ALTERNATIVE METHODS

Reactivity studies to complement the development of alternative methods for the prediction of skin sensitization: the case of formaldehyde and formaldehyde releasers

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Contact Dermatitis & Alternative Methods

Allergic diseases are increasing

Public health problem

Quality of life

Health system
hospitalization, sick leave,
drug prescriptions

Use of many chemicals
Fields of pharmaceuticals and cosmetics

Contact allergy is most prevalent

European regulations (2003/15/EC) prohibit the use of animals for toxicological evaluation of cosmetic products

Essential to have methods that can predict the allergenic potential of molecules

Necessary to develop alternative methods

Contact Dermatitis

2 associated immunological steps

Sensitization phase

↓

Formation of [hapten-protein] complex
Key step
Antigen formation

↓

Production of specific T cells

Elicitation Phase

↓

Formation of [hapten-protein] complex

↓

Activation of specific T cells

↓

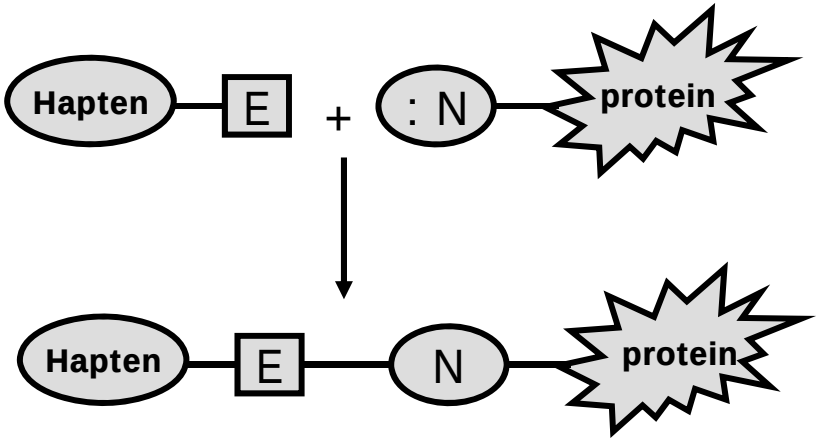
Allergic Reaction

Hapten-protein complex

Hapten:
small molecule (<10 kDa)
electrophilic and lipophilic
able to cross the skin barrier

Pre/Pro-hapten:
after air oxidation
or in vivo metabolism
molecule becomes reactive

Interaction with nucleophilic residues of proteins to form the complex [hapten-protein]



immune response

Antigenic complex

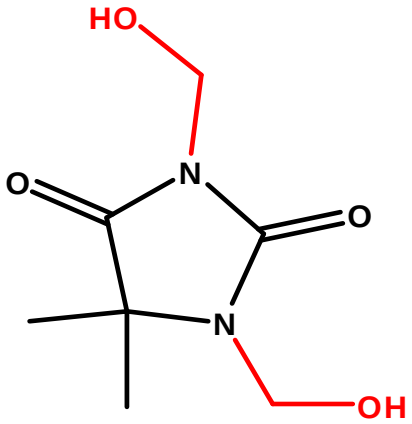
KEY STEP

relationship between structure & activity

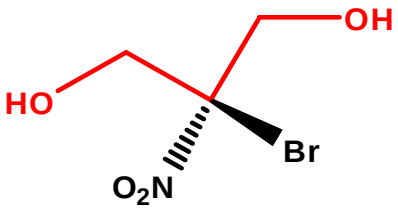
Contact Dermatitis to Preservatives

Preservatives are one of the major causes of contact allergy to cosmetics

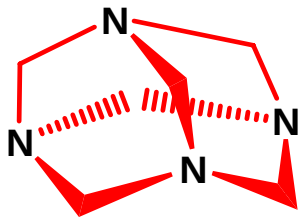
The most common used are formaldehyde releasers



DMDM Hydantoin



Bronopol



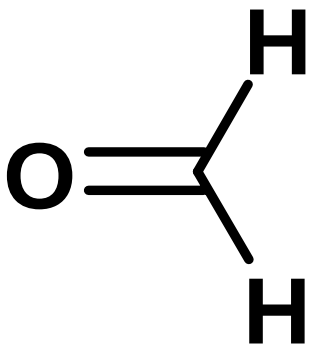
Methenamine

CD to formaldehyde releasers ? **No CD to formaldehyde**

Study of hapten-protein interactions

Database on the reactivity of formaldehyde with amino acids
Compare with reactivity of formaldehyde releasers

Formaldehyde



CAS [50-00-0]

Properties: bactericide and fungicide

Largely present in everyday life:
detergents, paints, polish, cosmetics …

CMR class 1
carcinogenic for human

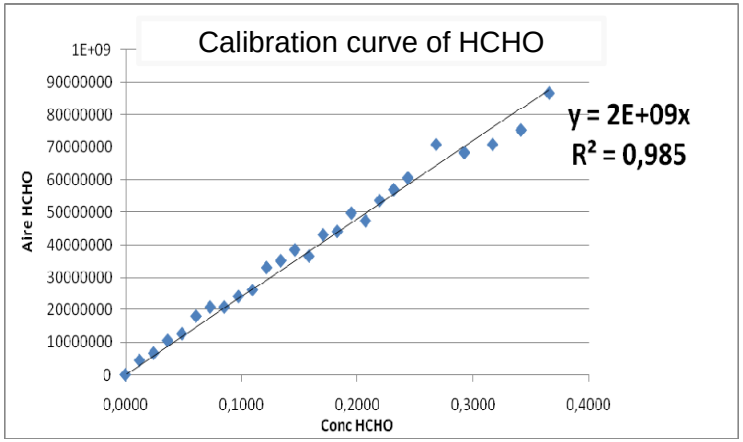
Strong skin sensitizer

Small molecule (<10 kDa)
Electrophile = Hapten

Present in cosmetics in diverse forms:

- **Free form (rare)**
- **Contained in compounds that can release it**

Kinetics of release of formaldehyde



Methodology:

Determination of free HCHO by extrapolation on a calibration curve

Analysis by NMR of 26 solutions with a known concentration of HCHO (0 to 0.37 mol/L)

The peak area is measured in order to obtain a curve: Area (peak HCHO) = f ([HCHO])

Area (peak HCHO) = 2 x 10^9 [HCHO]

Carbon NMR settings: T1 = 6s d1 = 30s NS = 200

FR	[FR]	[HCHO] in phosphate buffer D1	HCHO] in phosphate buffer D7
HCHO	0.146	0.146	0.146
DMDMH	0.289	0.121	0.122
Bronopol	0.273	0.017	0.020

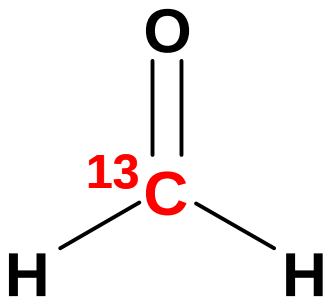
Bronopol D 40 [HCHO] free 0.104 mol/ L

Different behaviour for the release of HCHO

Reactivity with amino acids

Strategy:

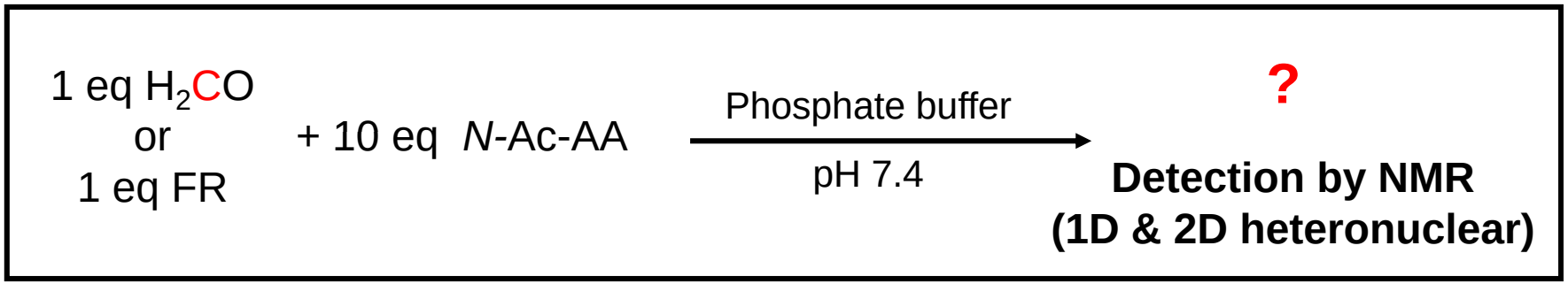
Method based on carbon 13 NMR



Labelling the reactive site with ^{13}C (gain of detection)

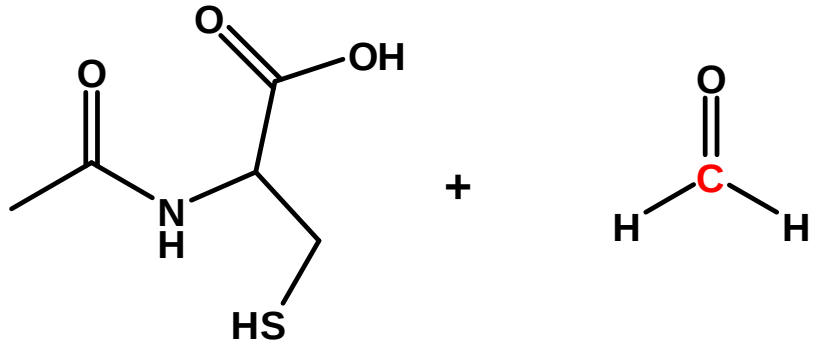
Study the interactions with nucleophilic *N*-acetylated amino acids

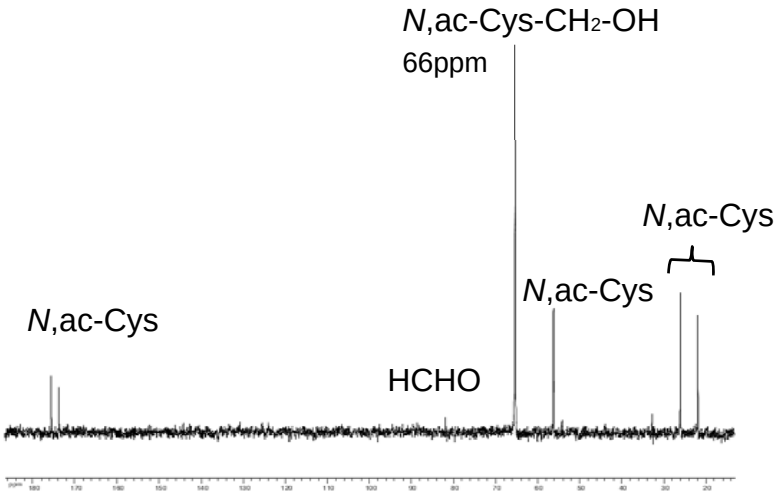
Methodology:

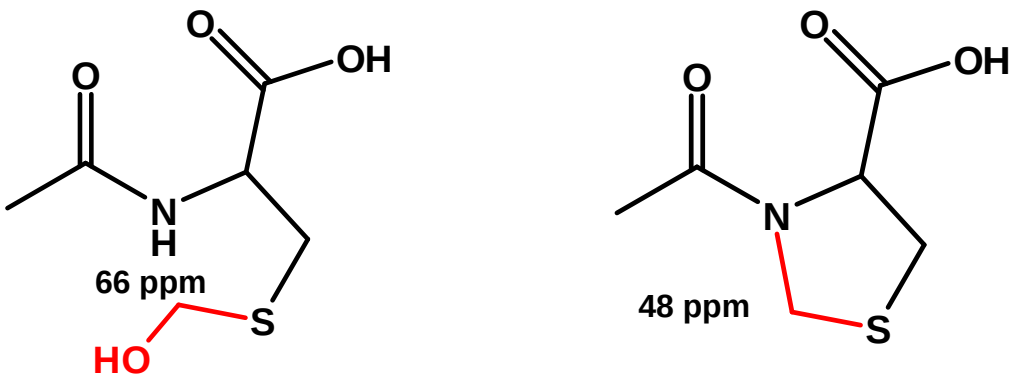


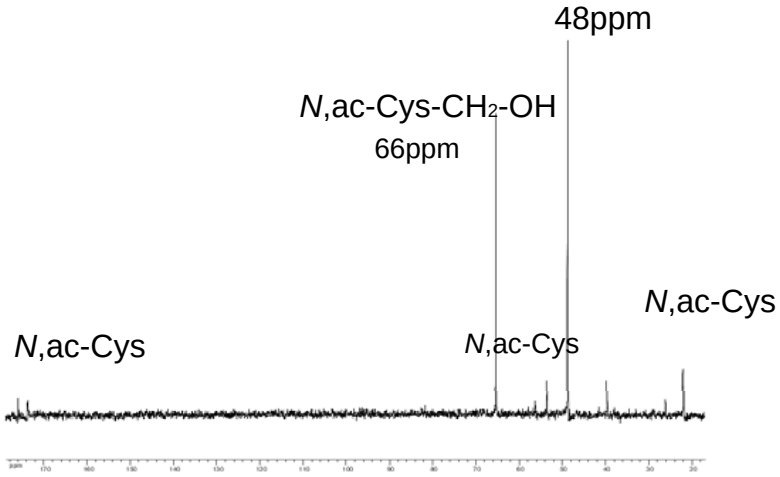
FR: ^{13}C labelled formaldehyde releaser

Reactivity of *N*-acetyl-cysteine + H_2CO





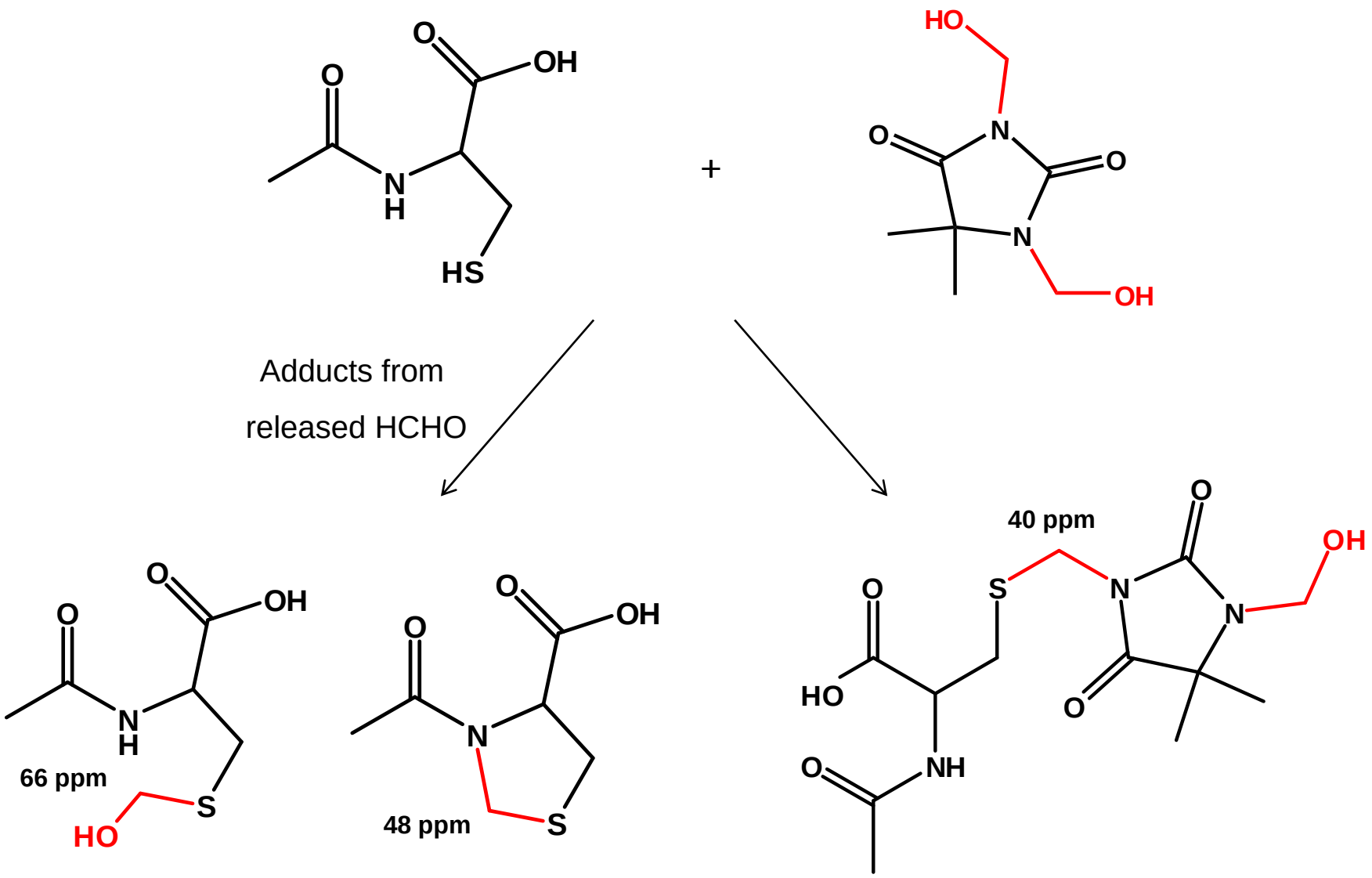




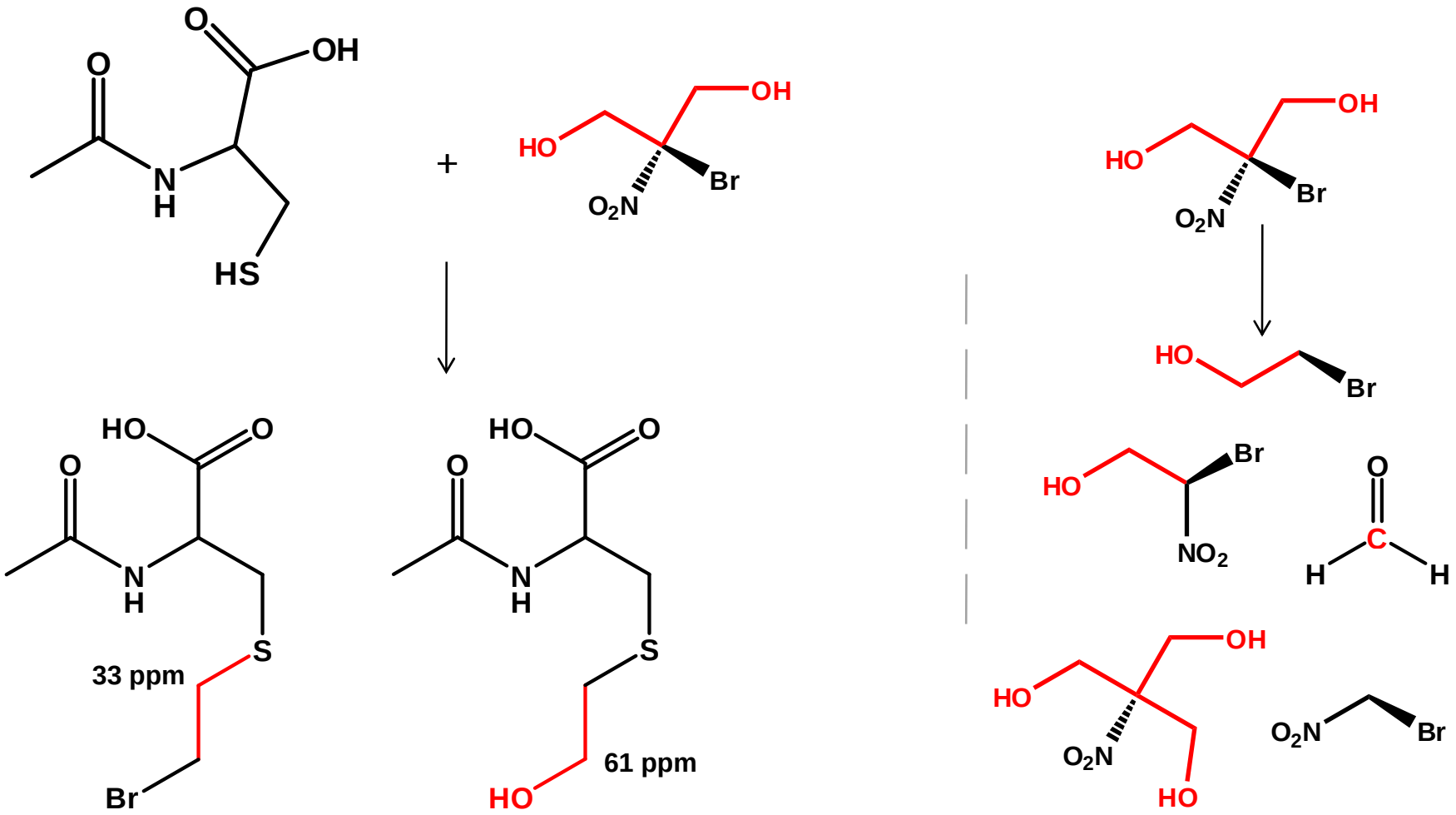
Formation of hydroxymethyl

Cyclization

Reactivity of *N*-acetyl-cysteine + DMDM Hydantoin



Reactivity of *N*-acetyl-cysteine + Bronopol



We do not observe adducts derived from a reactivity with released HCHO

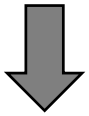
Conclusion

Reactivity of formaldehyde releasers with *N*-acetyl-cysteine

Formation of the hydroxymethyl adduct followed by cyclization

Formation of adducts by reactivity with released HCHO

Formation of adducts by direct reactivity with the formaldehyde releaser



This experiment could explain the CD cross reactions observed and why some people do not have CD to formaldehyde but to a formaldehyde releaser