

tion of platinum II compounds
307.

ment of mutation induced by

al molecules, in: T.A. Connors
Chemotherapy, Springer, New

$\text{cis-Pt}(\text{NH}_3)_2\text{Cl}_2$. An anti-tumor

d mutagenesis: dependence on
(77) 93-103.

Escherichia coli, Bacteriol. Rev., 40

Mutation Research, 77 (1980) 55-63
© Elsevier/North-Holland Biomedical Press

P6W

55

CYTOCHROME P-450 MEDIATED GENETIC ACTIVITY AND CYTOTOXICITY OF SEVEN HALOGENATED ALIPHATIC HYDROCARBONS IN *Saccharomyces cerevisiae*

DAVID F. CALLEN¹, C. ROLAND WOLF and RICHARD M. PHILPOT²

¹ School of Biological Sciences, Flinders University of S.A., Bedford Park, South Australia,
5042 (Australia) and ² National Institute of Environmental Health Sciences, P.O. Box
12233, Research Triangle Park, NC 27709 (U.S.A.)

(Received 28 May 1979)

(Revision received 7 August 1979)

(Accepted 14 August 1979)

Summary

Cells of *Saccharomyces cerevisiae*, harvested from log-phase cultures, contain cytochrome P-450 and are capable of metabolizing promutagens to genetically active products. The activities of 7 halogenated aliphatic hydrocarbons in the yeast system have been investigated. All of the compounds tested (methylene chloride, halothane, chloroform, carbon tetrachloride, trichloroethylene, tetrachloroethylene and *s*-tetrachloroethane) induced mitotic gene convertants and recombinants and, to a lesser extent, gene revertants when incubated with log-phase cells of the yeast strain D7. An examination of the difference spectra observed upon the addition of carbon tetrachloride, halothane and trichloroethylene to whole-cell or microsomal suspensions of yeast suggested that cytochrome P-450 mediated the metabolism of the hydrocarbons tested to cytotoxic and genetically active compounds.

Several halogenated aliphatic hydrocarbons are known to be carcinogenic. Epidemiological studies have shown that vinyl chloride is a carcinogen in humans [2]. Recent studies by the National Cancer Institute (U.S.A.) have established that chloroform, carbon tetrachloride, trichloroethylene and several other chlorinated hydrocarbons are carcinogenic [23]. These compounds generally induce tumours in the livers of mice but are only marginally carcinogenic in rats. It has been reported that operating room personnel have an increased risk of cancer [15] which may be due to exposure to anesthetic gases such as halothane (CF_3CHClBr).

The toxicity and potential carcinogenicity of the halogenated aliphatic hydrocarbons have been related to their metabolic activation by the hepatic

microsomal monooxygenase system. Metabolism to free-radical intermediates has been suggested for some halogenated alkanes [3].

The hepatic microsomal monooxygenase system has been incorporated into *in vitro* systems which are used for the determination of mutagenicity [12]. Such systems have established that most carcinogenic compounds are also mutagenic e.g. [13]. Some of the halogenoalkanes tested in these systems have been shown to be mutagenic [4,7]; however, tests with carbon tetrachloride [13,22], chloroform [18,22], and halothane [1,18,22] have consistently given negative results. Several of the halogenated alkenes tested, including vinyl chloride [2,8] and trichloroethylene [8,16], have also been shown to be mutagenic [8,11,16].

Most *in vitro* test systems for mutagenicity require mammalian hepatic microsomal fractions as a source of monooxygenase activity. In such systems active metabolites are generated outside of the indicator organism and may react before entering the cell and reaching the DNA and thereby lead to false negative results.

Yeast cells harvested from cultures grown on medium containing glucose contain a cytochrome P-450-dependent monooxygenase system [10] and are capable of activating a variety of promutagens [5,6]. Therefore, yeast cells can be used as an indicator organism for promutagens without the need for mammalian monooxygenase systems.

Some halogenated hydrocarbons are metabolized to products which interact with ferrous cytochrome P-450 to give characteristic spectra [3,14,19-21]. In order to obtain evidence that halogenated hydrocarbons enter yeast cells and are metabolized, the spectra formed on the addition of 3 of these compounds to suspensions of whole cells and microsomal preparations were recorded.

Materials and methods

Strain. D7 is a multipurpose diploid in which the frequencies of gene conversion at the *trp5* locus, mitotic recombination at the *ade2* locus, and gene conversion at the *ilv1* locus can be measured [27]. Mitotic gene conversion can be induced by a variety of mutagens and mutagen specificity is absent at these loci [25]. The D7 diploid used in these studies was constructed from the parental haploid strains. D7.1528B (*a ade2-40, trp5b, ilv1-92*) and D7.9A (*a, ade2-119, trp5a, ilv1-92*), which were kindly supplied by Dr. F.K. Zimmermann. This D7 strain had a cytochrome P-450 concentration of approximately 13 nmoles/g dry weight in cells harvested from log-phase cultures grown with glucose as carbon source. The strain D5 [26] was used for spectral studies because of its high cytochrome P-450 concentration (up to 38 nmoles/g dry weight). Experiments [5 and unpublished] have shown that these two strains can activate a variety of promutagens to genetically active products.

Media. MIN medium consistent of 0.6% Difco yeast nitrogen base without amino acids and 2% glucose supplemented with 5 mg/l of adenine, tryptophan (10 mg/l), or isoleucine (60 mg/l) as necessary. Log-phase cultures were grown on complete medium (2% peptone, 1% yeast extract and 2% glucose).

Treatment conditions. Cells were harvested from log-phase cultures growing on medium containing glucose as carbon source and resuspended in 0.1 M phos-

pho
cor
bat
Tre
sarr
buf
ior
wer
esti
ade
Eac
plat
surv
plat
plat
on t
isole
two
its
whit
from
Sp
tures
phos
divic
the a
prep
Amin
Cl
ethyl
stabil
purcl
burg,

Resul

Pre
the st
partic
frequ
only
eviden
attrib
geneti
ect
tant
eviden

ee-radical intermediates

been incorporated into
of mutagenicity [12].
ic compounds are also
ed in these systems have
ith carbon tetrachloride
| have consistently given
tested, including vinyl
been shown to be muta-

ire mammalian hepatic
activity. In such systems
ator organism and may
and thereby lead to false

dium containing glucose
ase system [10] and are
Therefore, yeast cells can
out the need for mamma-

roducts which interact
spectra [3,14,19-21]. In
ons enter yeast cells and
of 3 of these compounds
ons were recorded.

equencies of gene conver-
ade2 locus, and gene con-
ic gene conversion can be
city is absent at these loci
ructed from the parental
and D7.9A (*a*, *ade2-119*,
K. Zimmermann. This D7
proximately 13 nmoles/g
is grown with glucose as
tral studies because of its
les/g dry weight). Experi-
two strains can activate a

ast nitrogen base without
g/l of adenine, tryptophan
phase cultures were grown
and 2% glucose).

g-phase cultures growing
suspended in 0.1 M phos-

phate buffer, pH 7.0, at a cell concentration of approx. 3×10^7 /ml. The test compound was added directly to a 3-ml aliquot of cell suspension and incubated in a screw-capped 50-ml glass centrifuge tube at 37° for varying times. Treatments were terminated by the addition of 40 ml of ice-cold buffer. The samples were then centrifuged and the sedimented cells resuspended in 3 ml of buffer. These cell suspensions were then plated on appropriate media to allow for estimation of genetic activity.

Assessment of genetic activity. For D7, 0.1-ml aliquots of each treatment were spread on each of 5 plates of MIN + adenine + tryptophan to allow estimation of the *ilv1* reversion frequency, and on each of 5 plates of MIN + adenine + isoleucine to allow estimation of the *trp5* conversion frequency. Each treatment was diluted 10^4 -fold and 0.1-ml aliquots spread on each of 20 plates of MIN medium containing all 3 supplements to allow estimation of cell survival. Survivors and *trp5* convertants were scored after incubation of the plates for 2 days and *ilv1* revertants after incubation for 5 days at 30°. The plates were stored at 4° for 7 days and the mitotic recombinants scored, both on the 20 plates containing all 3 supplements and on the 5 MIN + adenine + isoleucine plates. The genetic alterations at the *ade2* locus were grouped into two classes: twin spots (red-pink and red-pink-white colonies, which arise from mitotic recombination) and other genetically altered colonies (red, pink, red-white, and pink-white). Data are given for one of two duplicate experiments from which similar results were obtained in all cases.

Spectral analysis. Cells of the D5 strain were harvested from log-phase cultures grown on complete medium containing glucose and were resuspended in phosphate buffer, pH 7.0, at 5×10^8 to 10^9 cells/ml. The cell suspension was divided between two cuvettes (3 ml) and the spectra recorded before and after the addition of the test ligand to the sample cuvette. Microsomal fractions were prepared as described previously [5]. All spectra were recorded with an Aminco DW2A spectrophotometer.

Chemicals. Halothane (Ayerst Labs, Inc., New York, NY) and tetrachloroethylene (Eastman Kodak Co., Rochester, NY) contained thymol, 0.01% as stabilizer. Methylene chloride, trichloroethylene, and s-tetrachloroethane were purchased from Fisher, Fair Lawn, NH; chloroform from J.T. Baker, Phillipsburg, NJ and carbon tetrachloride from Mallinckrodt, St. Louis, MO.

Results

Preliminary experiments established that 4-h treatment of log-phase cells of the strain D4 (used to monitor gene conversion, see ref. 5) or strain D7 with a particular dose of the various halogenated hydrocarbons resulted in increased frequencies of gene conversion per cell survivor (data not shown). However, only marginal increases in the observed numbers of convertant colonies was evident in the treatments as compared with the controls. Such a result could be attributed to differential survival of the various cell phenotypes rather than any genetic effect of the treatment. In subsequent experiments positive genetic effects (as assessed by at least a doubling in the observed numbers of convertant or mitotic recombinant colonies as compared with the control) were evident when log-phase cells of the strain D7 were treated for 1 h (Table 1).

TABLE 1

EFFECT OF VARIOUS HALOGENATED HYDROCARBONS ON STRAIN D7 OF YEAST

Cells were harvested from log-phase cultures of the strain D7 grown on medium with glucose as carbon source. Treatments were incubated at 37°C for 1 h.

Compound	Concentration (mM)	Survival		trp5 locus		2 locus
		Total number of colonies counted	%	Total number of revertants counted	Revertants/10 ⁵ survivors	Total number of revertants counted
Methylene chloride	0	1467	100	258	1.8	
	104	1128	77	314	2.8	
	157	617	42	660	10.7	
	209	1	<0.1			
Halothane	0	1201	100	171	1.4	
	47	1002	83	355	3.5	
	63	364	30	583	16.0	
	79	0	<0.1			
Chloroform	0	1423	100	246	1.7	
	21	1302	91	274	2.1	
	41	982	69	450	4.6	
	54	84	6	278	33.1	
Carbon tetrachloride	0	1454	100	285	2.0	
	21	1252	86	331	2.6	
	28	1120	77	350	3.1	
	34	152	10	506	61.7	
Trichloroethylene	0	1201	100	171	1.4	
	15	802	67	610	7.6	
	22	3	0.3			
Tetrachloroethylene	0	1546	100	221	1.4	
	4.9	1301	84	251	1.9	
	6.6	891	58	742	8.3	
	8.2	0	<0.1			
syn-Tetrachloroethane	0	1201	100	171	1.4	
	3.1	1127	94	213	1.9	
	5.2	426	35	741	17.4	
	7.3	1	0.1			

N.D., not determined.

The total number of colonies in the different classes represent total counts of colonies from 5 plates in the case of survival, conversion and revertant-frequency estimations. Mitotic recombination was estimated

When strain D4 was similarly treated, i.e. for 1 h, only marginal increases in the frequencies of gene conversion, were evident.

When cells of the strain D7, harvested from log-phase cultures, were treated with increasing concentrations of each of the halogenated hydrocarbons there were decreased cell survival and increased frequencies of gene conversion, mitotic recombination and, to a lesser extent, reversion. Although the toxicity of the 7 compounds differed considerably, there were no apparent differences among the genetic activities of the compounds when comparisons were made at similar levels of cell survival. A nonlinear correlation between cell survival and the concentration of the hydrocarbons in the incubation mixtures was apparent (Table 1).

from counts of
ing cells grew

In order
yeast cells
carbon tet
or microso
When c
with sodiu
observed (c
shift toward
whole cell
nm and a
order to o

IN D7 OF YEAST

medium with glucose as carbon

<i>trp5</i> locus		Total number of spots counted	Mitotic recombinants/ 10^4 survivors	Total number of genetically altered colonies counted	Total frequency of genetically altered colonies/ 10^3 survivors	<i>ilv1</i> locus	
Total number of revertants counted	Revertants/ 10^6 survivors						
258	1.8		3.1	21	3.3	39	2.7
314	2.8		1.9	20	3.9	50	4.4
660	10.7	1	44.9	53	14.0	36	5.8
171	1.4		3.5	13	3.3	43	3.6
355	3.5		17.0	29	6.1	63	6.3
583	16.0		22.9	43	16.4	29	8.0
246	1.7		1.6	6	1.0	61	4.3
274	2.1		1.7	11	1.9	46	3.5
450	4.6		4.1	43	8.9	81	8.2
278	33.1		44.8	47	52.7	50	60.0
285	2.0		1.6	11	1.7	38	2.6
331	2.6		5.3	19	3.4	41	3.3
350	3.1		5.8	16	3.1	57	5.1
506	61.7	1	40.1	65	33.3	11	7.2
171	1.4		2.5	13	3.3	43	3.6
610	7.6	1	36.0	53	12.0	59	7.4
221	1.4		3.3	18	3.0	45	2.9
251	1.9		5.3	27	4.7	49	3.8
742	8.3	2	52.6	102	26.8	N.D.	N.D.
171	1.4		2.5	13	3.3	43	3.6
213	1.9		2.0	19	3.9	26	2.3
741	17.4	14	56.5	60	18.8	64	15.0

counts of colonies from 5 plates in
mitotic recombination was estimated

from counts of colonies growing on a total of 30 plates, 20 plates containing medium on which all surviving cells grew and 10 plates containing medium on which only *trp5* convertants grew.

y marginal increases in the

nase cultures, were treated
nated hydrocarbons there
cies of gene conversion,
ion. Although the toxicity
re no apparent differences
comparisons were made at
between cell survival and
ion mixtures was apparent

In order to obtain evidence that these halogenated hydrocarbons entered the yeast cells and were metabolized the spectra obtained on the addition of carbon tetrachloride, halothane or trichloroethylene to whole-cell suspensions or microsomal preparations were recorded (Figs. 1 and 2).

When carbon tetrachloride was added to microsomal preparations reduced with sodium dithionite, an absorption peak with a maximum at 460 nm was observed (Fig. 1). The formation of the peak was time-dependent and a gradual shift towards 450 nm was observed. When carbon tetrachloride was added to whole-cell suspensions, two peaks were observed, one with a maximum at 450 nm and a second at 505 nm (Fig. 2). Sodium dithionite was not required in order to obtain these spectra. The formation of both peaks was time-depen-

SL 034301

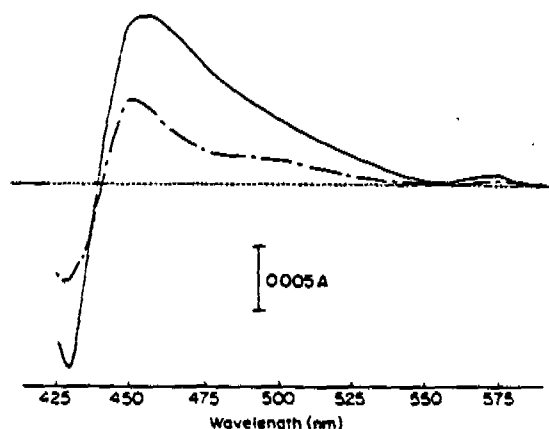


Fig. 1. Difference spectra obtained on the addition of carbon tetrachloride and halothane to microsomes prepared from the yeast strain D5. Microsomes were prepared from log-phase cells of the strain D5 harvested from medium containing glucose as carbon source. The concentration of cytochrome P-450 from the carbon monoxide difference spectrum was $0.66 \Delta A$ (450–490 nm)/ml. —, difference spectrum of dithionite-reduced microsomes 15 min after the addition of carbon tetrachloride, 10 mM, to the sample cuvette. - - -, difference spectrum of dithionite-reduced microsomes 6 min after the addition of halothane, 10 mM, to the sample cuvette., baseline.

dent. The peak at 505 nm was still observed after the test cuvette had been bubbled with carbon monoxide. On the addition of carbon monoxide to the cuvette, prior to the addition of carbon tetrachloride, no peak at 505 nm was observed.

Similar experiments with halothane resulted in the formation of a peak with a maximum at 450 nm in microsomal preparations (Fig. 1) and with peaks at 437 and 505 nm in suspensions of whole cells (Fig. 2). The addition of trichloroethylene to whole-cell suspensions gave peaks with maxima at 450 and 505 nm. The peak at 450 nm appeared to reach a maximum after approx.

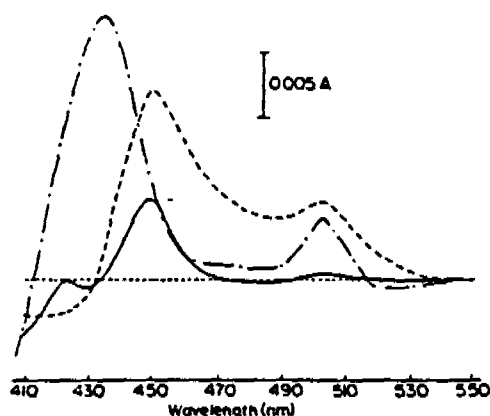


Fig. 2. Difference spectra obtained on the addition of carbon tetrachloride, trichloroethylene and halothane to cell suspensions of the yeast strain D5. Cells of the strain D5 were harvested from log-phase cultures grown on medium containing glucose as carbon source. The concentration of cytochrome P-450 from the carbon monoxide difference spectrum was $0.88 \Delta A$ (450–490 nm)/ml. —, difference spectrum 8 min after addition of trichloroethylene, 15 mM, to the sample cuvette. - - -, difference spectrum 15 min after the addition of halothane, 12 mM, to the sample cuvette., difference spectrum 15 min after the addition of carbon tetrachloride, 10 mM, to the sample cuvette., baseline.

5 min. After this action was observed, preparations after

Discussion

There was an increase in the number of carbon monoxide binding sites when incubated for 4 h. However, increased frequency suggests that these sites are to be expressed rapidly in the strain suspensions of the P-450 concentration of the D4 strain. The observed cells and test for toxic properties of metabolites, or a

When treatment of log-phase cells of mitotic recombinant was of induced metabolism has been reported of some halogenated alkenes to be epoxidized by yeast to these two

It has been suggested that trichloroethylene impurity is epichlorohydrin, a metabolic activation of these genetic effects active to log-phase Bronzetti, E. Zeiger

Several lines of evidence indicate that these metabolized by a cell to products which are compounds. Experimental chloroform were given with yeast cells harvested on galactose (such cells hydrocarbons were evidenced by the suspensions of whole cells observed, however, in preparations [14,19].

The addition of t

5 min. After this period an additional peak at 423 nm was observed. No interaction was observed on the addition of sodium dithionite to microsomal preparations after incubation with trichloroethylene and NADPH.

Discussion

There was an observed marginal genetic effect of these 7 halogenated hydrocarbons when incubated with log-phase cells of strain D4 for 1 or 4 h and strain D7 for 4 h. However, treatment of strain D7 for 1 h resulted in significant increased frequencies of mitotic gene conversion and recombination. This suggests that these compounds need metabolic activation for their genetic activity to be expressed and that the metabolism of these compounds may be more rapid in the strain D7. This latter result may not be unexpected since cell suspensions of this strain, harvested from log-phase cultures, had a cytochrome P-450 concentration up to 5 times higher than a similar cell suspension of the D4 strain. The observation of a marginal genetic activity when incubations of cells and test compounds were continued for 4 h could be the result of the toxic properties of these compounds masking the genetic effects of their metabolites, or a destruction of the metabolic system.

When treatment times were 1 h all 7 hydrocarbons tested were activated by log-phase cells of the strain D7, to products which induced gene conversion, mitotic recombination and gene reversion. The frequency of *ade2* mitotic recombinants was the most sensitive indicator of genetic damage; frequencies of induced *ilv1* reversion were low. Reversion of the *ilv1* gene in D7 diploids has been reported to be mutagen specific [27]. Although the active metabolites of some halogenoalkanes are thought to be free radicals [17] and those of alkenes to be epoxides [3], no difference was evident in the response of the yeast to these two classes of hydrocarbons.

It has been suggested [9] that the carcinogenic and mutagenic properties of trichloroethylene are due to the presence of impurities. However, the major impurity is epichlorohydrin, a direct-acting mutagen which does not need metabolic activation. Therefore, it is unlikely that this is an explanation for these genetic effects observed in yeast since trichloroethylene is not genetically active to log-phase cultures of the strain D4 or stationary cultures of D7 (G., Bronzetti, E. Zeiger and D. Frezza, personal communication).

Several lines of evidence strongly indicate that the hydrocarbons tested are metabolized by a cytochrome P-450-dependent yeast monooxygenase system to products which are both mutagenic and more toxic than the parent compounds. Experiments (data not shown) indicate that methylene chloride and chloroform were genetically inactive and significantly less toxic when tested with yeast cells harvested from log-phase cultures grown in medium containing galactose (such cells have a low level of cytochrome P-450 [5]). Halogenated hydrocarbons were also shown to interact with yeast cytochrome P-450 as evidenced by the spectra obtained on the addition of several compounds to suspensions of whole yeast cells or yeast microsomal preparations. The spectra observed, however, were often different from those observed with mammalian preparations [14,19,20,24].

The addition of trichloroethylene to suspensions of whole cells resulted in

nd halothane to microsomes
phase cells of the strain D5
ration of cytochrome P-450
nm)/ml. —, difference
on tetrachloride, 10 mM, to
microsomes 6 min after the

test cuvette had been
n monoxide to the
peak at 505 nm was

mation of a peak with
1) and with peaks at
. The addition of tri-
h maxima at 450 and
ximum after approx.

trichloroethylene and halo-
harvested from log-phase cul-
tivation of cytochrome P-450
nm)/ml. —, difference
cuvette. — — —, difference
cuvette., difference spec-
cuvette., baseline.

the formation of a spectrum which was similar to that observed in mammalian microsomes. It has been suggested that the spectrum, with a peak at 450 nm, results from the metabolism of trichloroethylene to carbon monoxide [19]. The peak of absorption at 505 nm which formed on the addition of either halothane or carbon tetrachloride to suspensions of whole yeast cells remains unexplained.

It is concluded that the halogenated hydrocarbons tested are metabolized to genetically active and cytotoxic products by a yeast cytochrome P-450-dependent monooxygenase system. Halothane, chloroform, carbon tetrachloride and tetrachloroethylene have been reported to be inactive in test systems which require the use of a mammalian monooxygenase system [13,18,22] even though they appear to be carcinogenic [23]. Thus, the yeast system described appears to be more sensitive than some other in vitro test systems in the detection of the activity of some compounds. The higher sensitivity of this yeast-activation system may result from the production of active metabolites in much closer proximity to the nucleus than is possible with liver S9 in vitro activation systems. The use of this yeast-activation system may be advantageous where the active metabolites are particularly unstable and reactive; as is likely for those of the halogenated hydrocarbons.

References

- 1 Baden, J.M., M. Brinkenhoff, R.S. Wharton, B.A. Hiff, V.F. Simmon and R.I. Mazze, Mutagenicity of volatile anesthetics: Halothane, *Anesthesiology*, 45 (1976) 311-318.
- 2 Bartsch, H., and R. Montesano, Mutagenic and carcinogenic effects of vinyl chloride, *Mutation Res.*, 32 (1975) 93-114.
- 3 Bonse, G., and D. Henschler, Chemical reactivity, biotransformation and toxicity of polychlorinated aliphatic compounds, *CRC Critical Rev. Toxicol.*, 4 (1976) 395-410.
- 4 Brem, H., A.B. Stein and H.S. Rosenkranz, The mutagenicity and DNA-modifying effect of haloalkanes, *Cancer Res.*, 34 (1974) 2576-2579.
- 5 Callen, D.F., and R.M. Philpot, Cytochrome P-450 and the activation of promutagens in *Saccharomyces cerevisiae*, *Mutation Res.*, 45 (1977) 309-324.
- 6 Callen, D.F., C.R. Wolf and R.M. Philpot, Cumene hydroperoxide and yeast cytochrome P-450: spectral interactions and effect on the genetic activity of promutagens, *Biochem. Biophys. Res. Commun.*, 83 (1978) 14-20.
- 7 Fishbein, L., Industrial mutagens and potential mutagens, 1. Halogenated aliphatic derivatives, *Mutation Res.*, 32 (1976) 267-308.
- 8 Greim, H., G. Bonse, Z. Radwan, D. Reichert and D. Henschler, Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation, *Biochem. Pharmacol.*, 24 (1975) 2013-2017.
- 9 Henschler, D., E. Eder, T. Neudecker and M. Metzler, Carcinogenicity of trichloroethylene: fact or artifact? *Arch. Toxicol.*, 37 (1977) 233-236.
- 10 Kawaguchi, K., K. Ishidate and K. Tagawa, Nonmitochondrial electron transfer system in anaerobically grown yeast cells, *J. Biochem.*, 74 (1973) 817-826.
- 11 de Lorenzo, F., S. Degl'Innocenti, A. Ruocco, L. Silengo and R. Cortese, Mutagenicity of pesticides containing 1,3-dichloropropene, *Cancer Res.*, 37 (1977) 1915-1917.
- 12 Mallin, H.V., Dimethylnitrosamine: formation of mutagenic compounds by interaction with mouse liver microsomes, *Mutation Res.*, 13 (1971) 425-429.
- 13 McCann, J., E. Choi, E. Yamasaki and B.N. Ames, Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals, *Proc. Natl. Acad. Sci. (U.S.A.)*, 72 (1975) 5135-5139.
- 14 Mansuy, D., W. Nastainczyk and V. Ullrich, The mechanism of halothane binding to microsomal cytochrome, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 285 (1974) 315-324.
- 15 Report of an Ad Hoc committee on the effect of trace anesthetics on the health of operating room personnel, *Anesthesiology*, 41 (1974) 321-340.
- 16 Shahin, M.M., and R.C. von Borstel, Mutagenic and lethal effects of α -benzene hexachloride, dibutylphthalate and trichloroethylene in *Saccharomyces cerevisiae*, *Mutation Res.*, 48 (1977) 173-180.

17 Sla:
18 Stu
nin-
19 Tay
mic
Cor
20 Uet
pro:
1-1
21 Uehl
oxira
22 Uehl
for
23 Weis:
(19"
24 Wolf
halog
25 Zimr
32"
26 Zimr
cross:
27 Zimr
mitot
386.

observed in mammalian
with a peak at 450 nm,
carbon monoxide [19].
the addition of either halo-
hole yeast cells remains

tested are metabolized to
cytochrome P-450-depen-
carbon tetrachloride and
ve in test systems which
system [13,18,22] even
he yeast system described
test systems in the detec-
r sensitivity of this yeast-
of active metabolites in
ble with liver S9 in vitro
system may be advanta-
nstable and reactive; as is

on and R.I. Mazze, Mutagenicity of
3.

of vinyl chloride, Mutation Res.,

and toxicity of polychlorinated

and DNA-modifying effect of halo-

ation of promutagens in *Saccha-*

ide and yeast cytochrome P-450:
mutagens, Biochem. Biophys. Res.

generated aliphatic derivatives, Muta-

Mutagenicity in vitro and potential
bolic oxirane formation, Biochem.

nicity of trichloroethylene: fact or

electron transfer system in anaerobi-

Cortese, Mutagenicity of pesticides
7.

compounds by interaction with mouse

carcinogens as mutagens in the Salmo-
n. (U.S.A.), 72 (1975) 5135-5139.
othane binding to microsomal cyto-
5-324.

on the health of operating room

of α -benzene hexachloride, dibutyl-
tion Res., 48 (1977) 173-180.

Slater, T.F., Free radical mechanisms in tissue injury, Pion Press, London, 1972.

Sturrock, J., Lack of mutagenic effect of halothane or chloroform on cultured cells using the azaguanine test system, Br. J. Anaesth., 49 (1977) 207-210.

Taylor, P.S., W. Nastainczyk and V. Ullrich, Conversion of trichloroethylene to carbon monoxide by microsomal cytochrome P-450, in: V. Ullrich, I. Roots, A. Hildebrandt, R.W. Estabrook and A.H. Conney, (Eds.), Microsomes and Drug Oxidations, Pergamon, Oxford, 1977, pp. 615-621.

Uehleke, H., K.H. Hellmer and S. Tabarelli, Binding of ^{14}C -carbon tetrachloride to microsomal proteins in vitro and formation of chloroform by reduced liver microsomes, Xenobiotica, 3 (1973) 1-11.

Uehleke, H., S. Tabarelli-Poplowski, G. Bonse and D. Henschler, Spectral evidence for 2,2,3-trichloroxirane formation during microsomal trichloroethylene oxidation, Arch. Toxicol., 37 (1977) 95-105.

Uehleke, H., T. Werner, H. Greim and M. Kramer, Metabolic activation of haloalkanes and tests in vitro for mutagenicity, Xenobiotica, 7 (1977) 393-400.

Weisburger, E.K., Carcinogenicity studies on halogenated hydrocarbons, Environ. Hlth. Perspect., 21 (1977) 7-16.

Wolf, C.R., D. Mansuy, W. Nastainczyk, G. Deutschmann and V. Ullrich, The reduction of polyhalogenated methanes by liver microsomal cytochrome P-450, Mol. Pharmacol., 13 (1977) 698-705.

Zimmerman, F.K., Induction of mitotic gene conversion by mutagens, Mutation Res., 11 (1971) 327-337.

Zimmerman, F.K., A yeast strain for visual screening for the two reciprocal products of mitotic crossing-over, Mutation Res., 21 (1973) 263-269.

Zimmerman, F.K., R. Kern and H. Rasenberger, A yeast strain for simultaneous detection of induced mitotic crossing-over, mitotic gene conversion and reverse mutation, Mutation Res., 28 (1975) 381-388.

