

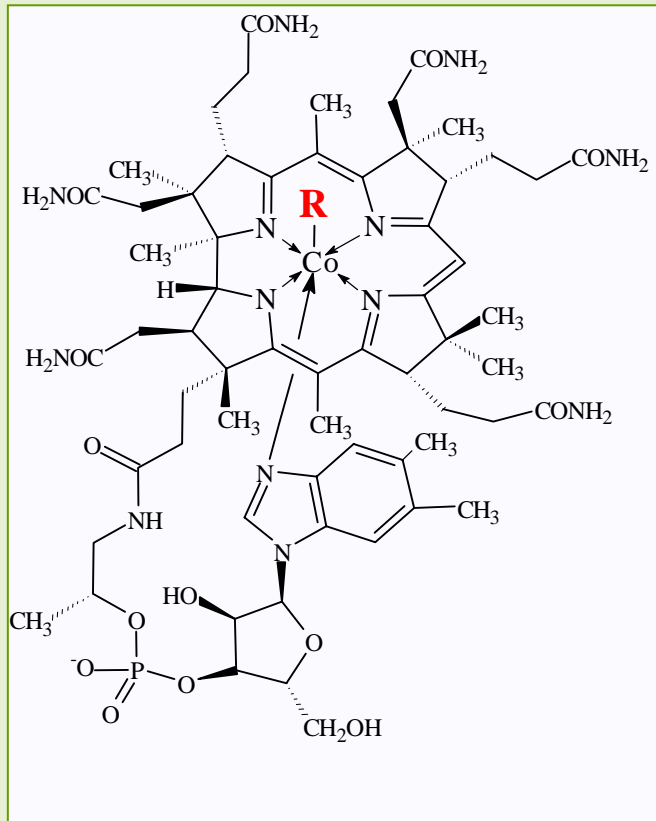


# Vitamin B<sub>12</sub> as an Analytical Tool: Application to Industrial, Pharmaceutical and Background Xenobiotics

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# Vitamin B<sub>12</sub>



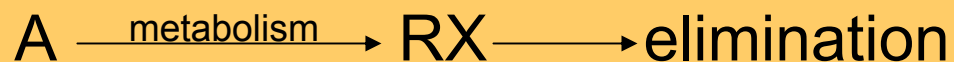
- 1926 'Anti-pernicious anæmia factor' found (Minot & Murphy)
- 1948 Isolation of a crystalline solid (Folkers et al; Lester Smith)
- 1958 Vitamin B<sub>12</sub> was structurally characterised (Hodgkin)
- 1972 Vitamin B<sub>12</sub> was synthesised (Woodward and Escenmoser)
- 1996 We tested Vitamin B<sub>12</sub> as analytical tool



# Industrial, Pharmaceutical and Background xenobiotica



A large part of compounds in the environment (in a broad sense) are or are transformed into metabolites that possess **electrophilic reactivity (RX)**



All macromolecules essential for biological functions contain **nucleophilic sites (Nu:)**

- Proteins
- Genetic material, DNA



# Basic parameters in risk assessment of electrophiles (genotoxic compounds)

- Detection and identification
- Clarification of electrophilic reactivity
- Quantification

Analytical problem: Their inherent reactivity  
Ensuing short half-life  
Low-molecular weight  
Hydrophilicity



# Nucleophiles for analysis of electrophiles

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Analysis of reaction products with proteins and DNA (in vivo)

Miller & Miller, 1947  
Brookes & Lawley, 1964  
Ehrenberg et al., 1974



Established methods used worldwide



Efforts to measure formation of reactive compounds using nucleophiles (in vitro)

Preussmann et al., 1969  
Göthe et al, 1974  
Nelis & Sinsheimer, 1981

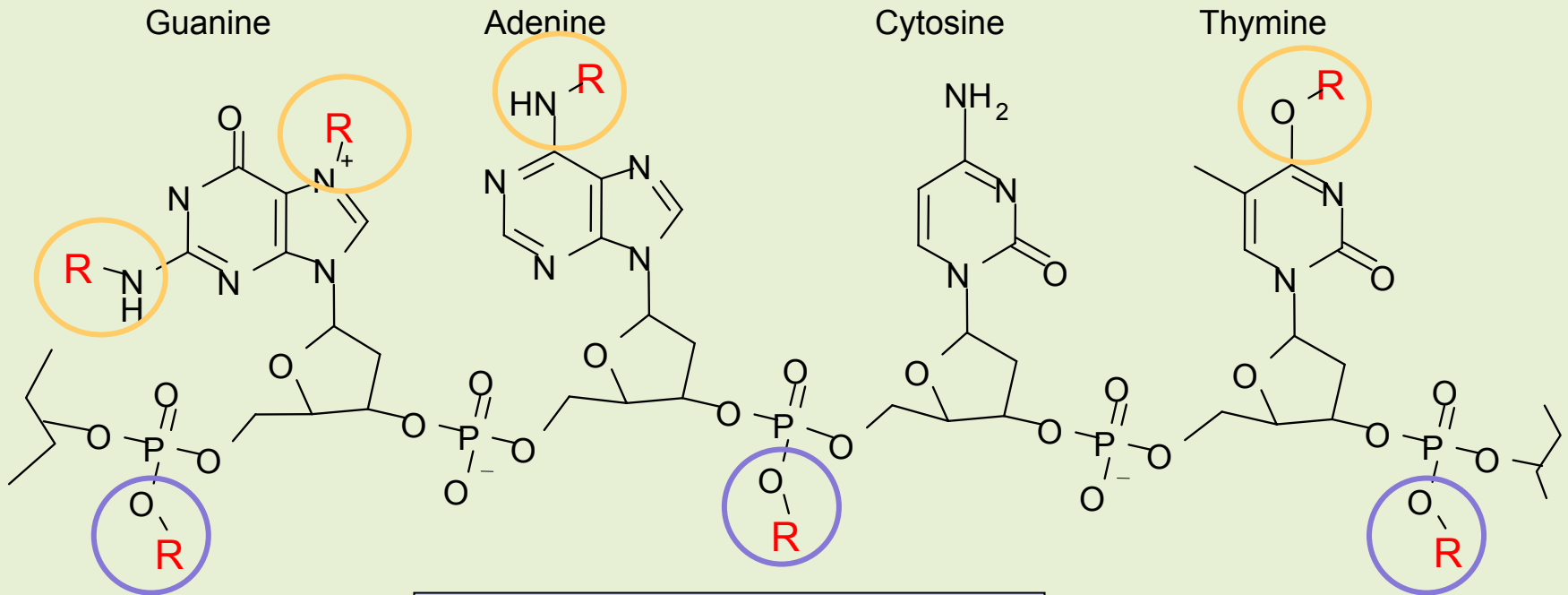


Limited usefulness



# DNA-adduct formation

## Base adducts: complex turnover kinetics

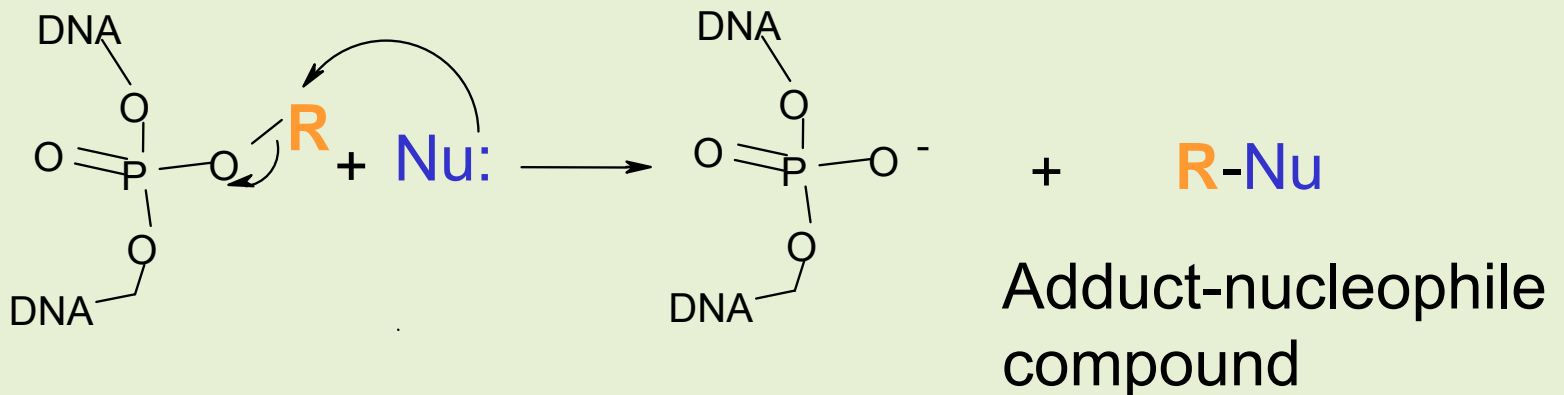
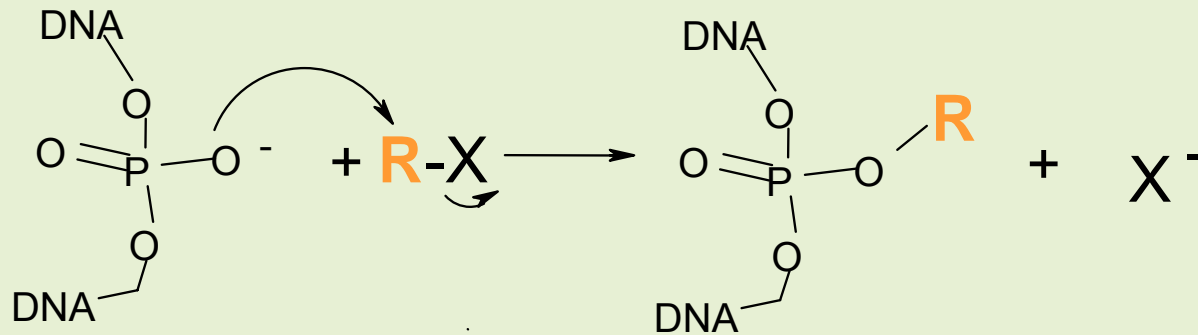


### Phosphate adducts:

- Stable at physiological pH
- No or little repair
- Weakly alkylating

# Development of the transalkylation method

Is it possible to transfer the DNA-phosphate adducts to a nucleophile?



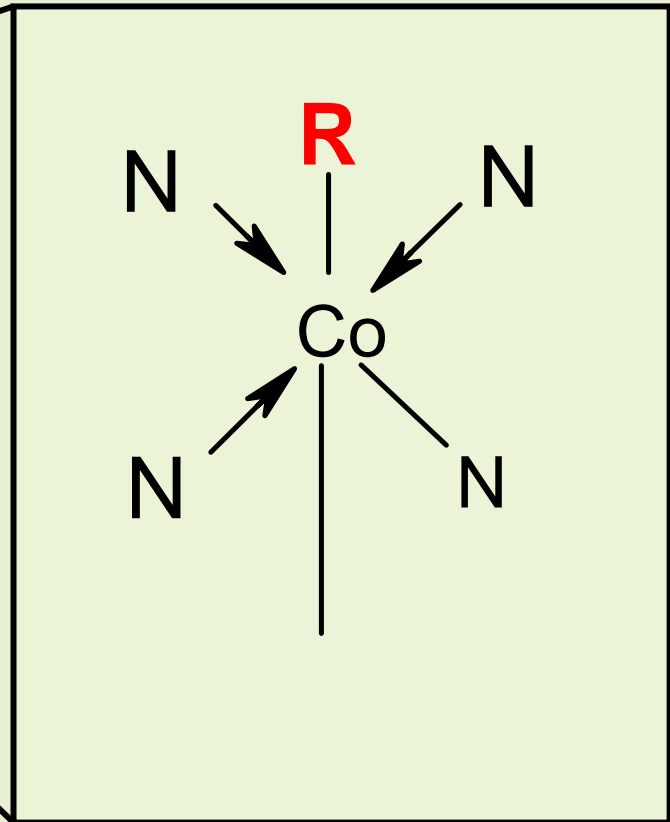
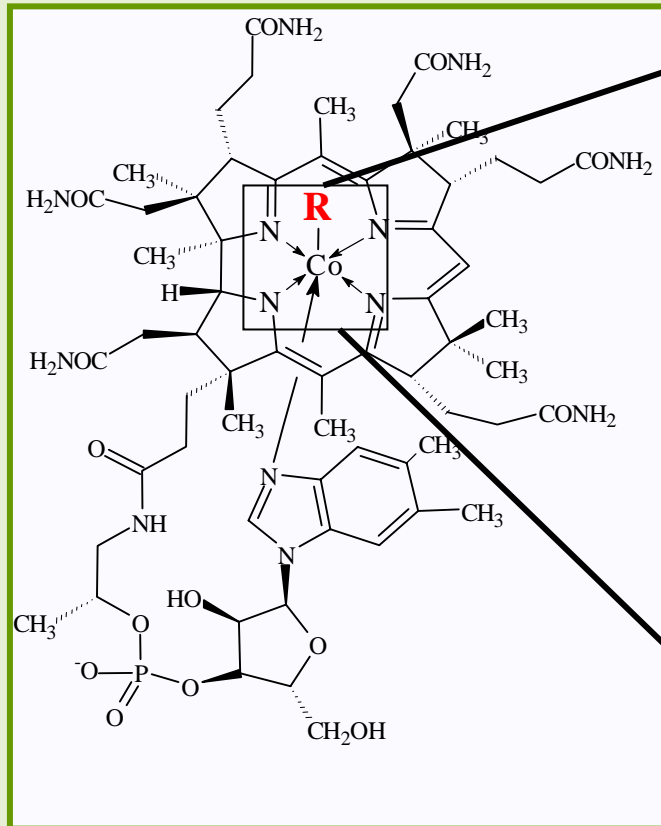
# Reaction kinetics: Results

Time required for complete transfer of methyl adducts using 0.1 M nucleophile

<b>Nucleophile</b>	<b>Time</b>
Aniline	2.5 months
Thiosulfate	1.8 days

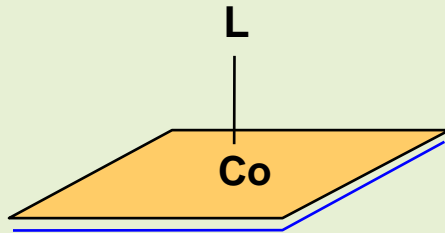


# Vitamin B<sub>12</sub>



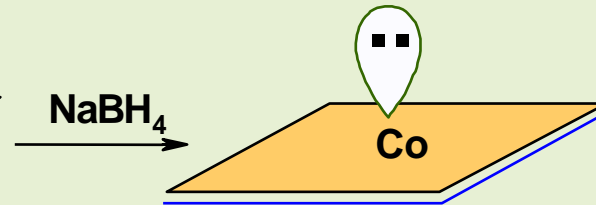
# Vitamin B<sub>12</sub> (cobalamin)

**Cobalamin**



**L = variable ligand**

**Cob(I)alamin**



**L = free electron pair**

**→ NUCLEOPHILE**



# Reaction kinetics: Results

Time required for complete transfer of methyl adducts using 0.1 M nucleophile

<b>Nucleophile</b>	<b>Time</b>
Aniline	2.5 months
Thiosulfate	1.8 days
Cob(I)alamin	27 seconds

This data indicates the power of cob(I)alamin as an analytical tool



# Properties of a trapping agent

Nucleophiles containing	Relative reactivity
Oxygen	1
Nitrogen	100
Sulphur	10,000
Cobalt	10,000,000

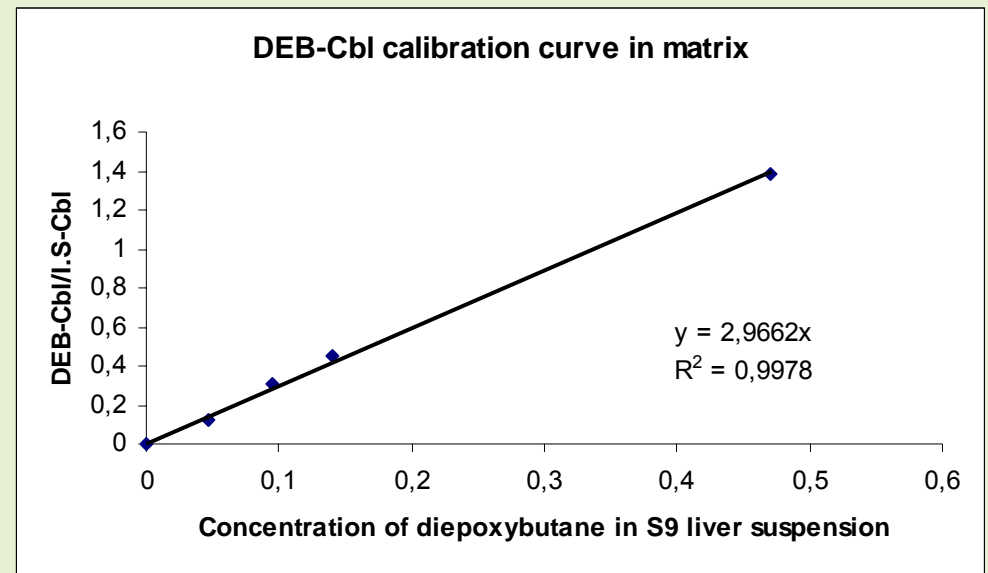
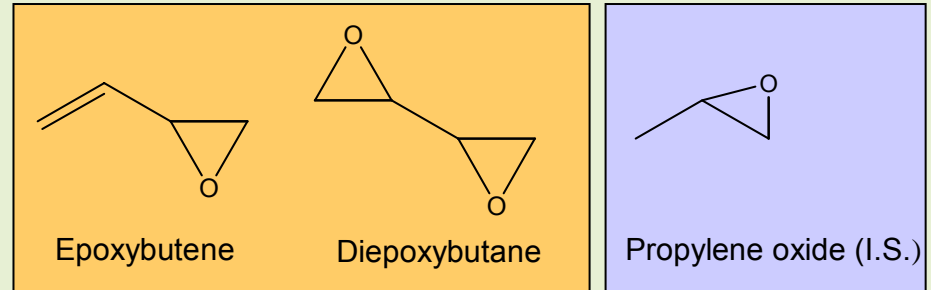
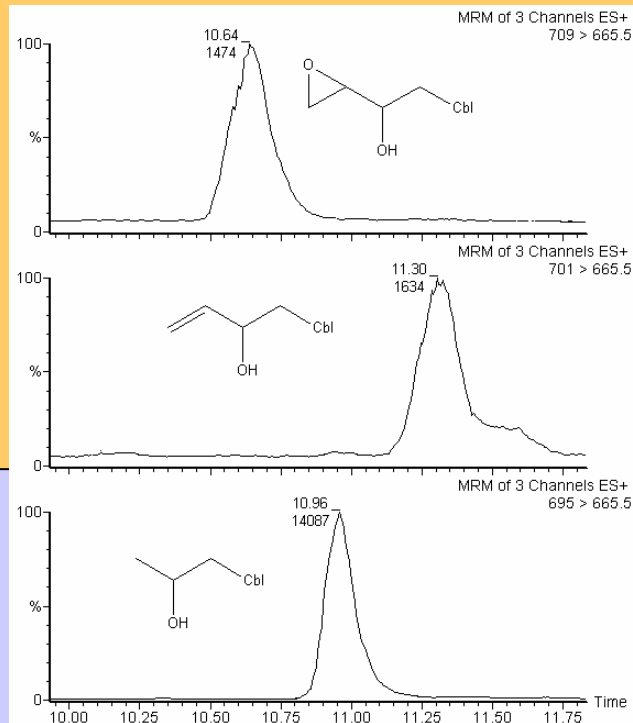
$$\frac{k_{n_1}}{k_{n_2}} = 10^{\Delta n}$$



# A new internal standard approach

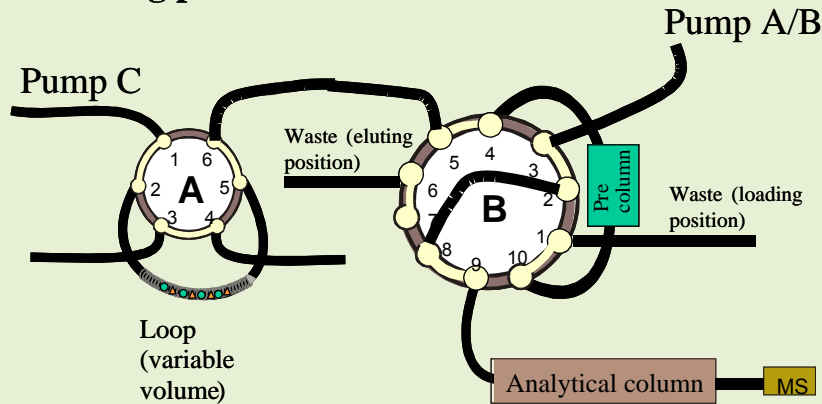
Internal standard: A chemical standard of a similar compound

## LC-MS/MS analysis of Epoxybutene and Diepoxybutane

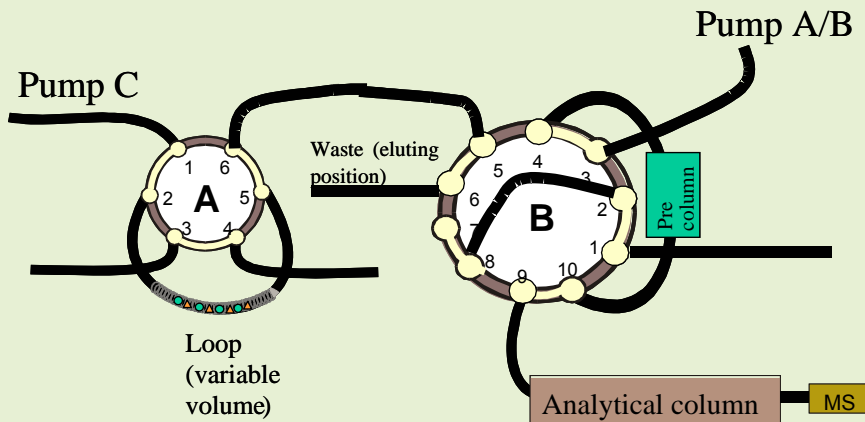


# Miniaturised LC-MS/MS column switching

## Loading position:



## Eluting position: Forward flush



- Excess of unmodified cobalamin to waste
- On-column focussing
- Increased concentration sensitivity
- No prior separation



# Research proposal: Cob(I)alamin as an analytical tool

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1. Further development of the transalkylation method
2. Studies of electrophilic reactivity
3. Trapping of reactive compounds in various matrices
4. Studies of metabolism in vitro



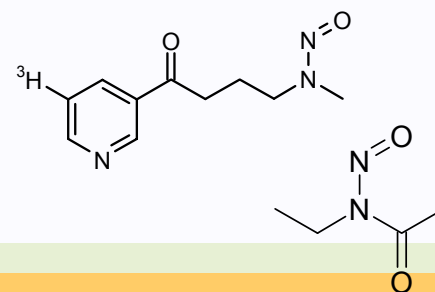
# 1. Further development of the transalkylation method

## State of the art

Confirmation of specific transfer<sup>1</sup>

Application to radiolabeled compounds in vivo<sup>2</sup>

Application to non-labeled compounds in vitro<sup>3</sup>



## Aims

Quantification in vitro and in vivo using the internal standard approach

Application to DNA from exposed humans

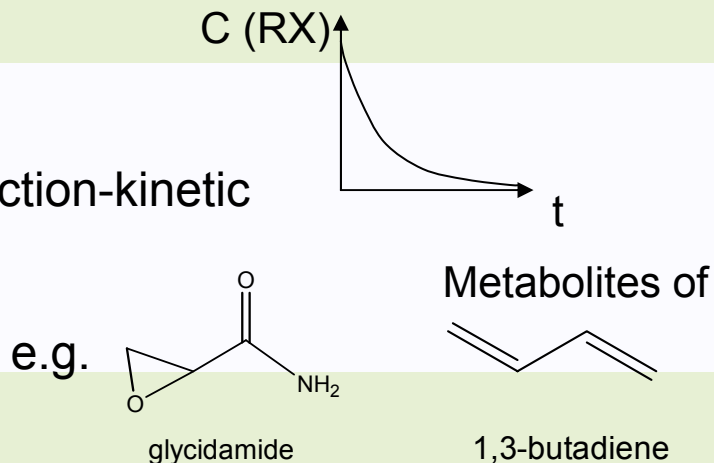
Dose distribution of chemotherapeutics



## 2. Studies of electrophilic reactivity

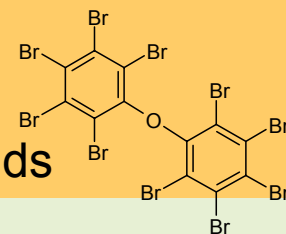
### State of the art

- Procedures have been developed for reaction-kinetic studies of reactive compounds<sup>1</sup>
- Several oxiranes have been studied<sup>1,2</sup>



### Aims

- Systematic study of electrophilically reactive compounds
- Comparison with data from mutagenicity tests
- Detection of electrophilic reactivity of semipersistent compounds



Decabromodiphenyl ether

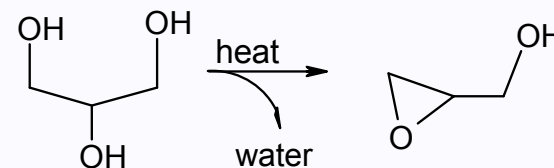
<sup>1</sup>Haglund et al., 2003; <sup>1,2</sup>Silvari, Haglund et al., ms



### 3. Trapping of reactive compounds in matrices

#### State of the art

- Procedures are being developed for trapping of reactive compounds in food stuffs.
- Studies of formation of reactive species in specific abiotic processes<sup>1</sup>.



#### Aims

- Further development of procedures for trapping different compounds in, e.g. food, water, air, blood
- Studies of other important abiotic processes
- Trapping of reactive metabolites in human blood

<sup>1</sup>Hindsö-Landin, Törnqvist et al., 1999



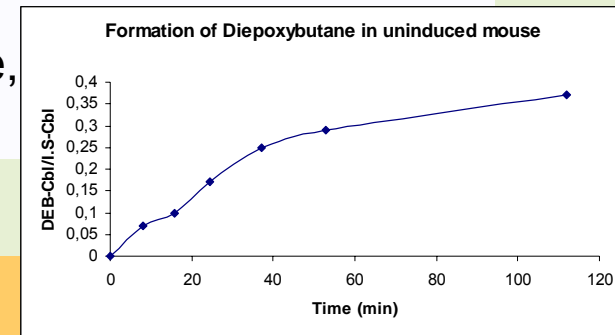
## 4. Studies of metabolism in vitro

### State of the art

- Internal standard approach has been developed<sup>1,2</sup>
- Studies of metabolic formation of diepoxybutane, metabolite of butadiene, has been initiated<sup>1,2</sup>

### Aims

- Further optimisation of the procedure
- Detection and identification of unknown metabolites
- Metabolic studies in human in vitro systems
- Comparison between species



<sup>1,2</sup>Fred et al., 2004, Haglund et al., manuscript



# Innovatory aspects and conclusions

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- Realisation of the need for a strong nucleophile for studies of reactive compounds
- Utilisation of supernucleophilicity of Cob(I)alamin in toxicology
- ONE reagent to solve analytical problems in a range of applications
- Quantification using a new approach suitable for all applications
- Sophisticated LC-MS/MS method developed useful in all applications
- The approach allows *in vitro* studies of genotoxicity
- Application to industrial, pharmaceutical and background compounds





## Collaboration and networks

### Stockholm University, Sweden

Assoc. Prof. Margareta Törnqvist  
Prof. em Lars Ehrenberg  
Dr. Charlotta Fred  
Dr. Virginia Silvari  
Dr. Tomas Alsberg

### University of Antwerp, Belgium

Prof. Eddy Esmans  
Dr. Filip Lemiére

### University of Newcastle, England

Prof. Bernard T. Golding  
Dr. Alistair Henderson

### Syngenta Central Toxicology

Laboratory, Cheshire, England  
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Dr. Toni Munter

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