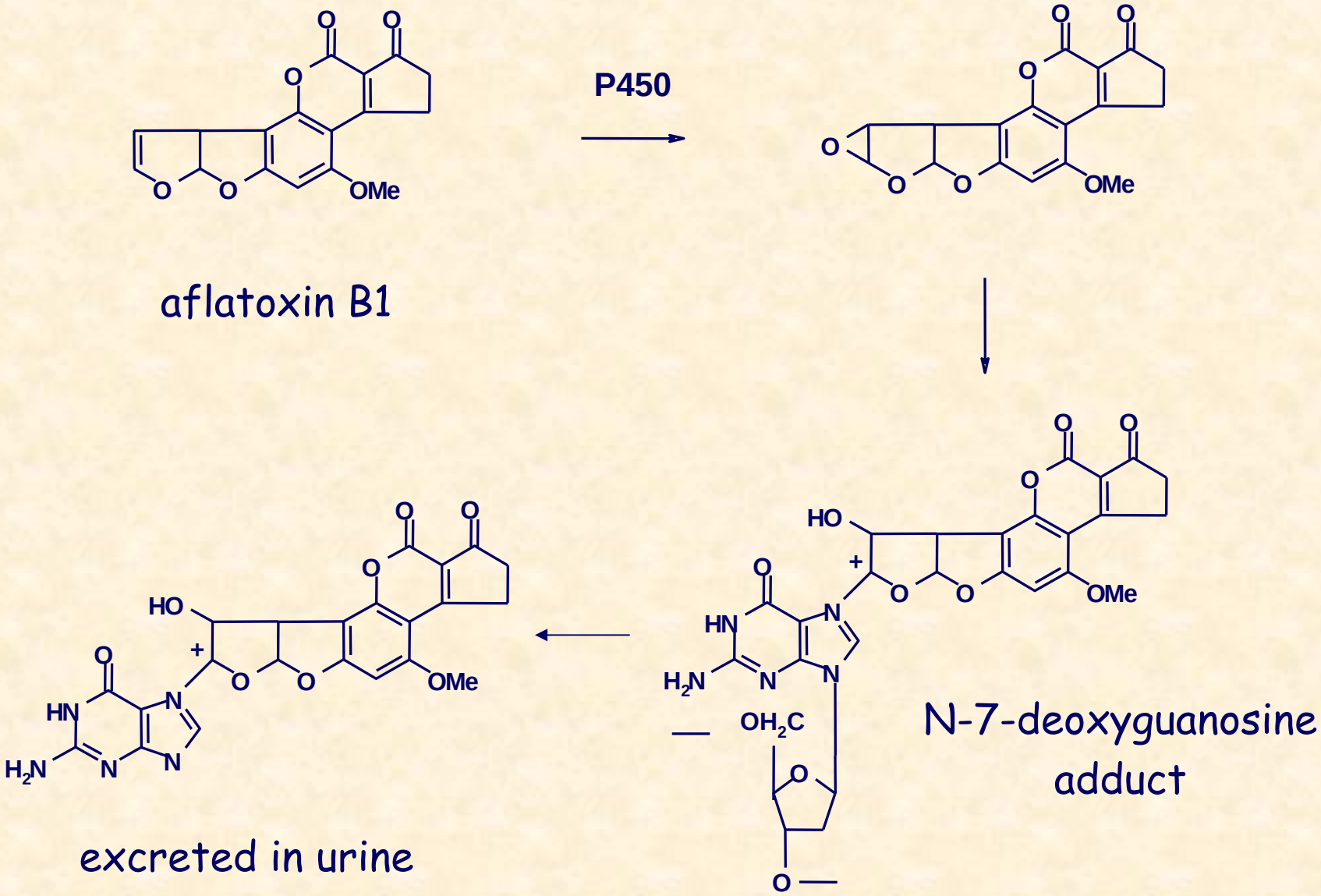
Dose-response relationships: threshold or no threshold?



Possible mechanisms for thresholds for effects of DNA-reacting compounds

Example of synergy

aflatoxin B1 urinary DNA adduct



Example of synergy

Combined effects of HBsAg positivity and aflatoxin biomarkers on hepatocellular carcinoma

HBsAg	Aflatoxin negative Relative risk (95% CI)	Aflatoxin positive Relative risk (95% CI)
Negative	1.0	3.4 (1.1, 10.0)
Positive	7.3 (2.2, 24.4)	59.4 (16.6, 212.0)

Qian et al Cancer Epidemiol. Biomark. Prev. 3 (1994) 3-10

Summary

- Analytical methods for detecting carcinogen metabolites or DNA damage often exceed the sensitivity of biological assays, i.e. need to know more about low level dose-response relationships to improve cancer risk estimates for environmentally exposed populations.

- 'Despite the substantial progress which has been achieved in the development of analytical methodologies, few biomarkers ……can be considered as adequately validated and mature for use in risk assessment'

S Kyrtopoulos ECNIS, 2007

Major aspects in interpretation of carcinogen biomarker data:

The analytical integrity of data

- *Is the answer right?*

The data's ability to describe exposure

- *Is the answer specific and selective?*

The relationship between the biomarker and effects

- *What is the biological relevance of the answer?*

An overall evaluation and weight of evidence

- *What is the risk assessment and what do we tell the subject?*

Guidance for the Interpretation of Biomonitoring Data
ECETOC, Document 44, 2005
Proposed framework for the evaluation of biomonitoring data

Purpose of study	**Required knowledge**			
	Analytical integrity	**Toxicokinetics**	**Health effects**	**Weight of evidence**
Trends in exposures	×			
Characterisation of exposures	×	×		
Investigation of health impacts	×	×	×	
Risk assessment and standard setting	×	×	×	×

Human Biomonitoring for Environmental Chemicals
The National Academies, USA, 2006

		Group						
Properties[1]		I	II	III	IV	V	VI	VII
Reproducible sampling/analytical methodology			R	R	R	R	R	R
External dose-[BM] relationship in animals[2]				R				
External dose-[BM] relationship in humans[2]					R		R	R
[BM] – biological effect relationship in animals							O	
[BM] – biological effect relationship in humans						R		R
External dose-response relationship in animals							O	
External dose-response relationship in humans							O	
Biomarker informs on	Internal Dose		✞	✞	✞	✞	✞	✞
	External Dose			✞	✞		✞	✞
	Biological effects[3]					✞	✞	✞
						Potential for risk assessment		

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