

8EHQ-0700-13687

COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

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8EHQ-96-13687

July 11, 2000

TSCA Document Control Office (7408)
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

Attention: TSCA 8(e) Coordinator

Re: 8EHQ-0796-13687 - Supplement

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2000 JUL 17 5:11:53

The American Chemistry Council Propylene Oxide Panel has received information from a study measuring DNA adducts of propylene oxide (PO, CAS No. 75-66-9) in workers reportedly exposed to PO. The information is being submitted in accordance with Section 8(e) of the Toxic Substances Control Act and supplements information previously reported by Panel member Lyondell Chemical Company (and its predecessor ARCO Chemical Company) on July 8, 1996, and November 4, 1999, (8EHQ-0796-13687). The Panel is submitting the information on behalf of all its member companies, which also include The Dow Chemical Company and Huntsman Petrochemical Corporation.

The attached information is a presentation made to the Panel on June 19, 2000, by Dr. Kamila Plna, a research assistant of Professor Dan Segbäck at the Karolinska Institute (Stockholm, Sweden). The study reported DNA adducts (1-(2-hydroxypropyl)adducts (HPA) in blood samples from seven out of eight workers reportedly exposed to PO at a manufacturing facility in China, while 8 control workers did not have detectable levels of HPA adducts.

This information is being submitted pursuant to current guidance issued by EPA indicating EPA's interpretation of Section 8(e) of the Toxic Substance Control Act. The Panel has made no determination as to whether a significant risk of injury to health or the environment is actually presented by the findings. This is the first study of which the Panel is aware to report DNA adducts in PO-exposed humans. The information is, however, incomplete, preliminary and of questionable significance. In particular, the sample set is very small, comprising only 8 workers each for control and exposed sample groups. Also, only minimal exposure information was provided for the eight exposed workers and no information was provided on exposures to other substances those workers may have experienced. In addition, contrary to expectations based on similar compounds, no detectable adducts were identified in control smokers, nor did the exposed smokers demonstrate higher levels of adducts; and there was no confirmatory data (e.g., cotinine levels) on smoking status of any control or exposed worker. Furthermore, the background incidence of HPA adducts in humans has not been well established nor have their biological relevance.

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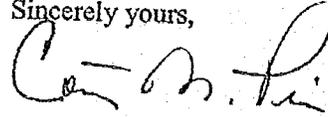
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The Panel will apprise the Agency of additional data as it is received. If there are any questions, please call the PO Panel Manager Anne LeHuray (703-741-5630).

Sincerely yours,



Courtney M. Price

Enclosure

cc: (w/att): Propylene Oxide Panel Members and TRTG

Minor adducts of PO
Useful as biomarkers of exposure?

- ³²P-Postlabelling of 1- and N⁶-substituted adenine and 3-substituted cytosine/uracil of propylene oxide: formation and persistence *in vitro* and *in vivo*.

Phase I study

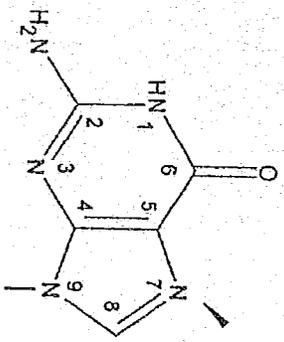
- Human exposure to propylene oxide

Procedure for analysis of PO-adducts

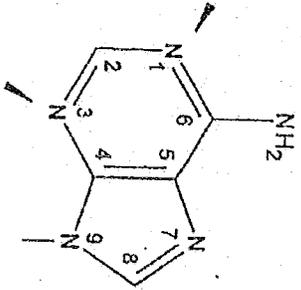
- *Preparation and characterization of standards*
 - 3'- and 5'-dNMP
 - characterized by UV, rearrangements, MS,
 - True adduct levels of *in vitro* modified DNA
 - Stability of adducts
- *Development of quantitative methods for the analysis of PO-adducts*
- ³²P-*postlabelling analysis of animal or human samples*
 - PO-DNA *in vitro* as external standard
 - 5'-dNMP adducts as internal UV standards

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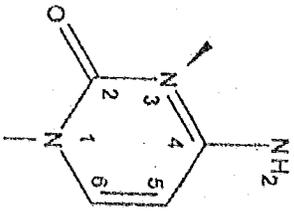
Adducts of PO in DNA *in vitro*



Guanine



Adenine



Cytosine

N7-guanine (100)

Half-life in DNA: 5 days
(depurination)

N1-adenine (4)

Half-life in DNA: 10 days
rearrangement to N⁶-adenine

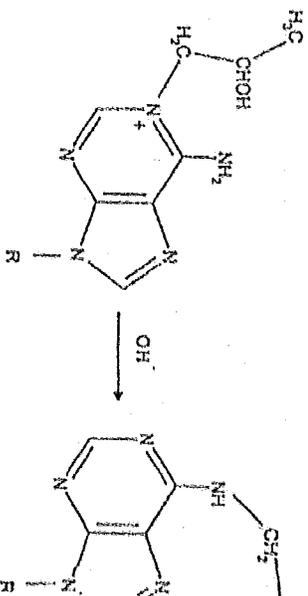
N3-cytosine (2)

Half-life in DNA: ~2 days
deamination to N3-uracil

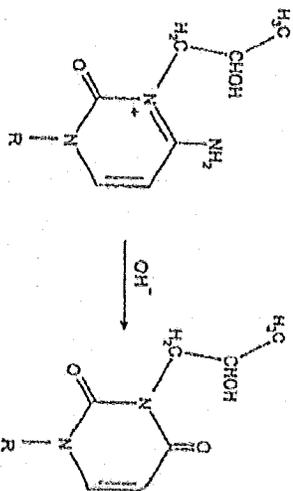
N3-adenine (10)

Half-life in DNA: ~1 day
(depurination)

Base-catalysed rearrangement of 1-(2-hydroxyprop-2-yl)adenosine-5'-monophosphate to N⁶-(2-hydroxyprop-2-yl)adenosine-5'-monophosphate; R = deoxyribose-5'-monophosphate



Base catalysed deamination of 3-(2-hydroxypropyl)-deoxyribose-5'-monophosphate to 3-(2-hydroxypropyl)-deoxyuridine-5'-monophosphate; R = deoxyribose-5'-monophosphate.



Scheme of the ³²P-postlabelling method

Carcinogen-adducted DNA
 [.....pNpNpNpXpNpNpN.....]

"Dinucleotide procedure"

▪ Initial digestion (enrichment)

NPI/PAP
 1-ADE, N⁶-ADE
 3-CYT, 3-URA
 ↓
 XpN + N + p_i

▪ Labelling

T4 kinase/ ³²P-ATP
 ↓
 *pXpN

▪ Final digestion

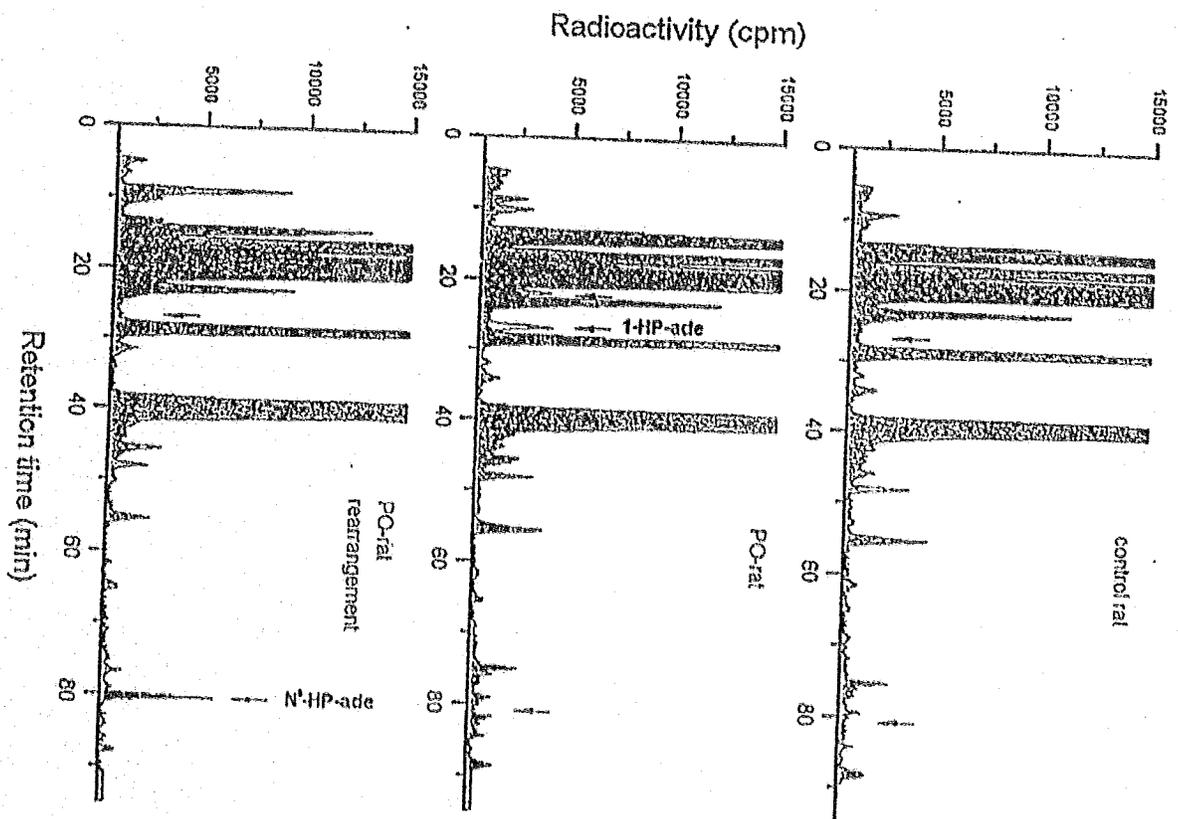
SVPD
 ↓
 *pX + p_i

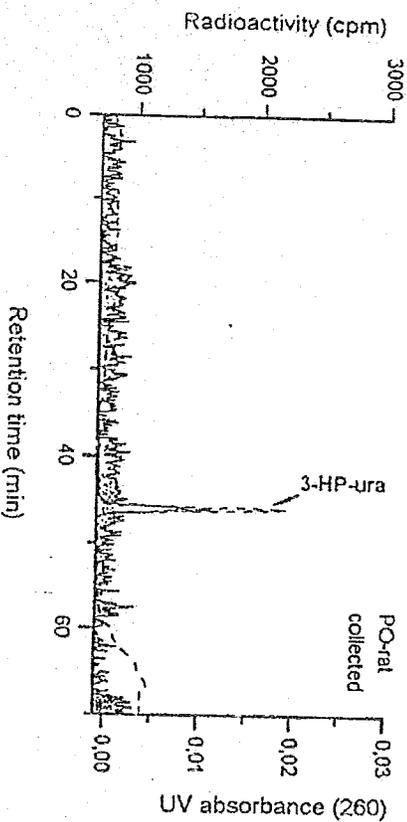
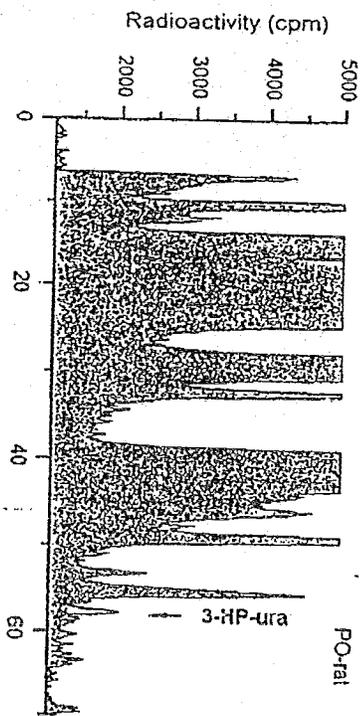
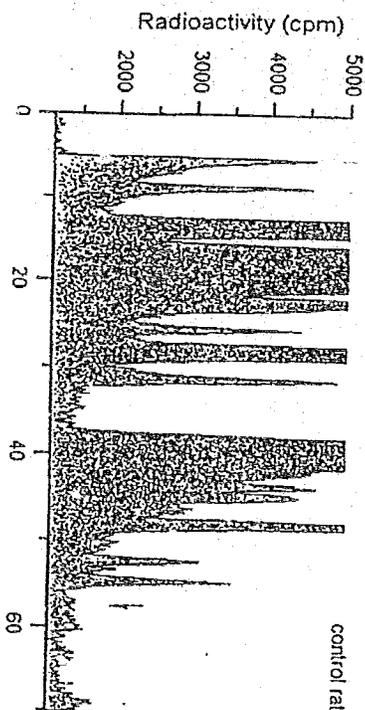
▪ Adduct separation (HPLC)

▪ Confirmation of adduct identity

▪ Quantification

³²P-HPLC analysis of 1-HP-5'-dAMP in postlabelled lung DNA





Relative amounts of different PO-adducts in DNA *in vitro* and *in vivo* (PO-rats sacrificed directly).

	<i>In vitro</i>	Res- mucosa	Lung	Lymph cytes
N ⁷ -guanine	100	100	100	100
N ¹ and N ³ -adenine	3	2	2	2
N ³ -cytosine/triplet	2	0.02	ND	ND

CONCLUSIONS I

IN VITRO

- 1-HP-adenine and 3-HP-cytosine and their rearrangement products to N⁶-adenine and N-3-uracil, respectively, could be detected with the nuclease P1 enrichment version of the postlabelling assay. Recoveries for all four adducts were higher than for 7-HP-guanine.
- The rearrangement of 1-HP-adenine to N⁶-HP-adenine was slow.
- The deamination of 3-HP-cytosine to 3-HP-uracil was faster than the rearrangement of 1-HP-adenine, but not as fast as indicated by earlier published data.

CONCLUSIONS II

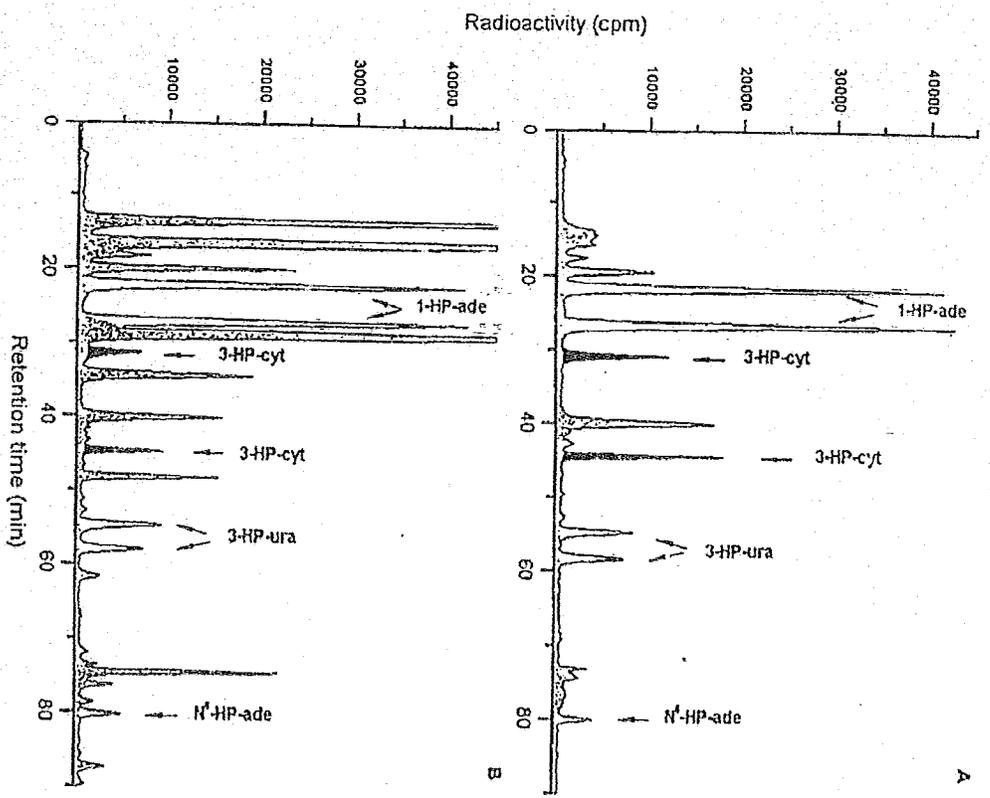
IN VIVO

- 1-HP-adenine was detected in PO-exposed rats and the adduct could be an alternative (to 7-HP-guanine) monitoring of PO-exposures.
- 3-HP-uracil was also detected in the nasal epithelia of P-exposed rats but at low levels.

IN VIVO REPAIR

- Adduct levels of 3-HP-cytosine/uracil in P-rats suggest repair of the cytosine and/or uracil adducts.
- 1-HP-adenine was not repaired (or repaired very slowly) in P-exposed rats
- 7-HP-guanine was not repaired in PO-exposed rats (loss adduct only due to depurination)

In vitro repair study



HPLC separation of nuclease P1 enriched and postlabelled, (A) propylene oxide reacted DNA; (B) propylene oxide reacted DNA incubated with protein extract from HeLa cells. 60% of 3-HP-cytosine was removed after 1 hour of incubation.

CONCLUSIONS III

IN VITRO REPAIR STUDY

- 3-HP-cytosine (not other adducts) was removed from DNA after incubation with the protein extract from HeLa cells.
- bacterial uracil glycosylase was not involved in repair of this adduct.

Human exposure to propylene oxide

- *Samples:* white blood cells: 8 exposed
8 controls
- *Exposed:* Huludao Chemical Plant in China (PO is produced)
working site: packing
concentrations of PO: 9-16 mg/m³ (4-7 ppm)
- *Controls:* Huludao Institute of Occupational Health
- *Biomarkers:* DNA adducts
Haemoglobin adducts
Chromosomal aberrations
- *Analysis:* 1-HP-adenine

Propylene oxide concentrations of working place (original data)

Code	Sites	Sample time (min)	Concentration (ug/m ³)
1	common room	60	2.1
2	polymerize	60	4.1
3	common room	60	2.7
4	packing	20	16.6
5	Packing	20	8.9
6	polymerize	60	3.1

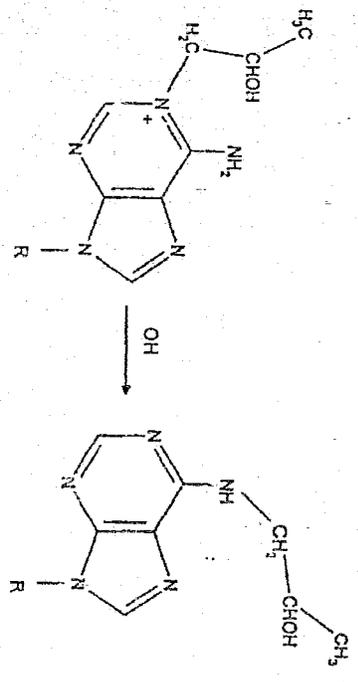
Analysis of 1-HP-adenine in human samples

"Dinucleotide procedure"

- Initial digestion (enrichment)
- ³²P-Labeling
- Final digestion

- HPLC separation of 1-HP-5'-dAMP
- Collection of adduct

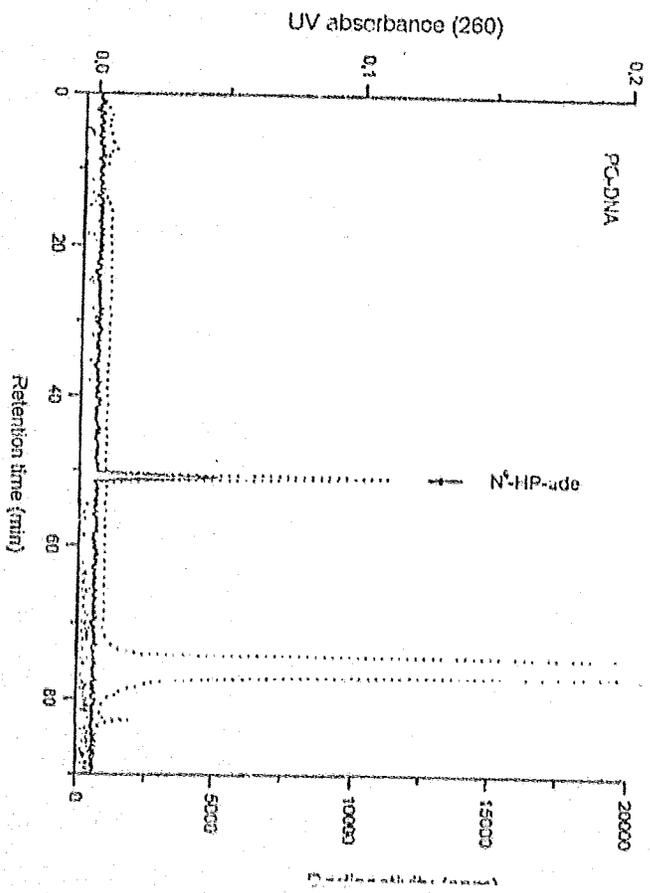
- Rearrangement of 1-HP-5'-dAMP to N⁶-HP-5'-dAMP (pH 12)



- HPLC separation of N⁶-HP-5'-dAMP

³²P-HPLC analysis of N⁶-HP-adenine in postlabelled PO-DNA

- PO-DNA
- Adduct recovery 54% (¹⁴C radiochromatography)
- Detection limit 0.1 adduct / 10⁹ dNp



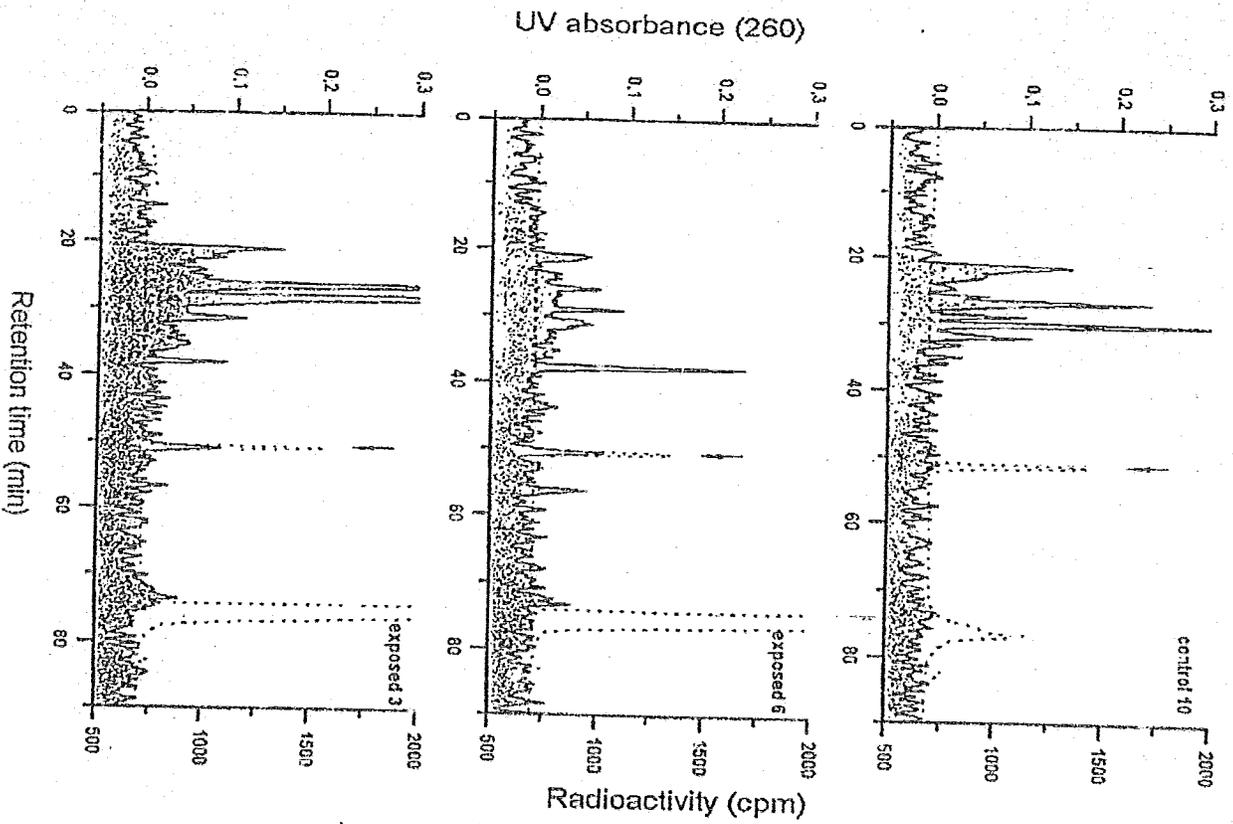


Table 1. Levels of 1-(2-hydroxypropyl)adenine (mol per 10⁹ mol of nucleotides) in workers occupationally exposed to propylene oxide

Individual no.	Smoking habit ^a	Exposure category	Adduct level ^b
1	NS	exposed	0.4
2	NS	exposed	< 0.1
3	NS	exposed	0.7
4	NS	exposed	1.0
5	S	exposed	0.97
6	NS	exposed	0.73
7	S	exposed	1.0
8	NS	exposed	0.45
Mean ± SD			0.66 ± 0.34
9	NS	control	< 0.1
10	S	control	< 0.1
11	S	control	< 0.1
12	NS	control	< 0.1
13	S	control	< 0.1
14	NS	control	< 0.1
15	NS	control	< 0.1
16	S	control	< 0.1
Mean ± SD			< 0.1

^aS, smoker; NS, non-smoker.

1-HP-adenine levels in white blood cells of propylene oxide-exposed workers

	Exposed	Controls
N	7 (8)	0 (8)
Findings/10 ⁵	0.4 - 1	<0.1

Mann-Whitney two sample test

- Significant difference between exposed and controls ($P = 0.0012$)

CONCLUSIONS

- Study demonstrates for the first time the presence of PO-induced DNA adducts in PO-exposed workers.
- 1-HP-adenine is one of few specific adducts detected in human tissues.
- Because of persistence *in vivo* (shown in rats) as well as the high sensitivity and specificity of the method, 1-HP-adenine is a suitable biomarker for monitoring low-level occupational exposure to PO.