

8EHQ-1199-13687



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Newtown Square, Pennsylvania 19079-2387
Telephone: 610.359.2000
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1999 NOV -8 AM 11:47

Health Sciences and Regulatory Programs

November 4, 1999

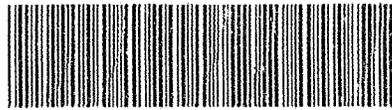
TSCA Document Control Office (7408)
Office of Pollution Prevention and Toxics
U. S. Environmental Protection Agency
401 M Street, S.W.
Washington, D. C. 20460

PR 22316

Contain NO CBI

Attention: TSCA 8(e) Coordinator

RE: 8EHQ-0796-13687 - Supplement



8EHQ-96-13687

Dear Sir or Madam:

As a member of the Propylene Oxide Panel of the Chemical Manufacturers Association, Lyondell Chemical Worldwide, Inc. has received preliminary data from a study measuring DNA adducts of propylene oxide (PO), CAS number 75-56-9. The information obtained is being submitted in accordance with Section 8(e) of the Toxic Substances Control Act (TSCA) and EPA's 1991 Section 8(e) Reporting Guide. This information supplements that reported on July 8, 1996 (8EHQ-0796-13687).

Please note that, since submission of the initial 8(e) report, ARCO Chemical Company has been acquired by Lyondell Chemical Company and now is operating as a wholly-owned subsidiary under the name of Lyondell Chemical Worldwide, Inc.

A research update meeting was held on October 26-27, 1999 to report progress on the research program to measure propylene oxide adducts with DNA and toxicity as evaluated by cell proliferation measurement. Preliminary results reported by program researchers indicate that: 1) PO adducts with rat nasal respiratory epithelial DNA may be detected after exposures to 5 ppm PO vapors, 6 hours/day, 5 days/week for either three days or for four weeks; 2) increases in cell proliferation in male rat nasal respiratory epithelium and in respiratory epithelium lining the nasopharyngeal duct could be detected at exposure levels as low as 300 ppm PO vapors 6 hours/day for three days or for 20 days total exposure.

While we continue to believe that these findings do not represent any new significant risk for humans, this is, to our knowledge, the first report of this finding in F344 rat nasal and nasopharyngeal tissues and supports the observation that, with PO, tumors are being seen only at the sites of cell damage. Charts describing the recent preliminary data are attached.

This research is part of a voluntary program sponsored by PO producers to better understand the mechanism of animal nasal tumor formation and its implications for hazard assessment. Results of the research program will be reported in the form of peer-review publications at the discretion of the researchers. The research being reported in this letter is being conducted by Dr. James Swenberg, University of North Carolina, Chapel Hill, NC.

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U. S. Environmental Protection Agency
Propylene Oxide
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The Agency will be apprised of ongoing data from this program as a supplement to this notice.

Sincerely,



L. S. Andrews, Ph.D.

Attachments

CC: Ms. Bette Moran
Associate Director, CHEMSTAR
Manager, Propylene Oxide Panel
Chemical Manufacturers Association
1300 Wilson Boulevard
Arlington, VA. 22209

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Propylene Oxide

Laboratory of Mutagenesis
and Molecular Carcinogenesis
University of North Carolina at Chapel Hill

Publications:

Ríos-Blanco, M.N., K. Pina, T. Faller, W. Kessler, K. Håkansson, P.E. Kreuzer, A. Ranasinghe, J.G. Filser, D. Segerbäck and J.A. Swenberg (1997) Propylene Oxide: mutagenesis, carcinogenesis and molecular dose *Mutation Research* 380, 179-197.

Ríos-Blanco, M.N., T. Faller, J. Nakamura, W. Kessler, P.E. Kreuzer, A. Ranasinghe, J.G. Filser and J.A. Swenberg DNA and hemoglobin adducts of propylene oxide in F344 rats and the quantitation of DNA apurinic/apyrimidinic sites in tissues after inhalation exposure. *In preparation*

Presentations/Abstracts:

M.N. Ríos-Blanco and J.A. Swenberg (1996) Characterization of the major propylene oxide guanine adduct and the development of a method for its detection by high resolution mass spectrometry. *Fundamental and Applied Toxicology*, vol. 30 (1) Abstract #1207 35th Society of Toxicology Annual Meeting, Anaheim, CA

M.N. Ríos-Blanco and J.A. Swenberg (1997) Molecular dosimetry of N7-(2-hydroxypropyl)guanine in F344 rats exposed to propylene oxide by the inhalation route. *Fundamental and Applied Toxicology*, vol. 36 (1) Abstract #484 36th Society of Toxicology Annual Meeting, Cincinnati, OH

M.N. Ríos-Blanco, T. Faller, A. Ranasinghe, J.G. Filser and J.A. Swenberg (1998) DNA versus Hemoglobin adducts as molecular dosimeters of propylene oxide exposure: Implications for risk assessment. *Toxicological Sciences*, vol. 42 (1-S) Abstract # 888 37th Society of Toxicology Annual Meeting, Seattle, WA

M.N. Ríos-Blanco, H. Koc, T. Faller, J.J. Solomon, J.G. Filser and J.A. Swenberg (1998) Measurement of N3-(2-hydroxypropyl)deoxyuridine, a propylene oxide DNA adduct, by liquid chromatography tandem mass spectrometry (LC/MS/MS). International Congress of Toxicology, Paris, France

M.N. Ríos-Blanco, M. Dhawan-Robl, T.H. Faller, W. Kessler, H.R. Oberste-Frielinghaus, R. Schoonhoven, J.G. Filser and J.A. Swenberg (1999) Changes in cell proliferation in rat nasal respiratory epithelium after inhalation exposure to propylene oxide. *Toxicology Letters*, vol. 109 (Suppl.1) Abstract # 47 37th European Congress of Toxicology, Oslo, Norway

M.N. Ríos-Blanco, A. Ranasinghe, J. Nakamura, M.S. Lee, M. Dhawan-Robl, J.G. Filser and J.A. Swenberg Exposure-dependent accumulation of N7-(2-hydroxypropyl)guanine (7-HPG) in DNA of tissues of F344 rats after inhalation exposure to propylene oxide (PO). To be presented at the 39th Society of Toxicology Annual Meeting, Philadelphia, PA

Level of 7-HPG (pmol adduct/ μ mol guanine)^a in tissues of male F344 rats exposed to 500 ppm PO (6 hr/day; 5 days/week for 4 weeks)

Tissue	At the end of exposure	3 days after end of exposure
Nasal Respiratory	606.2 \pm 53.0 (n = 3)	393.3 \pm 57.0 (n = 4)
Nasal Olfactory	297.5 \pm 56.5 (n = 4)	222.7 \pm 29.5 (n = 4)
Lung	69.8 \pm 3.8 (n = 3)	51.5 \pm 1.2 (n = 3)
Spleen	43.0 \pm 3.8 (n = 3)	26.7 \pm 1.0 (n = 3)
Lymphocytes	39.6 ^b	ND
Liver	27.5 \pm 2.4 (n = 4)	18.0 \pm 2.6 (n = 4)
Testis	14.2 \pm 0.7 (n = 3)	10.4 \pm 0.1 (n = 3)

^a mean \pm std. dev., ^b DNA pooled from four individual rats, n = number of animals, ND - not determined

Number of apurinic/aprimidinic (AP)

(AP sites / 10^6 nucleotides \pm std. dev.) in DNA of rats exposed by inhalation to 0 or 500 ppm propylene oxide (6 hr/day; 5 days/week for 4 weeks)

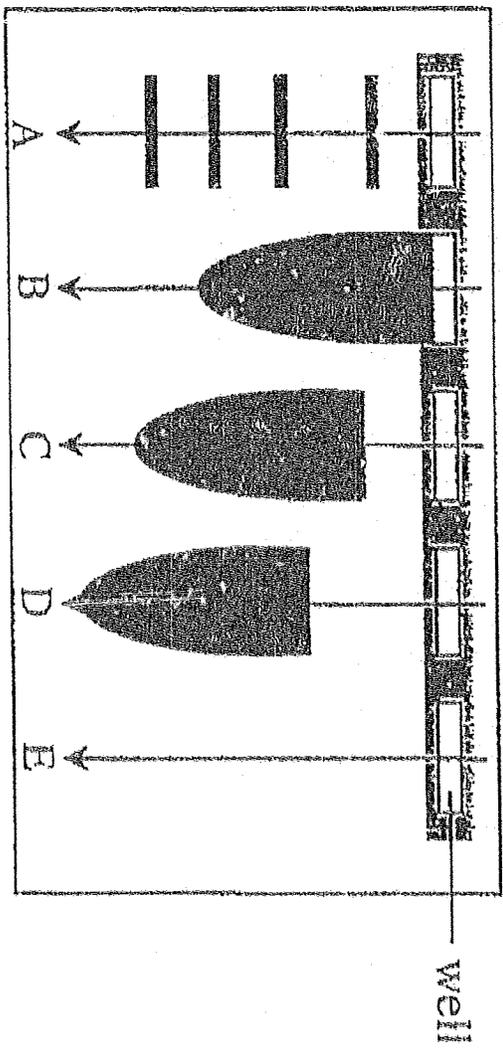
Tissue ^a	Control (0 ppm PO)	Exposed (500 ppm PO)
Nasal Respiratory Epithelium	12.5 \pm 3.1	10.6 \pm 0.7
Lung	14.5 \pm 1.7	13.8 \pm 0.4
Liver	10.2 \pm 1.1	8.8 \pm 0.6
Testis	10.1 \pm 0.8	11.3 \pm 1.8

^a The number of animals examined for each tissue was five. None of the differences between control and 500 ppm were statistically significant (p<0.01)

Rate of adduct loss in rats exposed to
500 ppm propylene oxide

Tissue	Adduct loss/ 10^6 nucleotides/day
Nasal Respiratory Mucosa	15.8
Nasal Olfactory Mucosa	5.5
Lung	1.4
Spleen	1.2
Liver	0.7
Testis	0.3

Agarose gel electrophoresis and Image analysis



↓ : Scanning direction (measurement of density)

A: Molecular marker

B: Control

C: Low dose

D: High dose

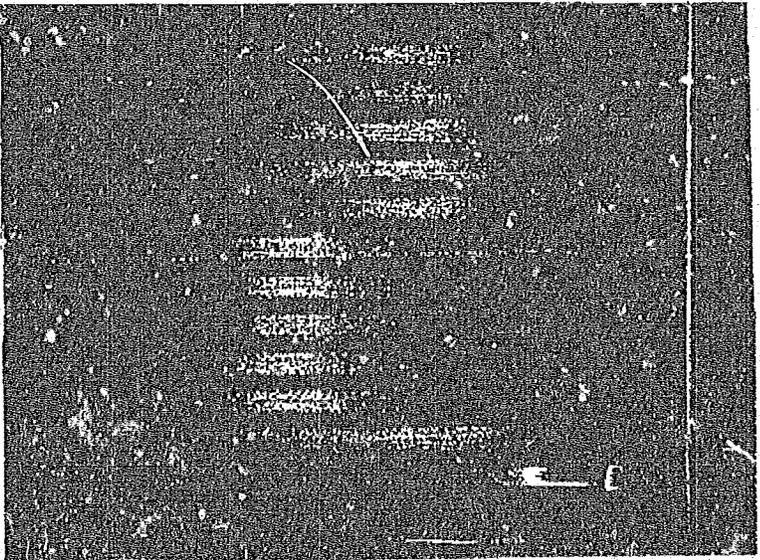
E: Blank (evaluation for back ground)

■ : Positive reaction of single strand DNA

▬ : Back ground related with well

Measurement of single strand breaks in DNA from lung and liver of control and PO exposed (500 ppm/20 days) male F344 rats by glyoxal gel electrophoresis

Positive Control

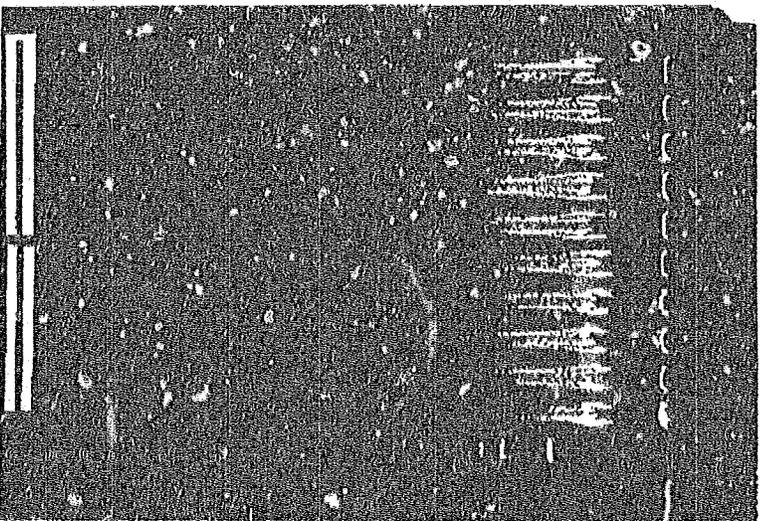


control
methanol/CuII

PCB catechol

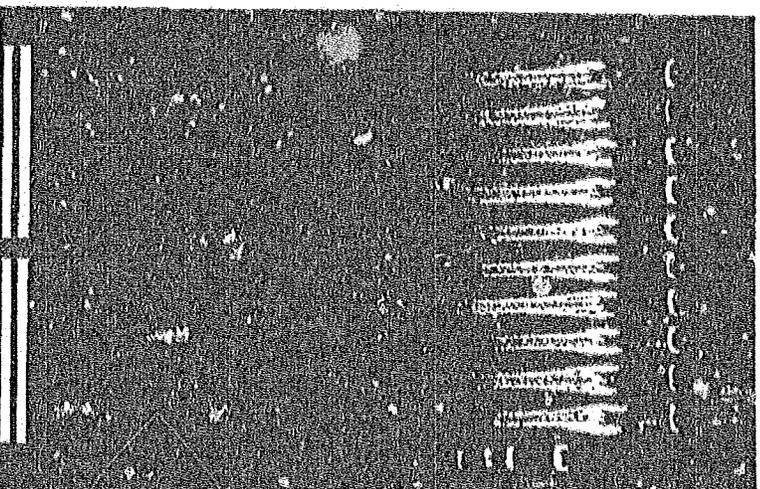
non-treated commercial CTDNA

Liver



exposed control

Lung



exposed control

Level of N-(2-hydroxypropyl)valine (HPV₂) (pmol/mg globin) in globin of F344 rats exposed to 500 ppm PO (6 hr/day; 5 days/week for 4 weeks)

GROUP	pmol adduct/mg globin ^a
At end of exposure	90.2 ± 11.4 (n = 24)
3 days after end of exposure	89.9 ± 8.9 (n = 22)

^a mean ± standard deviation

n = number of determinations (2-3 determinations per animal)

Dose Response Studies

Exposure doses

0, 5, 25, 50, 300 and 500 ppm

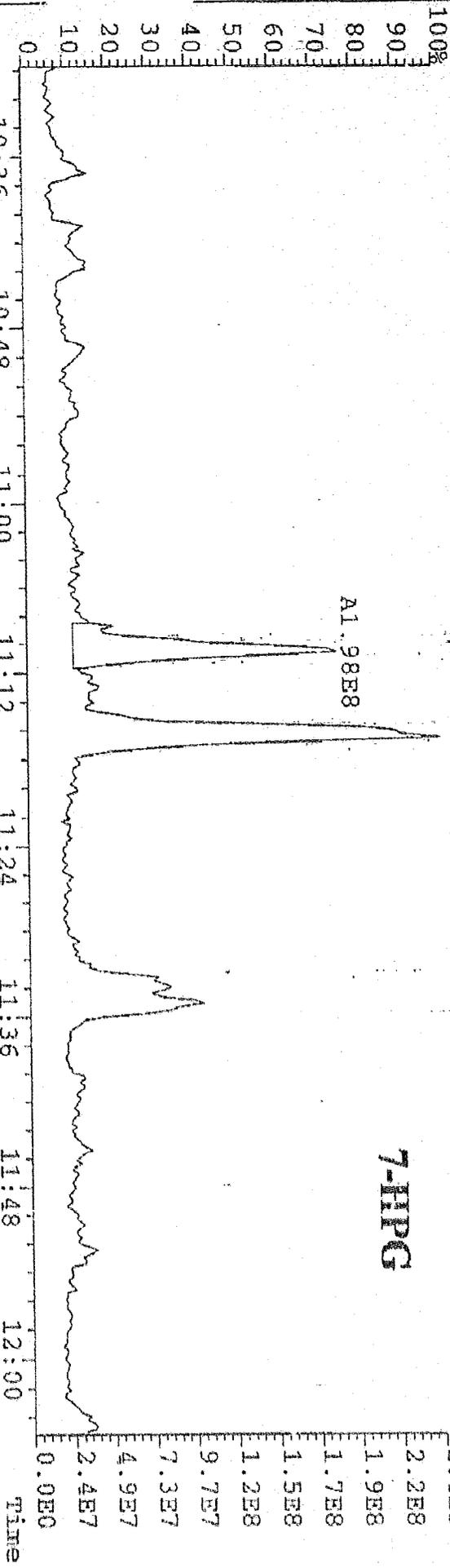
Length of exposure

3 days and ²⁰5 days

(6 hr/day; 5 days/wk)

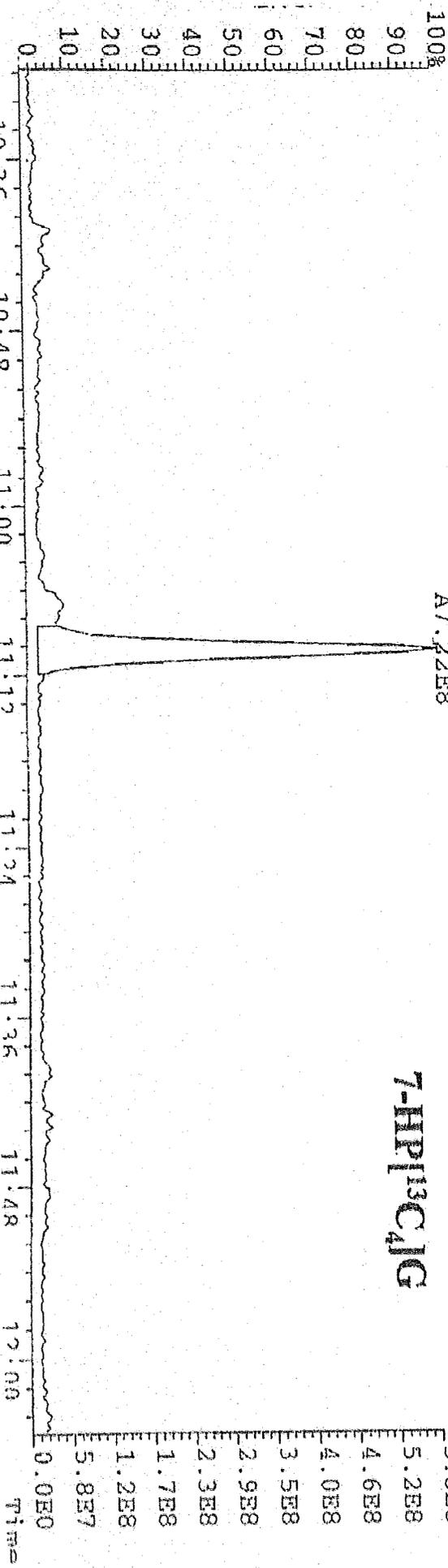
Representative chromatogram of 7-HPG found in nasal respiratory epithelium DNA of a male rat exposed to 5 ppm PO for 20 days

569.0671 S:2 Exp:7-HPG-FC43
 Sample Text:5/2-22/NR/JULY98 File Text:MEIVA-7HPG

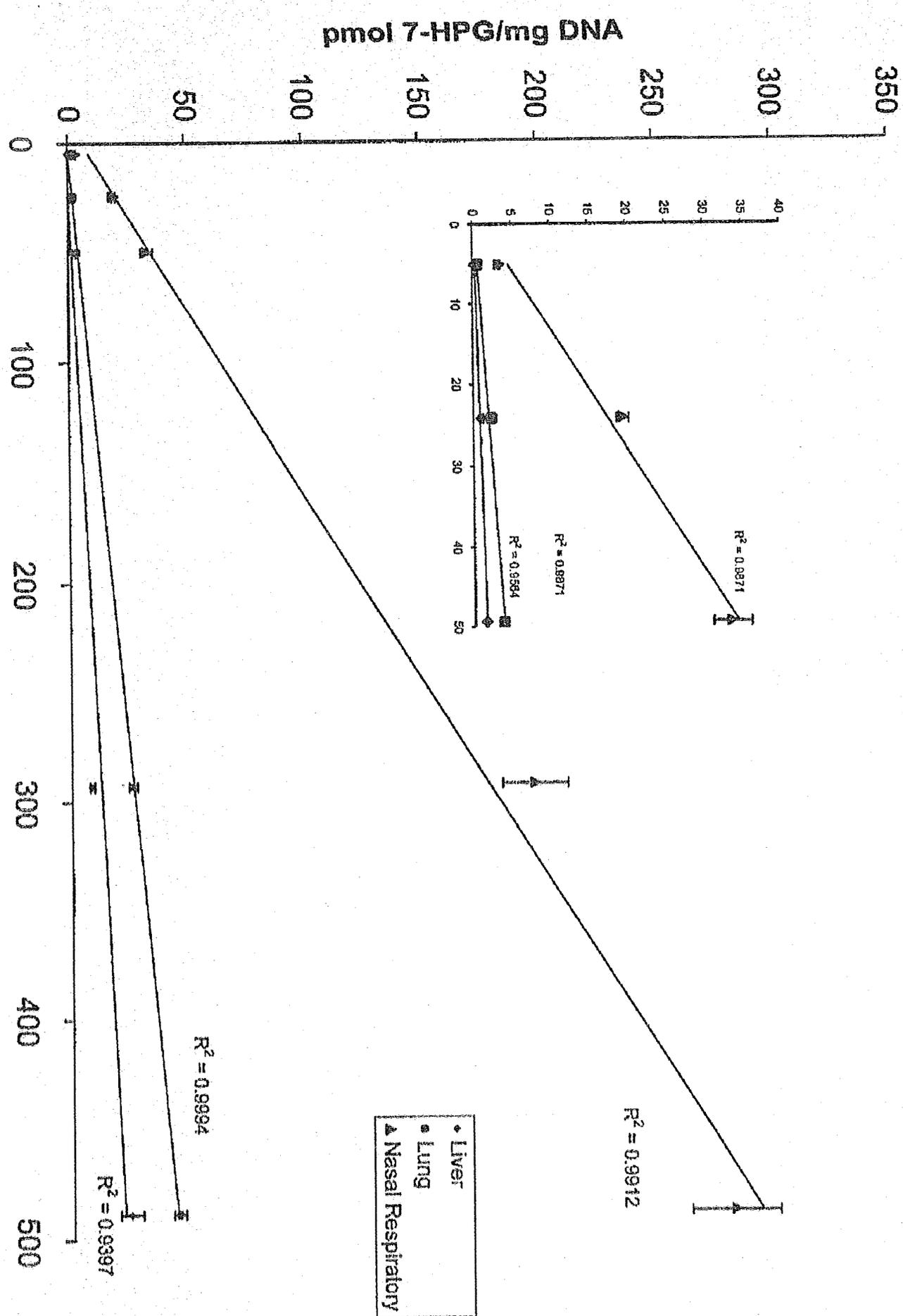


File:C1197 #1-3096 Acq:29-SEP-1999 15:30:41 GC CI- Voltage SIR 70SE0
 573.0807 S:2 Exp:7-HPG-FC43
 Sample Text:5/2-22/NR/JULY98 File Text:MEIVA-7HPG

7-HPG¹³C₄D₄G

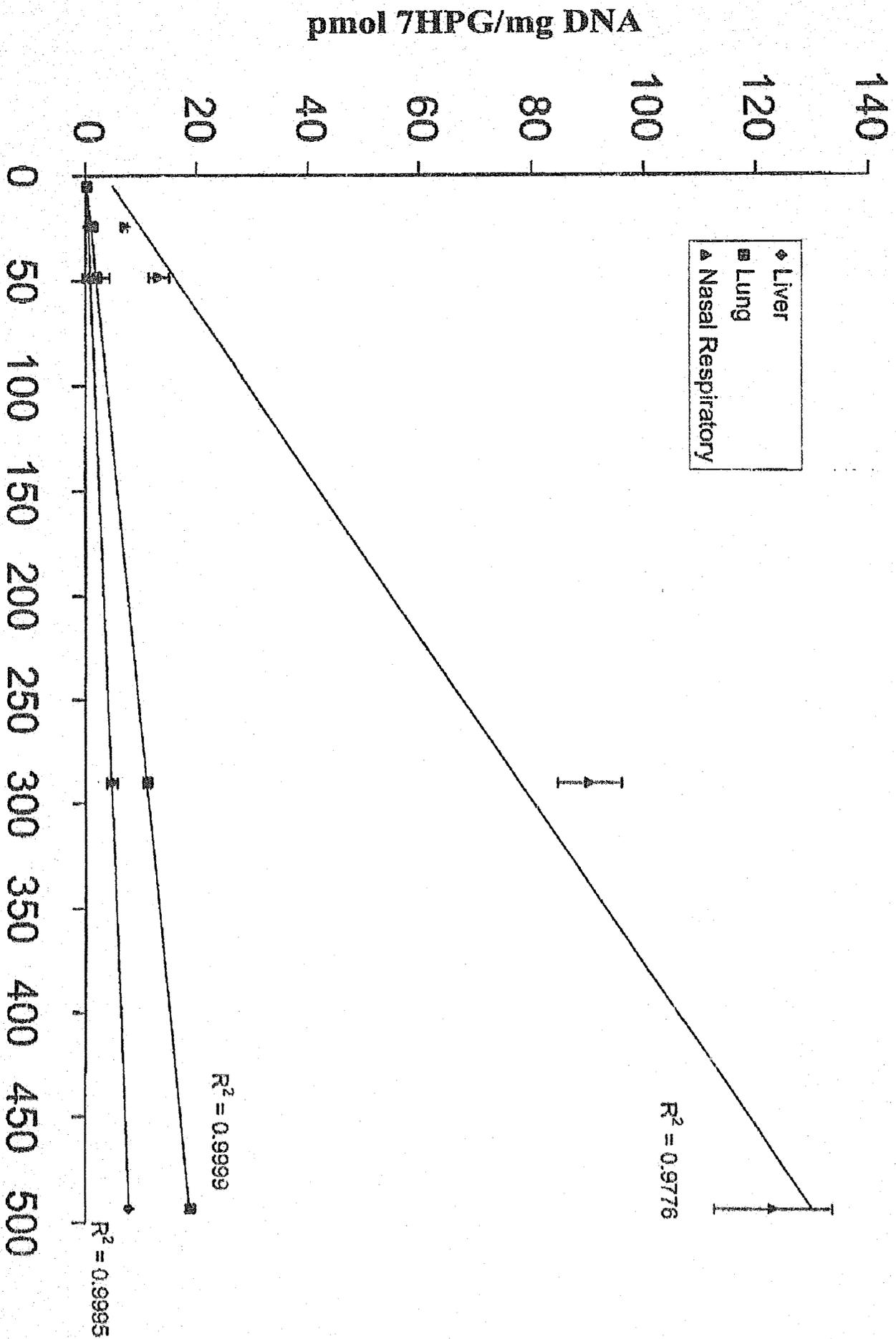


Amount of 7-HPG (pmol/mg DNA) in tissues of rats exposed to PO by the inhalation route for 4 weeks



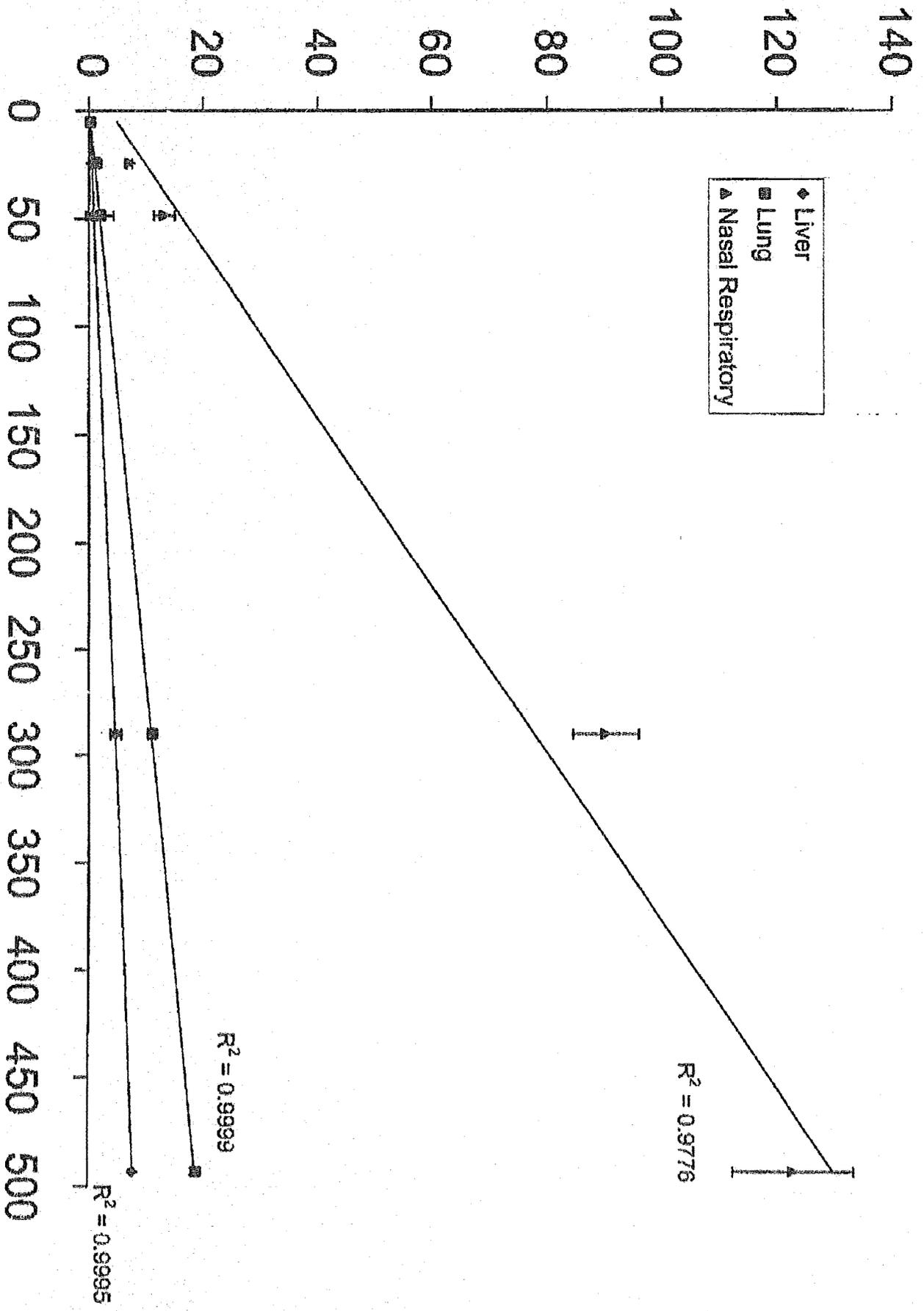
- ◆ Liver
- Lung
- ▲ Nasal Respiratory

PO by the inhalation route for 3 days

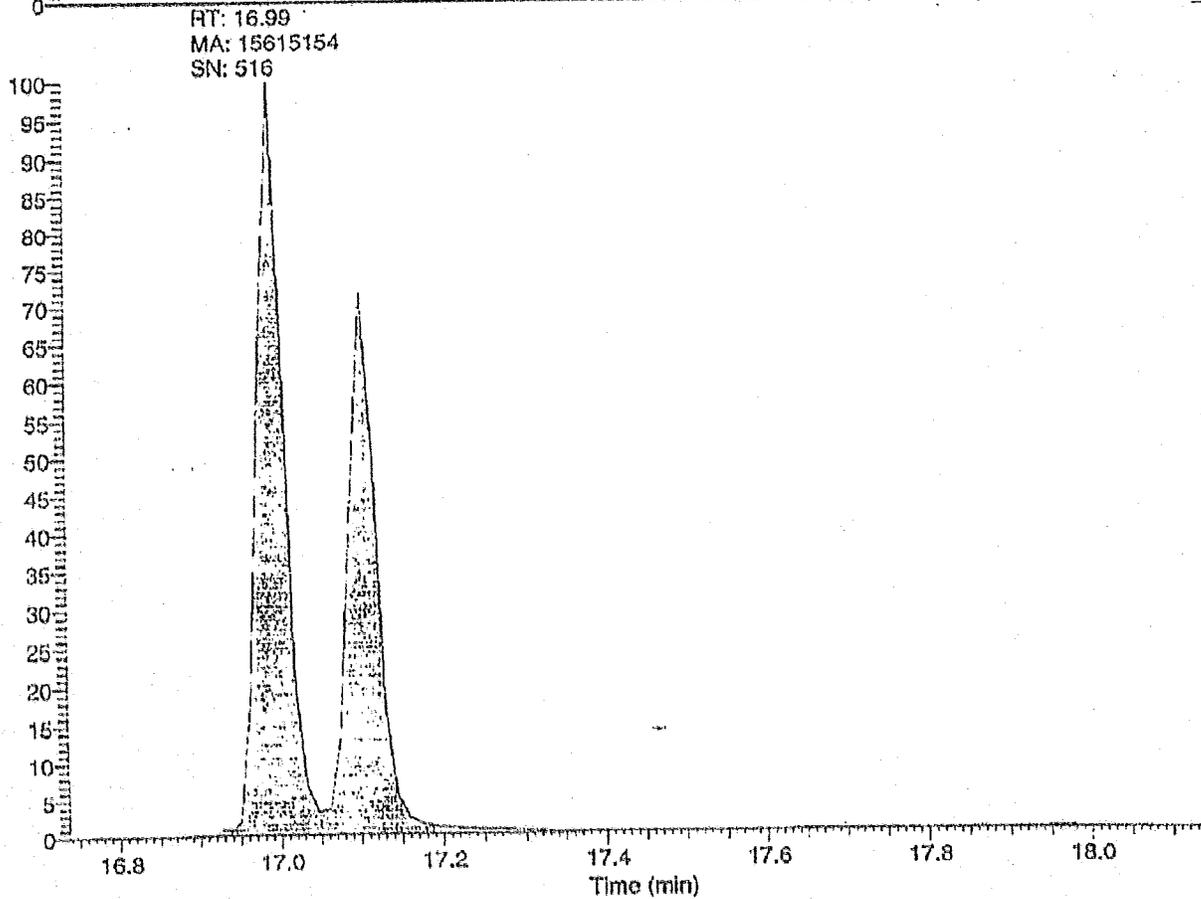
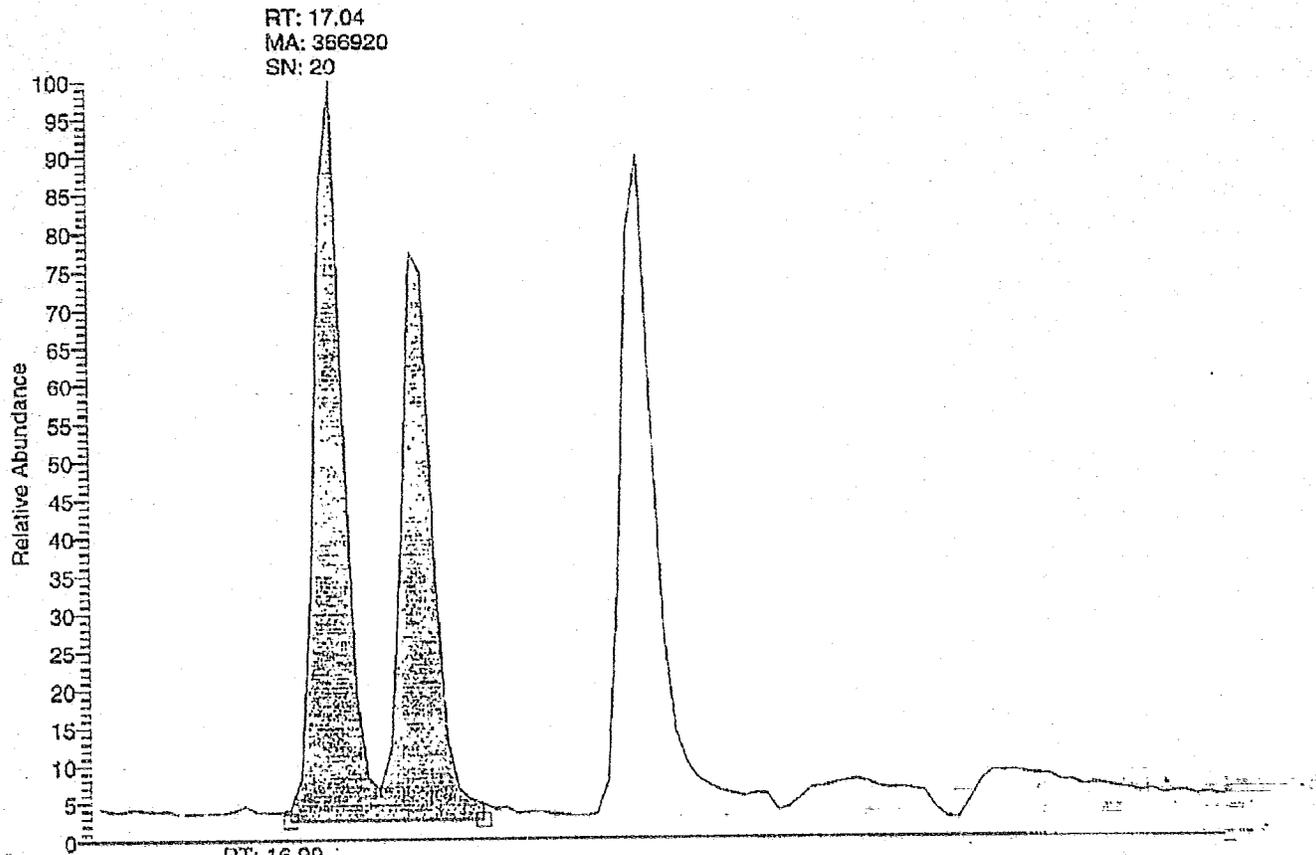


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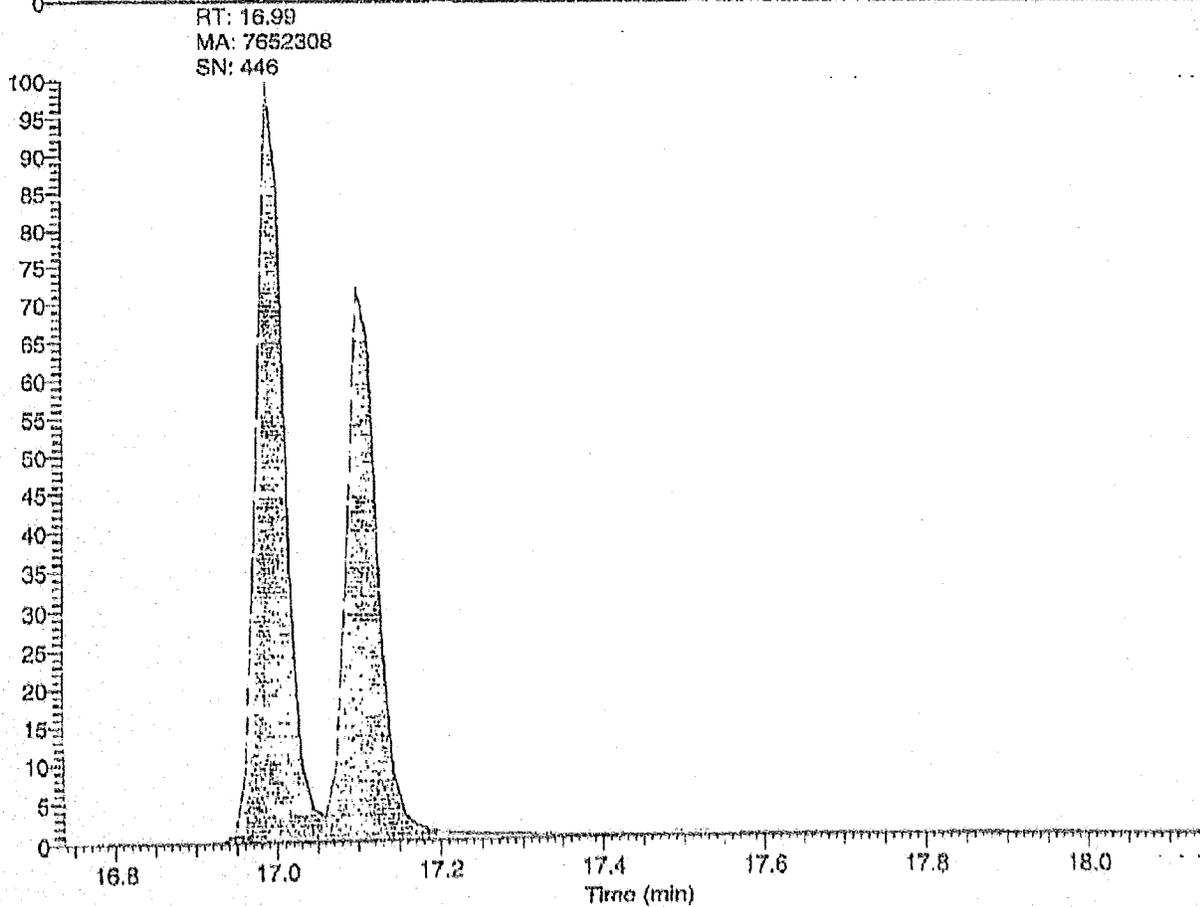
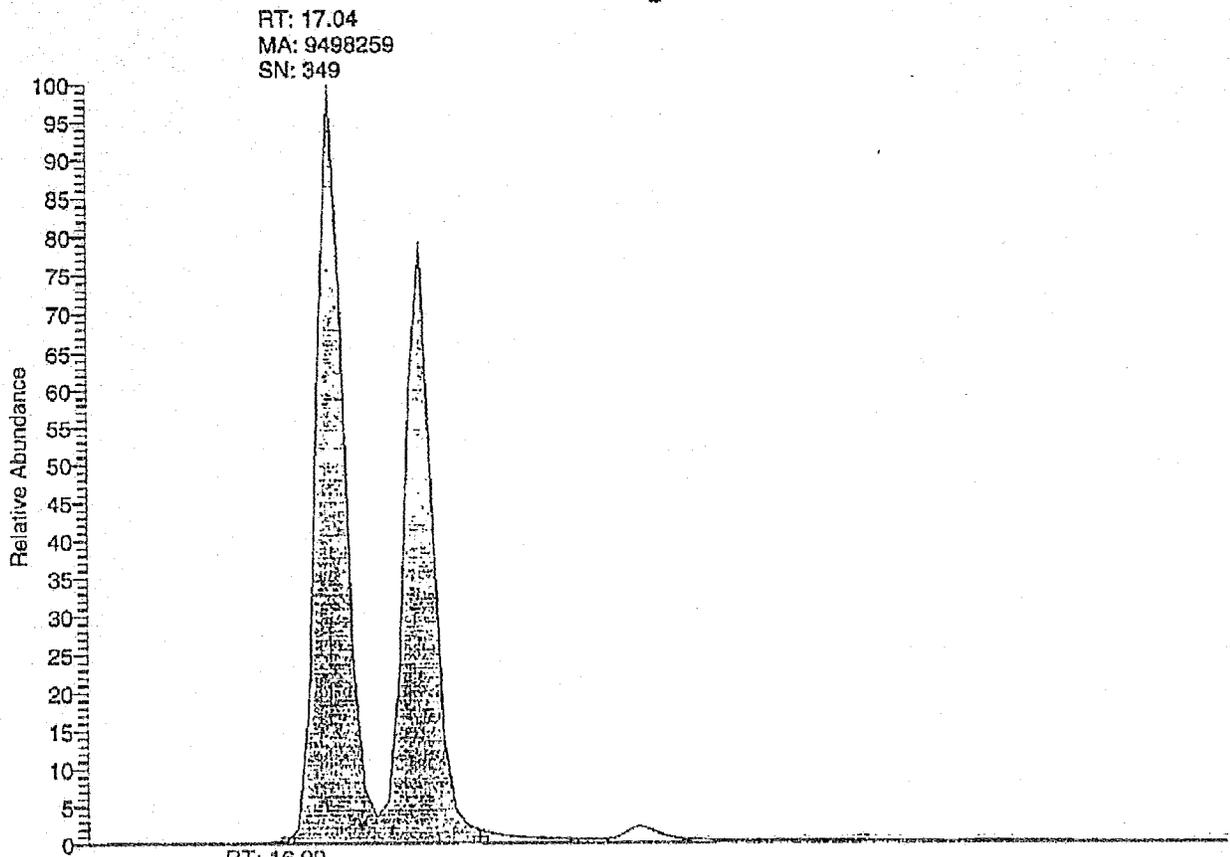
Amount of 7HPG (pmol/mg DNA) in tissues of rats exposed to PO by the inhalation route for 3 days



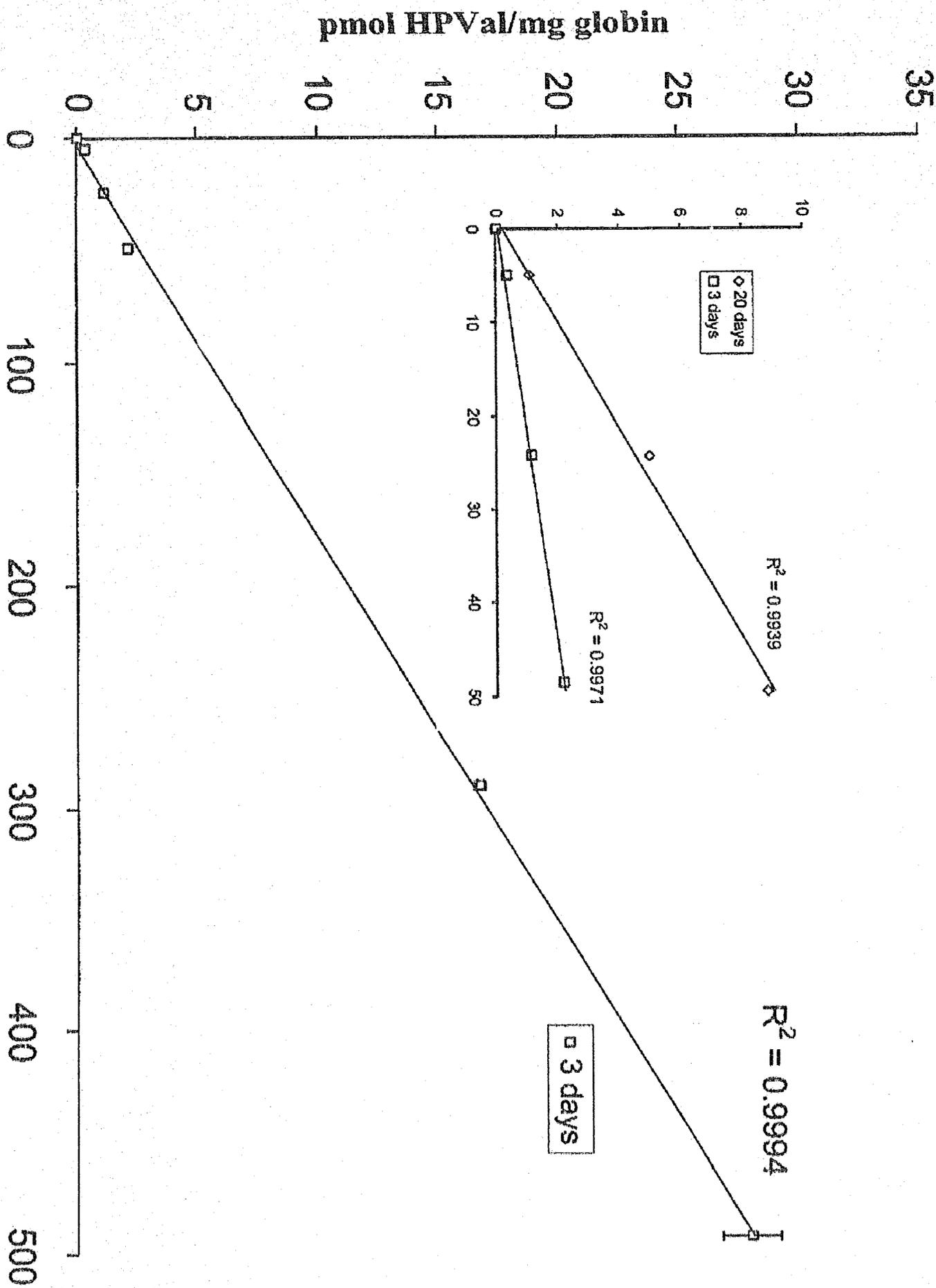
Representative GC-MS/MS chromatogram of HPVal in rat control globin (70 mg)



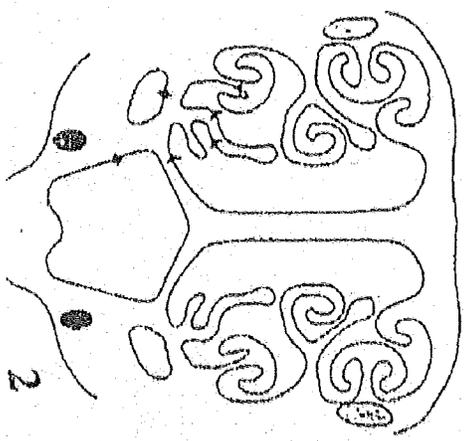
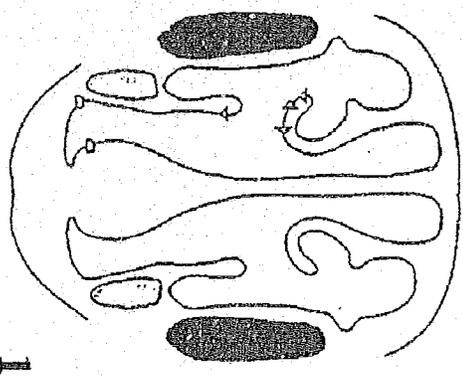
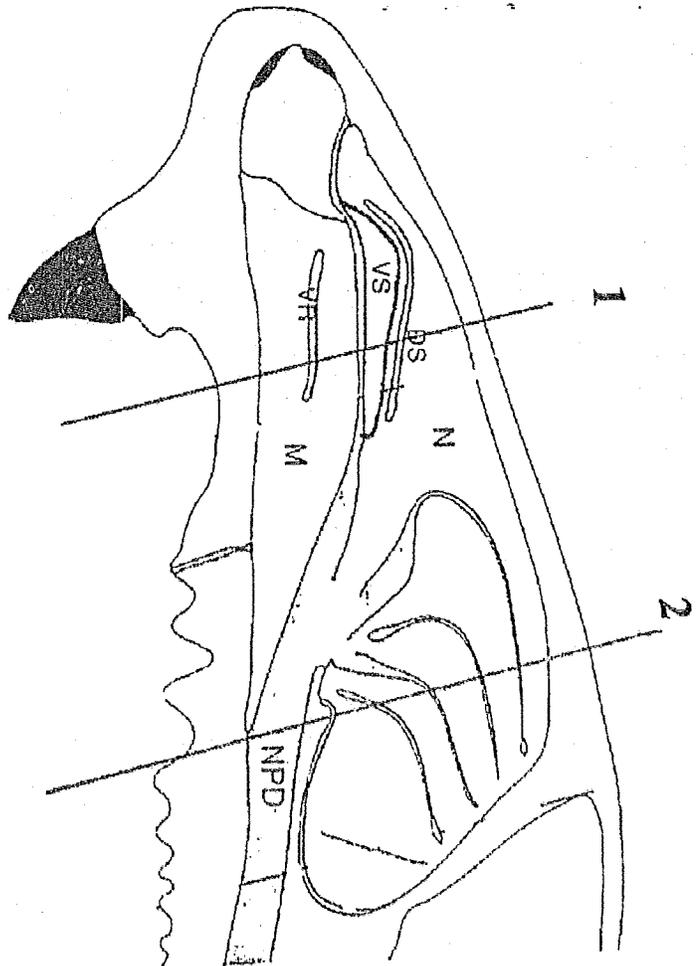
Representative GC-MS/MS chromatogram of HPVal in globin (50 mg) of a male F344 rat exposed to 5 ppm PO for 20 days



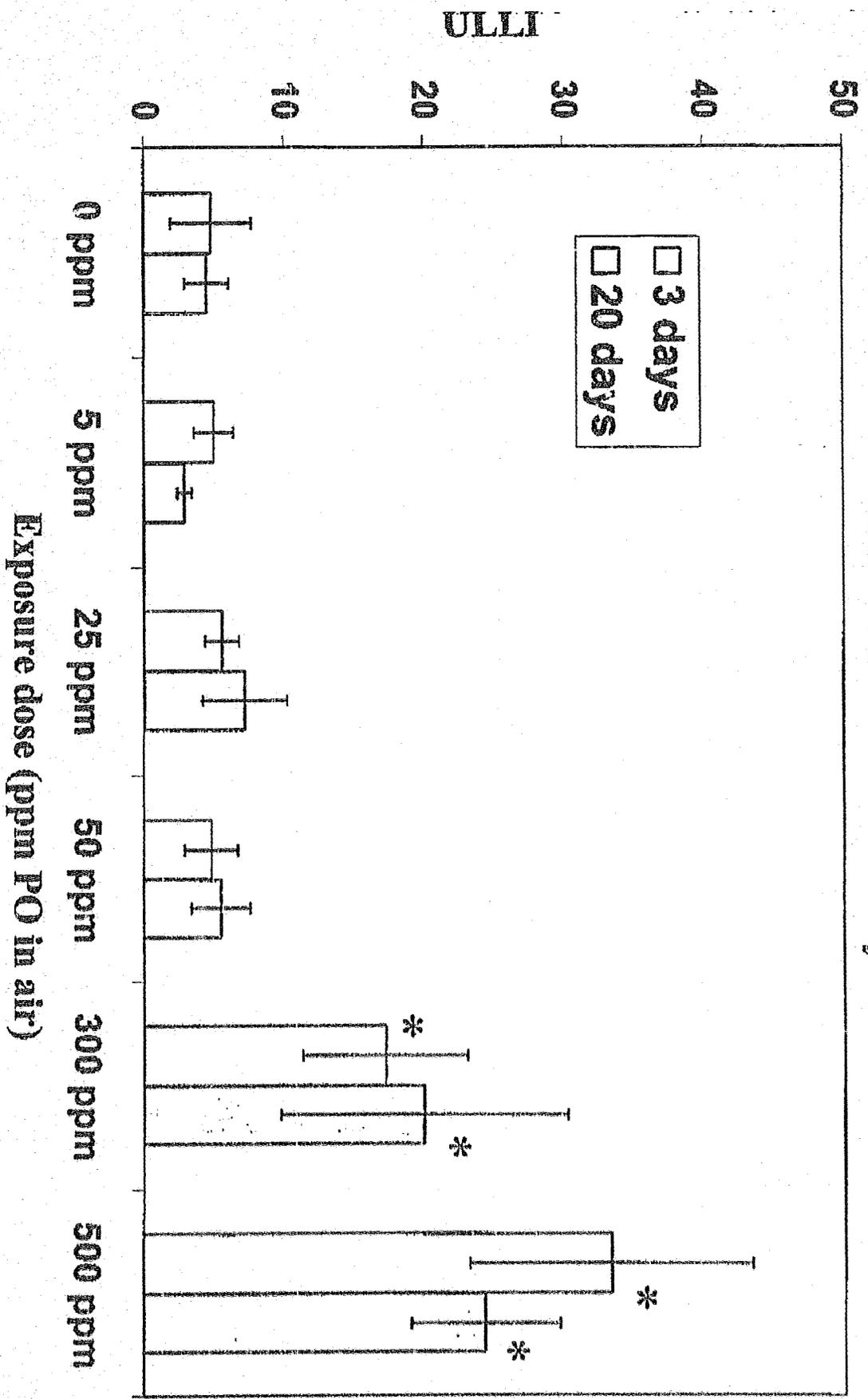
Amount of N-(2-hydroxypropyl)valine (HPVal) (pmol/mg globin) in F344 rats exposed to propylene oxide by the inhalation route



Midsagittal section of the nasal passages of a F344 rat.
Straight vertical lines indicate section levels selected for
transverse diagrams

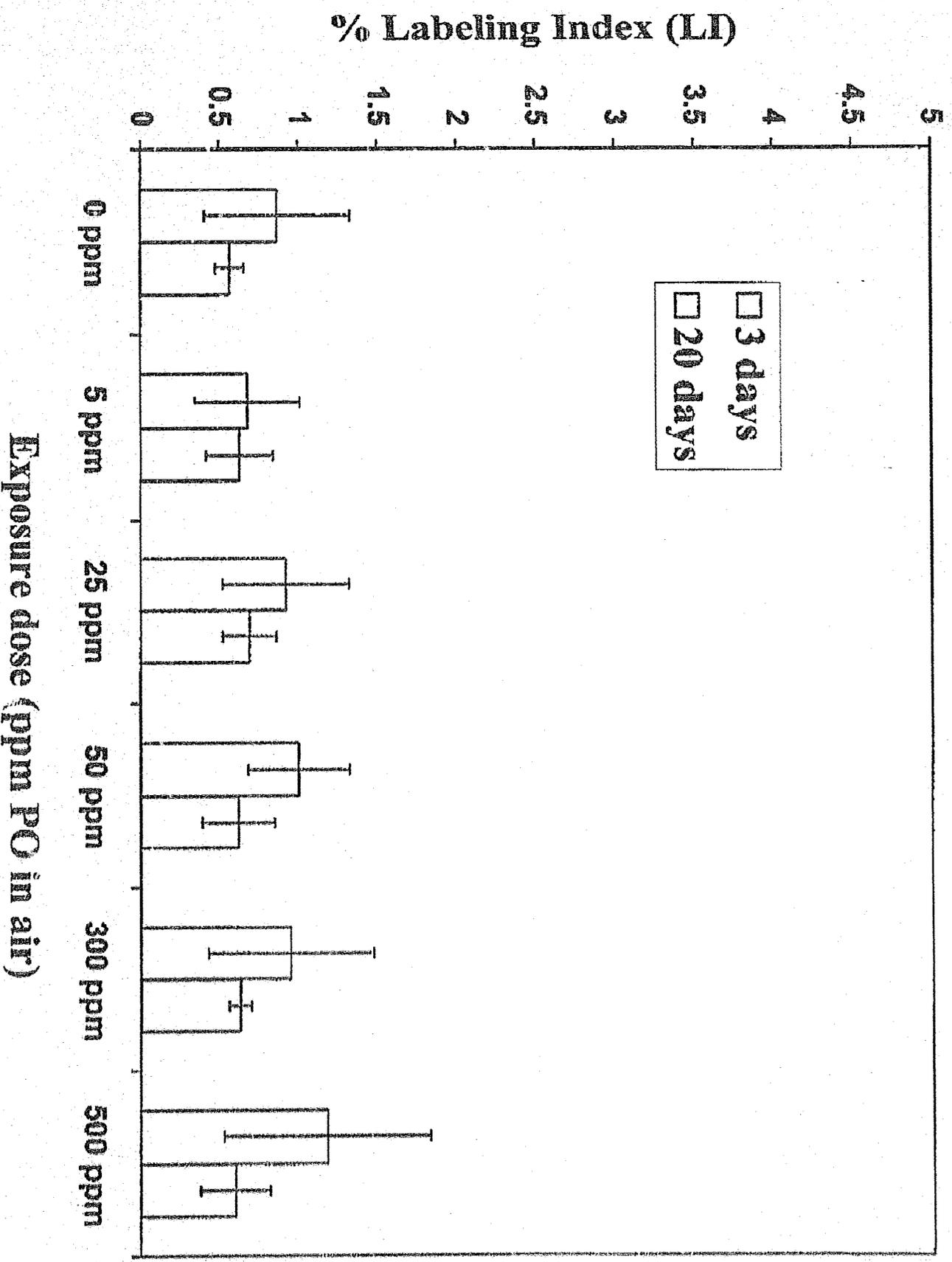


Cell proliferation measurements in the respiratory epithelium lining the septum and medial surface of the nasoturbinates in the anterior nasal passages of male F344 rats exposed to PO by inhalation for 3 or 20 days



* significantly different from control, p < 0.01

Cell proliferation measurements in liver of male F344 rats
exposed to PO by inhalation for 3 or 20 days



Summary

1. The mean value of 7-HPG in tissues of male F344 rats exposed to 500 ppm PO for 20 days followed the order:

nose >>> lung > spleen > liver > testis

2. Analysis of 7-HPG in tissues (nose, lung, spleen, liver and testis) of rats killed after three days of recovery demonstrated similar rates of adduct disappearance in all tissues, with 62 - 75 % of the adducts persisting

3. The target tissue for carcinogenesis (nose) has a greater level of alkylation of DNA than non-target tissues (lung, spleen, liver, testis).

4. The mean value of HPV a1 found in globin from exposed and exposed-recovery rats was 90.1 ± 0.2 pmol/mg globin. No differences in HPV a1 concentration were found between the two groups.

5. There was no increase in abasic site number in nasal respiratory epithelium, lung, liver and testis of rats exposed to 500 ppm PO for 20 days, suggesting that this is not a mechanism of mutagenesis for 7-HPG.

6. Exposure-dependent accumulation of 7-HPG in nasal respiratory epithelium, lung and liver was determined in male F344 rats exposed to PO (0, 5, 25, 50, 300 or 500 ppm) by the inhalation route for 3 days (6 hr/day) or 20 days (6 hr/day; 5 days/week).

7. Preliminary results showed a linear response in 7-HPG accumulation for all three tissues and both lengths of exposure.

8. The nose had the highest concentration of adducts followed by lung and liver, with linear regression slopes $1/6$ and $1/15$ that of the nose, respectively. These differences are thought to be due to greater amounts of PO reacting with nasal tissue and a combination of direct and systemic exposure in the lung.

9. Preliminary results showed a linear response in HPV-al accumulation in globin for both lengths of exposure.

10. Cell proliferation in the respiratory epithelium lining the septum and medial surface of the nasoturbinates of rats significantly increased (3.6 and 7 fold increase, 300 ppm and 500 ppm, respectively) over control after PO inhalation exposure for 3 days. Significant increases in cell proliferation (4.4 and 5.4 fold increase, 300 and 500 ppm, respectively) over control in the same area were also observed in rats exposed to PO by inhalation for 20 days.

11. The increase in cell proliferation in nasal respiratory epithelium of rats exposed to PO for 20 days is slightly lower than for the corresponding exposure group in the 3 days exposure period. This observation could be due to the role of adaptive mechanisms in the nasal tissue of rats over extended periods of exposure.

12. Changes in cell proliferation (~3 fold increase) were also observed in the nasal respiratory epithelium lining the nasopharyngeal duct. The lesser effect of PO in this region could be related to a lower concentration of the chemical reaching this site and/or more effective detoxication.

13. No changes in cell proliferation were observed in liver of exposed rats.

14. These studies demonstrate that the target organ for carcinogenesis (nose) is a sensitive site for toxicity-induced compensatory cell proliferation after inhalation exposure to PO.