Vitamin B_{12} as an Analytical Tool: Application to Industrial, Pharmaceutical and Background Xenobiotics

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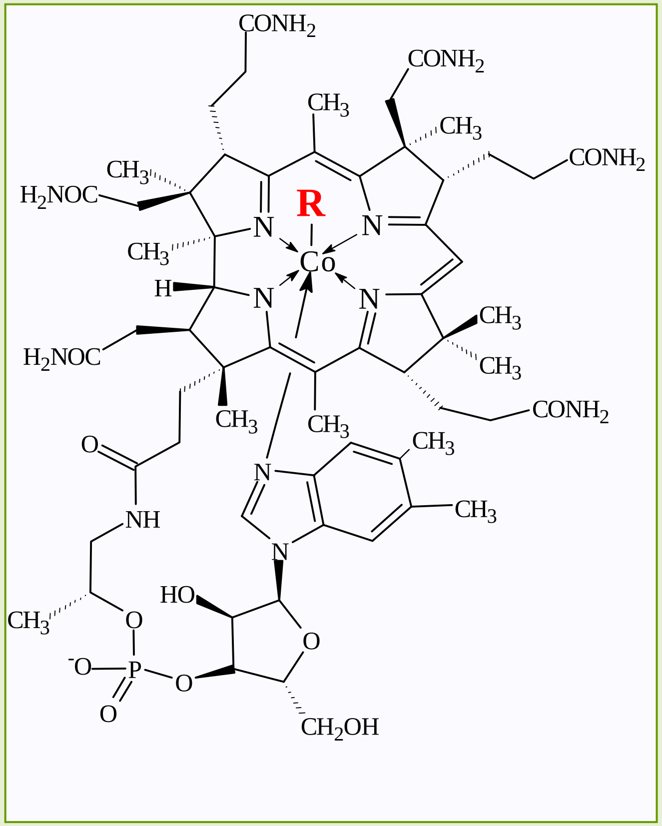
Vitamin B_{12}



1926	'Anti-pernicious anæmia factor' found (Minot & Murphy)
1948	Isolation of a crystalline solid (Folkers et al; Lester Smith)
1958	Vitamin B_{12} was structurally characterised (Hodgkin)
1972	Vitamin B_{12} was synthesised (Woodward and Escenmoser)
1996	We tested Vitamin B_{12} 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)**

$$A \xrightarrow{\text{metabolism}} RX \longrightarrow \text{elimination}$$

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

- Proteins
- Genetic material, DNA

$$RX + Nu: \longrightarrow R\text{-}Nu + X$$

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

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

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

- Established methods used worldwide

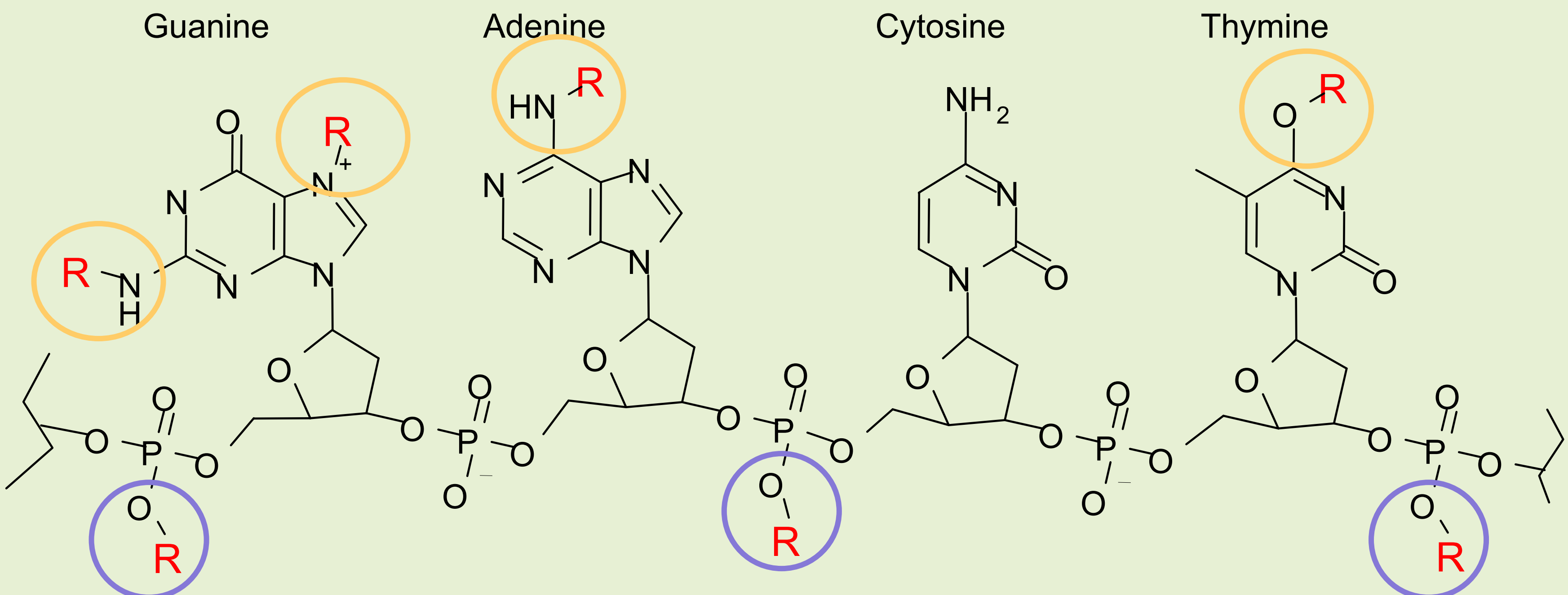
- Efforts to measure formation of reactive compounds using nucleophiles (in vitro)

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

- Limited usefulness

DNA-adduct formation

<u>Base adducts</u>: complex turnover kinetics

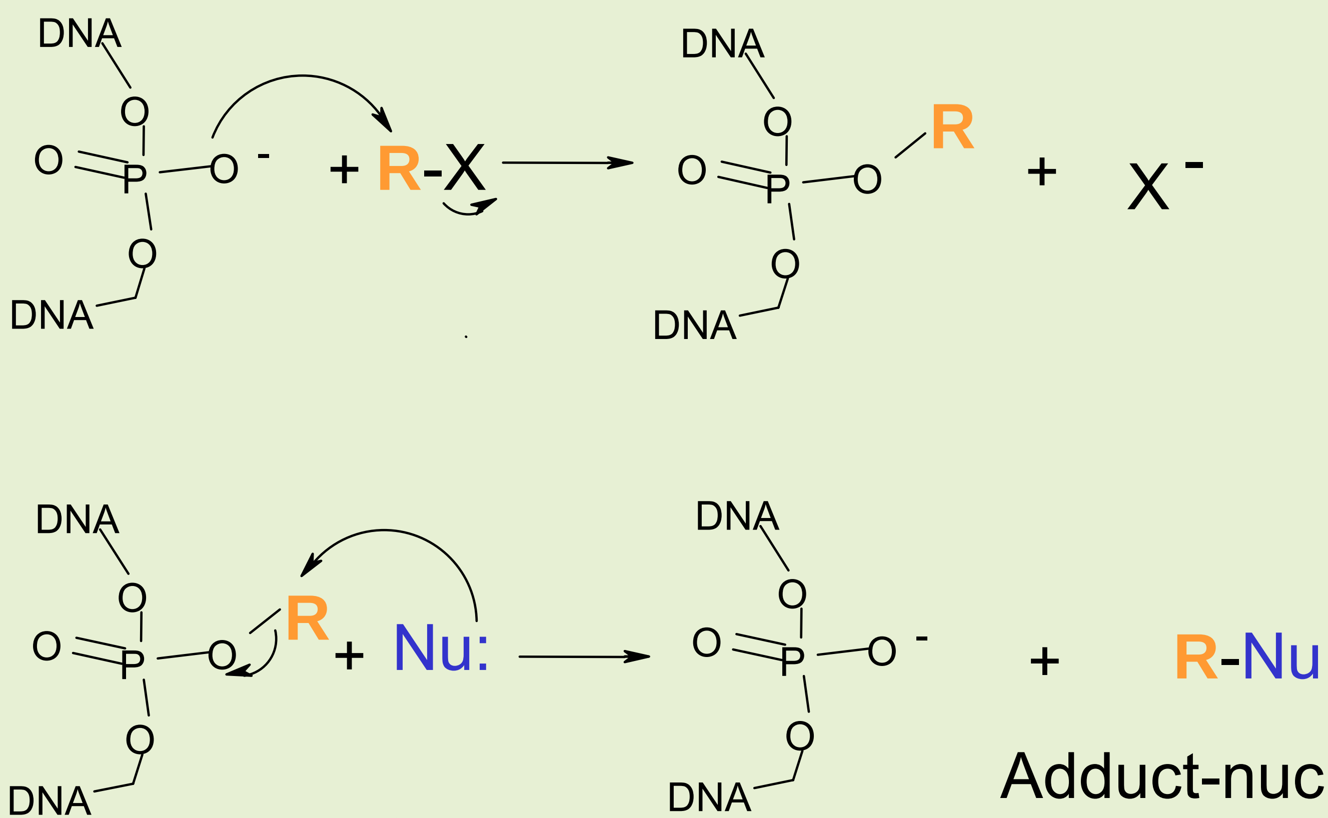


<u>Phoshate adducts</u>:

- 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?



Adduct-nucleophile compound

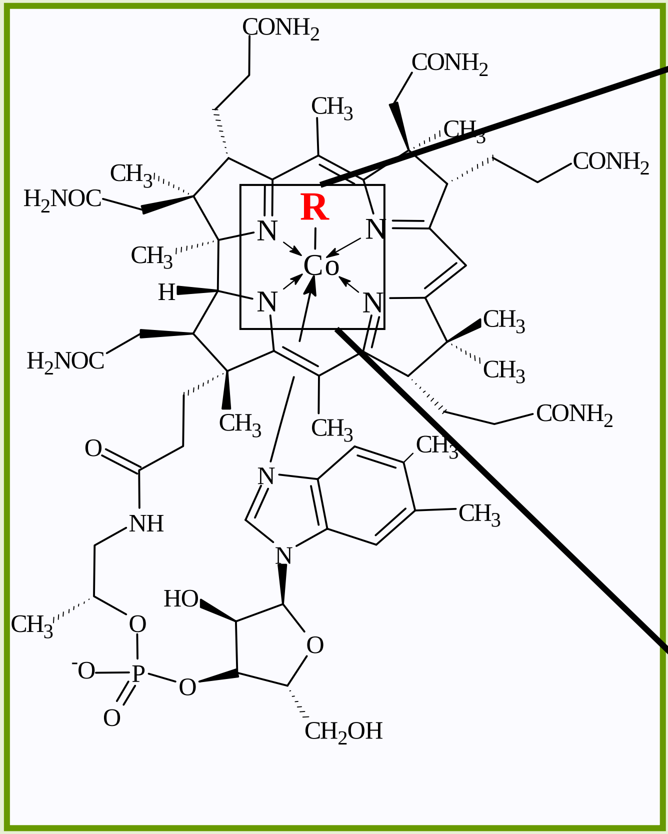
Reaction kinetics: Results

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

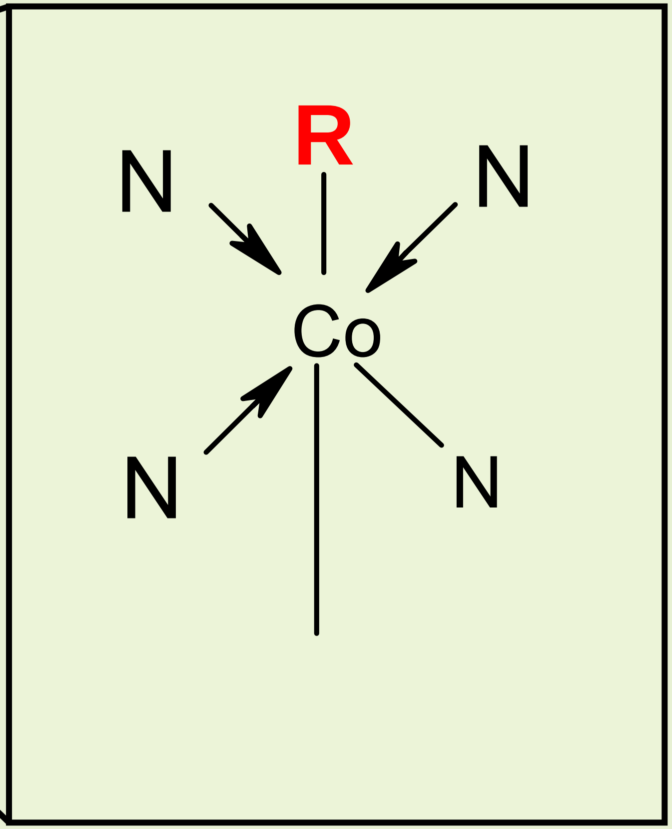
Nucleophile	Time
Aniline	2.5 months
Thiosulfate	1.8 days

Haglund et al., 1997

Vitamin B_{12}

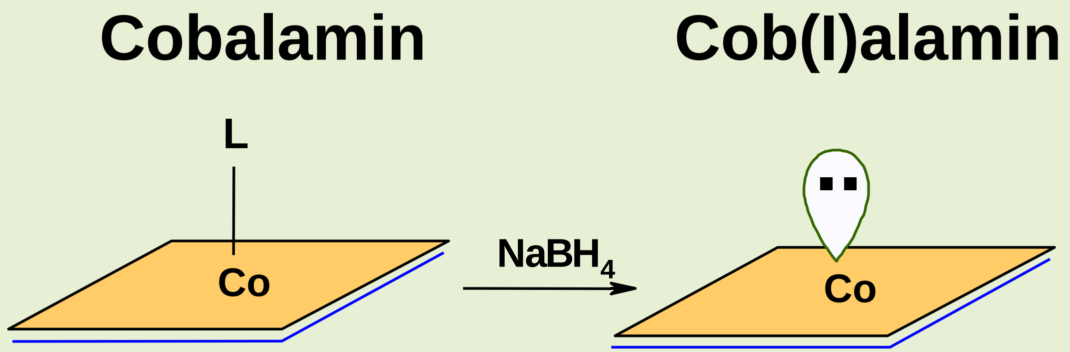


CONH2
CONH2
CH3
CH3
CONH2
CH3
H2NOC
R
N
N
Co
N
N
CH3
H
CH3
H2NOC
CH3
CH3
CH3
CONH2
CH3
CH3
O
N
NH
CH3
N
HO
CH3
O
-O
P
O
O
O
CH2OH



R
N
N
Co
N
N

Vitamin B_{12} (cobalamin)



L = variable ligand

L = free electron pair

→ **NUCLEOPHILE**

Reaction kinetics: Results

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

Nucleophile	Time
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

Haglund et al., 2000

Properties of a trapping agent

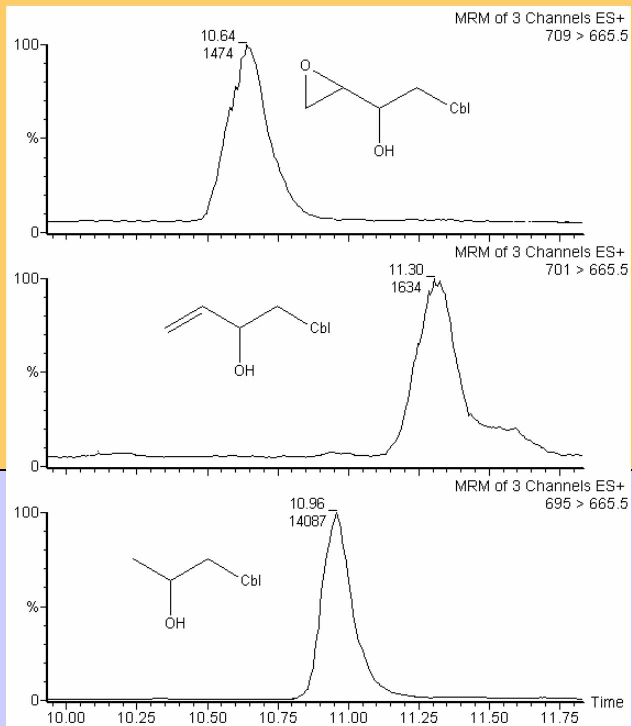
Nucleophiles containg	Relative reactivity
Oxygen	1
Nitrogen	100
Sulphur	10,000
Cobalt	10,000,000

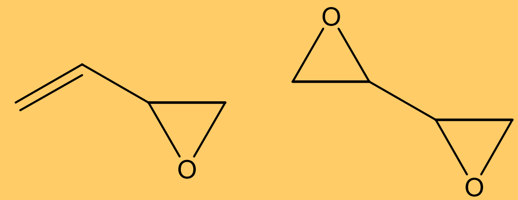
$$\frac{k_{n_1}}{k_{n_2}} = 10^{\Delta n}$$

A new internal standard approach

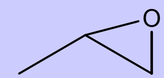
Internal standard: A chemical standard of a similar compound

LC-MS/MS analysis of Epoxybutene and Diepoxybutane

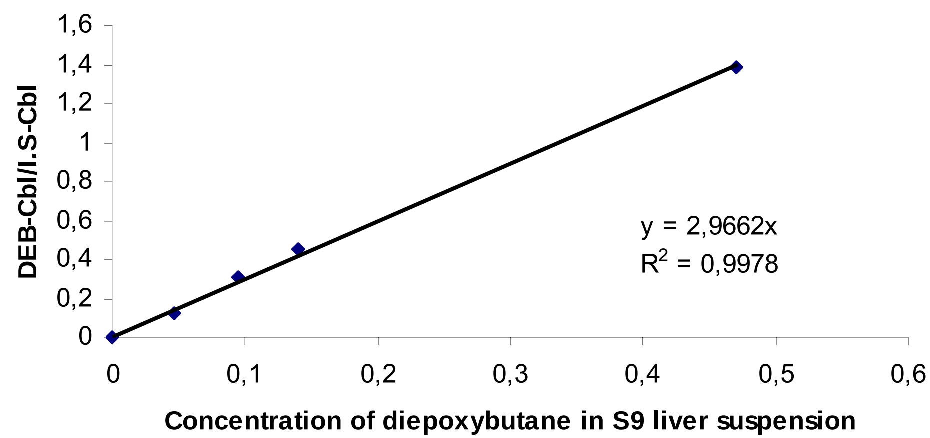




Epoxybutene Diepoxybutane



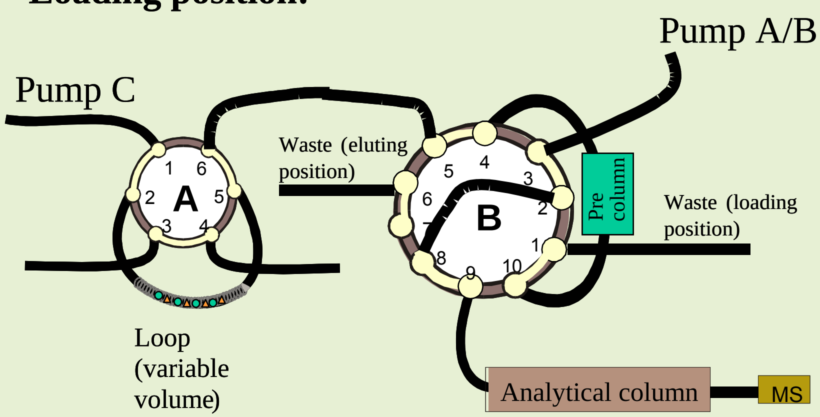
Propylene oxide (I.S.)



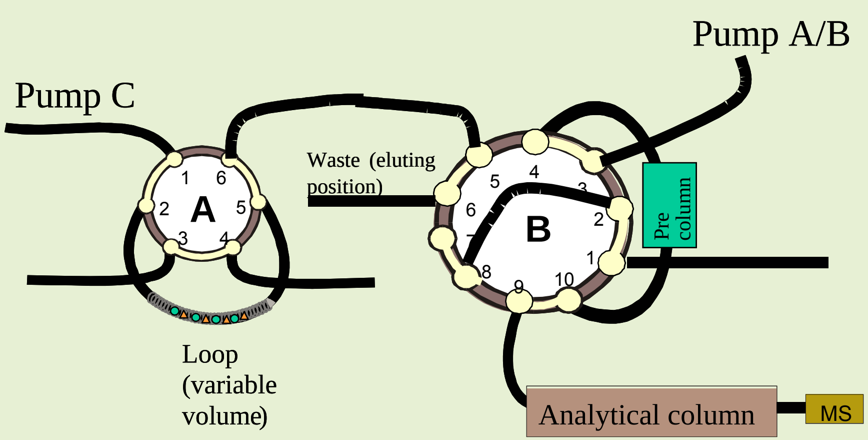
Fred et al., 2003; Haglund et al., ms

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

Alsberg et al., 2002; Haglund et al., 2004

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

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

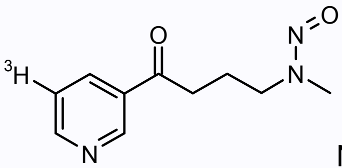
1. Further development of the transalkylation method

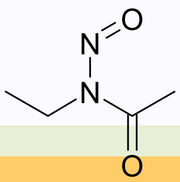
State of the art

Confirmation of specific transfer[1]

Application to radiolabeled compounds in vivo[2]

Application to non-labeled compounds in vitro[3]





Aims

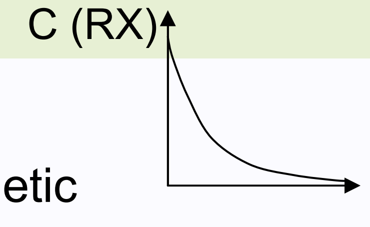
Quantification in vitro and in vivo using the internal standard approach

Application to DNA from exposed humans

Dose distribution of chemotheraputics

Haglund et al., 1997[1], 2000[1], 2002[2], 2004[3] and ms[3]

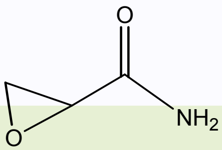
2. Studies of electrophilic reactivity



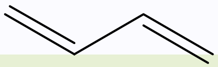
State of the art

- Procedures have been developed for reaction-kinetic studies of reactive compounds[1]
- Several oxiranes have been studied[1,2]

Metabolites of

e.g. 

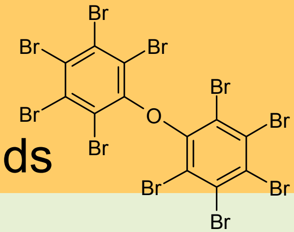
glycidamide



1,3-butadiene

Aims

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



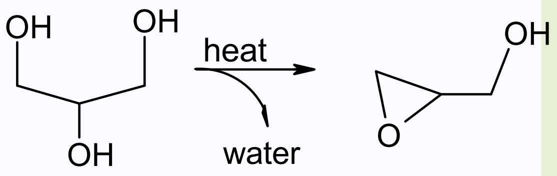
Decabromodiphenyl ether

[1]Haglund et al., 2003; [1,2]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[1].



Aims

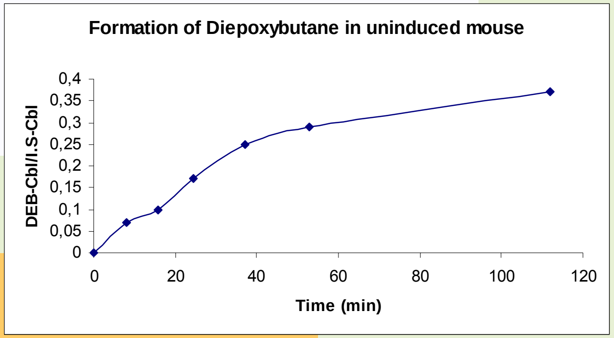
- Further development of procedures for trapping different compounds in, e.g. food, water, air, blood
- Studies of other important abiotic processes
- Trapping of reactive metabolites in human blood

[1]Hindsö-Landin, Törnqvist et al., 1999

4. Studies of metabolism in vitro

State of the art

- Internal standard approach has been developed[1,2]
- Studies of metabolic formation of diepoxybutane, metabolite of butadiene, has been initiated[1,2]



Aims

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

[1,2]Fred et al., 2004, Haglund et al., manuscript

Innovatory aspects and conclusions

- Realisation of the need for a strong nucleophile for studies of reactive compounds
- Utilisation of supernucleophilicity of Cob(I)alamin in toxicolgy
- 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

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Syngenta Central Toxicology
Laboratory, Cheshire, England
Dr. William P Watson
Dr. Toni Munter

Work partly supported by EU project: HPRT-CT-001538