

Mutagenicity of fume particles from stainless steel welding

by AGNETA HEDENSTEDT, B.Sc.,¹ DAG JENSSEN, B.Sc.,¹ BRITT-MARIE LIDESTEN, B.Sc.,¹ CLAES RAMEL, D.Sc.,¹ ULF RANNUG, B.Sc.,¹ and RICHARD M. STERN, Dr.Rer.Nat.²

HEDENSTEDT, A., JENSSEN, D., LIDESTEN, B.-M., RAMEL, C., RANNUG, U. and STERN, R. M. Mutagenicity of fume particles from stainless steel welding. *Scand. j. work environ. & health* 3 (1977) 203—211. Welding fume particles collected from different welding procedures were tested for mutagenicity in *Escherichia coli*, with the inhibition zone in pol A⁻ as compared to pol A⁺, and in *Salmonella typhimurium*, TA 100 strain. While no mutagenicity was found with mild steel welding, a mutagenic effect was established with samples from stainless steel welding. This mutagenicity was particularly associated with manual metal arc (MMA) welding, and less so with metal inert-gas welding. A decrease in or an elimination of the effect occurred with a liver microsomal metabolizing system (S-9 mix). The MMA samples produced the strongest mutagenic effect. More-detailed investigations on these samples showed that the mutagenic agent(s) is water soluble. An increased mutagenicity, which also revealed the induction of frame shift mutations, was found with TA 98. The same welding fume sample was used for a mutagenicity test (resistance to 6-thioguanine) with V 79 hamster cells. Because of the high toxicity of these welding fume particles on the cells, only very low concentrations could be tested, but the increase of mutations, when compared to the negative control, was significant. It is suggested that hexavalent chromium may be involved in the mutagenic effect of the welding fumes.

Key words: cancer, Chinese hamster cells, chromium, *Escherichia coli*, mutations, *Salmonella typhimurium*, welding fumes.

The screening for mutagenic and carcinogenic chemicals has usually been performed with pure chemicals. From an experimental point of view such an approach simplifies many calculations, procedures and extrapolations, but it is quite artificial and does not reflect what actually happens in real life. An alternative is to perform tests on samples actually encountered in

occupational exposure — which often contain highly complex chemical mixtures. The further identification of industrial processes or components as being hazardous can be done by mutagenicity and carcinogenicity screening in combination with chemical fractionation and analyses. Such an approach, however, requires a large series of tests, and for this purpose short-term screening methods are essential. Bacterial tests with microsomal activation have turned out to be particularly useful for this purpose, as exemplified by tests on tobacco smoke condensate (3) and waste products from the vinyl chloride industry (8).

The present report deals with a similar situation concerning fume particles from metal arc welding. Welding is performed

¹ Environmental Toxicology Unit, Wallenberg Laboratory, University of Stockholm, Stockholm, Sweden.

² The Danish Welding Institute and Physics Laboratory, Royal Danish College of Pharmacy, Copenhagen, Denmark.

Reprint requests to: Prof. C. Ramel, Wallenberg Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden.

by a large number of workers all over the world, and it is of great importance to obtain information as to which of the various welding techniques may constitute an especially high risk of exposure to mutagenic and carcinogenic compounds.

That such a risk probably occurs has been indicated by the fact that an increased incidence of throat cancer was recently reported for welders and other workers in a machine factory in Copenhagen (11). When tested in the present investigation, a sample from one of the welding procedures used in this factory had the strongest mutagenic effect of all the samples studied.

Preliminary spot tests using the histidine revertant test system of Ames et al. on *Salmonella typhimurium* and S-9 liver fraction (1) were performed with a number of samples of welding fume particles. In order to widen the experimental base, we also tested the samples on *Escherichia coli*, using the spot test system with polymerase A⁺ and A⁻ according to Rosenkranz and coworkers (10).

Some of the samples produced a mutagenic response with the base substitution strain TA 100, and therefore a more thorough quantitative investigation involving dose-effect response was undertaken.

The sample which was the most active in the bacterial mutagenicity test was also tested for genetic mutations in Chinese hamster cells.

MATERIAL AND METHODS

Welding processes and material

The welding process can be varied in a large number of ways with respect to the procedure and the material used. Those processes which give rise to the most significant fume exposure and which were examined in the present investigation can be divided into two main groups.

Manual metal arc welding. In manual metal arc (MMA) welding the electrodes are coated with chemical mixtures that vary in composition according to the manufacturer and the purpose. Coated electrodes are used to protect the welding

material from oxidation. The fume particles formed during MMA welding are almost entirely respirable (90 % less than 1 μ). Both their chemistry and structure vary and are complex. MMA welding accounts for over 50 % of the activity of the about 150,000 welders in the Scandinavian countries.

Metal inert-gas welding. In metal inert-gas (MIG) welding a continuously fed iron wire is used, and shielding is performed by means of a gas (argon, carbon dioxide, oxygen or mixtures of these). The particles formed are frequently crystalline and smaller than those produced in MMA welding.

The welding material can be separated into the following two main groups: (a) mild steel (MS), i.e., low alloyed iron with about 1 % silicon, 1 % carbon and 1 % manganese, and (b) stainless steel (SS), i.e., high alloyed metal varying in composition, but typically containing 15–25 % chromium, 8–15 % nickel and up to 3 % molybdenum.

Samples of fume particles

The collection of welding fume particles was performed according to a system described by Stern (12). A robot was used to produce automatically bead-on-plate welding in order to eliminate possible variation in the aerosol produced. The fume was collected via a long vertical stack connected to a variable speed fan, and the particles were collected on 240 mm Whatman 41 paper filters, the result being approximately 300 mg of particles per filter paper.

The welding fume samples tested are listed in table 1.

Mutagenicity testing

Metabolizing system. Male Sprague-Dawley rats were pretreated with a polychlorinated biphenyl mixture (Clophen A 50, 500 mg/kg, i.p.) five days before being killed. Livers from three animals were pooled. The postmitochondrial liver fraction (S-9), as well as the NADPH-generating system, was prepared according to Ames et al. (1). The proportion of S-9 in the complete metabolizing system (S-9 mix) was fixed at 10 %.

Table 1. Characteristics of the welding fume samples tested. (a, b and c indicate the same kind of sample, collected on different occasions)

Sample	Type of welding ^a	Chemical analysis of welding material	Usual source of sample
1 a, b, c	MMA/SS	0.15 % carbon 29 % chromium 9 % nickel 1.5 % manganese	Austenitic/ferritic electrode during the joining of alloy steels or the welding of steels with poor welding quality (crack resistant)
2	MMA/SS	< 0.04 % carbon ≤ 0.9 % silicon 1.5 % manganese 17–20 % chromium	Low hydrogen electrode, with especially low carbon content, during the welding of corrosion resistant Cr/Ni/Mo steel with approx. 18 % chromium, 12 % nickel and 2–3 % molybdenum and cold drawn austenitic steel
3	MMA/SS	< 0.03 carbon ≤ 1.5 % silicon ≤ 1.6 % manganese 17–20 % chromium 10.5–13 % nickel 2.5–3 % molybdenum	Rutile coated electrode during the welding of corrosion resistant Cr/Ni/Mo steel with a low carbon content
4	MMA/SS	< 0.03 % carbon ≤ 0.9 % silicon ≤ 1.5 % manganese 17–20 % chromium 11–13 % nickel 2.5–3 % molybdenum	Rutile electrode during the welding of austenitic stainless steel (18/12 + Mo) (acid resistant)
5 a, b, c	MIG/SS	< 0.08 % carbon 19.0 % chromium 9.5 % nickel 3.5 % molybdenum	Solid wire during the joining of austenitic with ferritic steel, high temperature steel use (500°C), mangan-hard steel, etc.
6	MIG/SS	< 0.025 % carbon 0.85 % silicon 1.4 % manganese ≤ 0.015 % phosphorus ≤ 0.015 % sulfur 18.5 % chromium 10.5 % nickel 2.7 % molybdenum	Solid wire during the welding of austenitic acid resistant steel (18/8 + Mo)
7	MMA/MS	0.09 % carbon ≤ 0.4 % silicon ≤ 0.5 % manganese	Universal rutile electrode during general construction or the welding of tanks or ship plates
8	MMA/MS	0.1 % carbon 0.6 % silicon 0.9 % manganese	All around, basic electrode, especially for vertical profiles, during general construction or the welding of ship plates or cast-iron (high quality welding)
9	MIG/MS	0.1 % carbon 0.6 % silicon 1.0 % manganese	Universal solid wire (CO ₂) during the welding of construction steel or tank plates (low hydrogen content)
10	MIG/MS	0.1 % carbon 0.3 % silicon 1.6 % manganese	Powder-filled rutile-type all purpose wire during the welding of unalloyed steel
11	MIG/MS	< 0.1 % carbon ≤ 0.5 % silicon 1.2 % manganese	Powder-filled rutile-type wire during general use with unalloyed steel

^a MMA = manual metal arc welding; MIG = metal inert-gas welding; SS = stainless steel; MS = mild steel.

Test organisms and test procedures. *Salmonella typhimurium* strains TA 100 and TA 98, derived from the histidine auxotrophs TA 1535 and TA 1538, respectively, by introducing an R factor plasmid (ampicillin resistance pKM 101) (5), were kindly provided by Prof. B. N. Ames, Berkeley, California, U.S.A.

The quantitative tests with TA 100 and TA 98 were performed as described by Rannug and Ramel (8) using aqueous solutions of the fume samples, scraped from the filter papers. One series with ethanol as the solvent was also made.

Spot tests with TA 100 were made with or without the addition of 0.5 ml of the S-9 mix in the soft agar overlay containing the bacteria. Discs (6 mm in diameter) were stamped from the sample filters and placed in the middle of the agar plates. Each sample was tested in duplicate.

The media, bacterial cultures, etc., were the same as in the quantitative test procedure. An accumulation of mutant colonies near the zone of inhibition was regarded as a positive effect when the plates were scored after two days of incubation.

The screening procedure for detecting agents that modify cellular DNA using *E. coli* W 3110 (pol A⁺) and its pol A⁻ derivative (*E. coli* p3478) was performed according to Slater et al. (10).

The discs with the samples were placed in the middle of the plates, and the zone of inhibition was measured after incubation at 37°C for 16–20 h.

The screening for mutagenicity and toxicity in V 79 Chinese hamster cells was performed according to the optimal protocol described by van Zeeland and Simons (13). The cells were cultured in Eagle's Minimum Essential Medium supplemented with 1.8 mM L-glutamine, 90 units/ml penicillin, 90 mcg/ml streptomycin and 10 % heat inactivated fetal calf serum (Flow Laboratories) and incubated at 37°C in 5 % carbon dioxide in air. The medium for selection was prepared with 6-thioguanine (5 µg/ml) (Sigma) which was five times the concentration needed to kill HGPRT⁺ cells in the actual serum batch used. The cells were found to be negative in a mycoplasma contamination test (9). The welding fume particles of sample 4 were dissolved in Hanks' balanced salt solution at con-

Table 2. The mutagenic effect of welding fume samples on *Escherichia coli*, as indicated by the inhibition zone of pol A⁻ as compared to pol A⁺ according to Slater et al. (10), and on histidine revertants in spot tests on *Salmonella typhimurium* TA 100. The numbers are based on the average of two plates.

Sample ^a	<i>Escherichia coli</i>		<i>Salmonella typhimurium</i>
	± S-9 mix	Diameter of zones, inhibition (mm) Pol A ⁻ /Pol A ⁺	Mutagenic effect on TA 100
MMA/SS			
1 a	—	18.3/10.0 ^b	+
	+	24.0/12.8 ^b	+
b	+	18.5/ 0	+
c	—	38.0/22.5	+
	+	30.0/23.5	—
2	—	14.3/ 0 ^b	+
	+	14.5/ 0 ^b	+
3	—	19.5/10.0 ^b	+
	+	18.3/ 0 ^b	+
4	—	28.5/12.5	+
	+	19.0/11.5	+
MIG/SS			
5 a	+	0 / 0	—
b	+	0 / 0	—
c	—	0 / 0	—
	+	0 / 0	—
6	—	11.5/ 0	+
	+	0 / 0	+
MMA/MS			
7	—	0 / 0	—
	+	0 / 0	—
8	—	0 / 0	—
	+	0 / 0	—
MIG/MS			
9	—	0 / 0	—
	+	0 / 0	—
10	—	0 / 0	—
	+	0 / 0	—
11	—	0 / 0	—
	+	0 / 0	—

^a MMA = manual metal arc welding; MIG = metal inert-gas welding; SS = stainless steel; MS = mild steel.

^b Indicates the average of four plates.

centrations of 5, 10 and 15 µg/ml. Negative and positive (ethyl methanesulfonate, 600 µg/ml) controls were used. Three experiments were performed. The mutagenicity data are based on six plates per dose and the survival on three plates per dose. Statistical evaluations were performed with *t* tests, and the combined probability analysis in table 3 according to Fisher (2).

RESULTS

The results of the spot tests on *Salmonella* with the histidine revertant strain TA 100 and with *E. coli* pol A⁺ and pol A⁻ strains are shown in table 2. Although all the samples were not investigated with the liver S-9 mix, the two test systems agree and indicate a mutagenic effect for all the MMA/SS samples. The mutagenic effect occurred both with and without the liver microsomal fraction, with the exception of one sample (1 c), for which no mutagenic effect was observed with liver microsomes. This occurrence pointed to some detoxication of the active compound. Such a detoxication was verified in the quantitative soft agar tests on *Salmonella*.

One of the MIG/SS samples likewise gave a positive response both with *Salmonella* and *E. coli*, but in the latter case the inhibition zone was eliminated in the presence of the liver microsomal fraction. None of the mild steel samples exhibited mutagenic activity.

On the basis of these spot test results it could be concluded that the mutagenic effects of the samples of welding fume particles are connected with stainless steel and, apparently, are especially significant for fumes from the MMA technique of welding. A quantitative investigation of histidine revertants in *Salmonella* after exposure in plating experiments with the soft agar technique was therefore performed on these welding fume samples. Tests were done with and without the liver postmitochondrial fraction (S-9 mix); the series with S-9 mix were performed with and without NADP. The dose effect curves in the experiments with SS samples are presented in fig. 1. With the liver microsomal fraction, but without NADP, there was a dose-related response for all samples, the strongest effect occurring with sample 4. The mutagenic effect of the samples were, however, diminished or eliminated by the complete S-9 mix with NADP, and a detoxification of the active compound(s) in the liver was therefore indicated. The series without S-9 mix are not shown as they gave the same result as the series with S-9 mix but without NADP. The requirement of the NADPH-generating system for the detoxifying

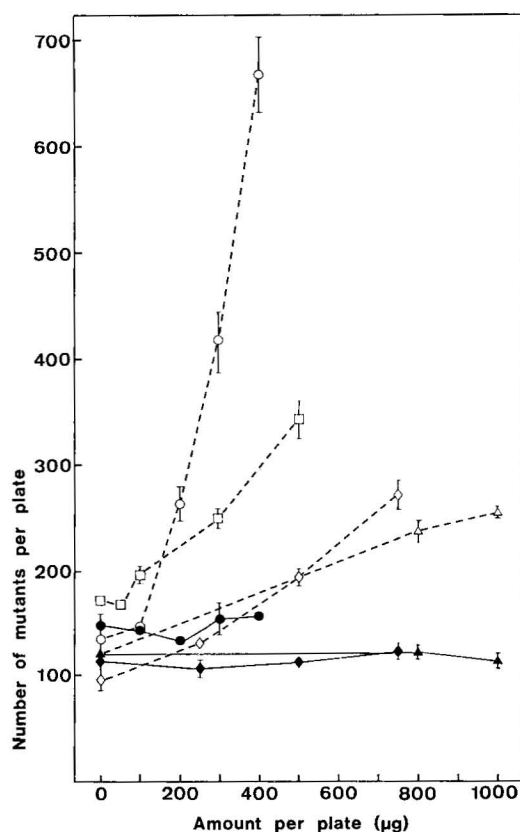


Fig. 1. The mutagenic effect on *Salmonella typhimurium* TA 100 of different samples of welding fumes from manual metal arc welding on stainless steel. The presence of a complete metabolizing system with S-9 mix and NADP is indicated with filled symbols and S-9 mix without NADP with open symbols. (Sample 1a □, 2. ◇, 3. △ and 4. ○)

action of the liver microsomes indicates that the mixed function oxygenase system is involved.

As sample 4 showed a particularly strong mutagenic effect, it was used for additional investigations. Water and ethanol were compared as solvents of the material. As shown in fig. 2, no or little mutagenic action was obtained after the use of ethanol, while dissolution in water gave a clear dose-related mutagenic response. The mutagenic agent(s) therefore must be water soluble.

Sample 4 was also used for an experiment with TA 98 in the study of the effect on frame shift. As is shown in fig. 3, the positive mutagenic response found in this

case was also counteracted by the presence of liver microsomes and NADP.

The test on one of the MIG welding samples with stainless steel (sample 6) gave a positive mutagenic response both in spot tests (table 2) and in the two quantitative experiments shown in table 3. Although the effect was rather small, the data indicate a dose-dependent increase. There seems to be a similar effect with and without microsomal fraction; therefore another type of compound, as compared to the MMA welding of stainless steel, is possibly operating to cause the mutations.

Sample 4 was finally tested in three series for genetic mutations (6-thioguanine resistance) in V 79 hamster cells (table 4). The toxicity of the fume particles is apparently considerably higher in this system than in the *Salmonella* tests, and therefore only very low concentrations

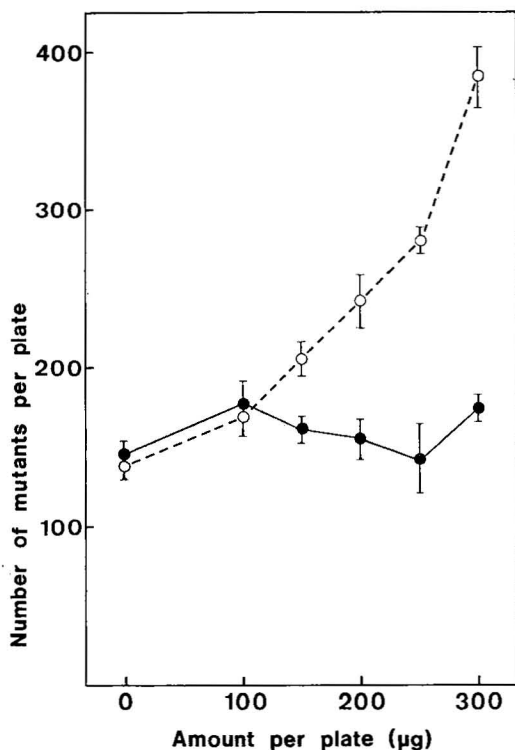


Fig. 2. Difference in mutagenic effect of sample 4 (manual metal arc/stainless steel) on *Salmonella typhimurium* TA 100 with ethanol (●) and water (○) as solvents. No metabolizing system added.

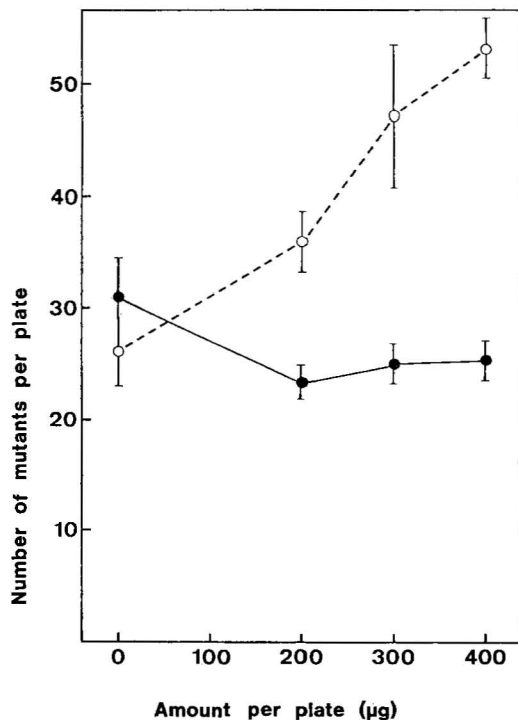


Fig. 3. The mutagenic effect of sample 4 on TA 98 with S-9 mix with (●) and without (○) NADP.

could be used. The frequency of mutations was slightly higher for 5 and 10 µg/ml when compared with the control, and the difference was significant at a 1 % level when a combined probability test was applied for the cell cultures at 10 µg/ml. From 10 to 15 µg/ml there was a drop in the mutation frequency that was presumably associated with low survival. There is no reason to believe that the high toxicity of the fume particles to the cells was caused by an effect on DNA. Because of this toxicity an extensive number of cell culture experiments would be required to establish a mutagenic effect at operative dose levels more firmly.

DISCUSSION

The present results show that the welding of stainless steel produces fume particles that are mutagenic, particularly in connection with MMA welding. This finding

Table 3. Mutagenic effect of sample 6 (metal inert-gas/stainless steel) on *Salmonella typhimurium* TA 100 with S-9 mix, with and without NADP.

Experiment	Micrograms per plate	S-9 mix $\pm$ NADP	Survival (%)	Number of mutants per plate $\pm$ standard error
I	0	— ^a	100	101.8 $\pm$ 10.7
	250	— ^a	90	96.4 $\pm$ 5.0
	500	— ^a	80	123.0 $\pm$ 9.1
	750	— ^a	88	129.0 $\pm$ 2.8 *
	1,000	— ^a	85	158.4 $\pm$ 7.5 **
II	0	—		122.8 $\pm$ 1.8
	0	—		115.0 $\pm$ 5.7
	750	—		132.2 $\pm$ 6.6
	750	—		129.4 $\pm$ 11.7
	1,000	+		159.6 $\pm$ 13.5 *
	1,000	+		135.2 $\pm$ 8.5
	1,250	+		139.0 $\pm$ 6.7
	1,250	+		143.3 $\pm$ 6.2 *

^a No S-9 mix added.

* = $P < 0.05$; ** = $P < 0.01$.

Table 4. The frequency of genetic mutations and survival in V 79 Chinese hamster cells after 4 h of treatment with different doses of welding fume particles (sample 4) and ethyl methanesulfonate (positive control).

Experiment	Compound ^a	Dose in $\mu\text{g/ml}$	% survival $\pm$ standard error	Number of 6-thioguanine resistant mutants per 10^5 cells $\pm$ standard error
I	None	0	100 $\pm$ 6	0.9 $\pm$ 0.36
	Sample 4	5	87 $\pm$ 2	1.4 $\pm$ 0.26
		10	63 $\pm$ 3	1.8 $\pm$ 0.47
		15	47 $\pm$ 0	0.3 $\pm$ 0.19
	EMS	600	79 $\pm$ 1	31.0 $\pm$ 0.79 ***
II	None	0	100 $\pm$ 6	1.4 $\pm$ 0.54
	Sample 4	5	82 $\pm$ 2	2.6 $\pm$ 0.83
		10	45 $\pm$ 11	2.7 $\pm$ 0.88
		15	6 $\pm$ 0	1.5 $\pm$ 0.66
	EMS	600	120 $\pm$ 2	21.7 $\pm$ 3.35 ***
III	None	0	100 $\pm$ 12	1.2 $\pm$ 0.74
	Sample 4	5	107 $\pm$ 2	1.3 $\pm$ 0.19
		10	65 $\pm$ 7	4.0 $\pm$ 0.56 *
		15	42 $\pm$ 4	1.5 $\pm$ 0.51
	EMS	600	124 $\pm$ 3	26.9 $\pm$ 1.32 ***
I, II and III combined	None	0	100	1.2
	Sample 4	5	92	1.8
		10	58	2.8 **
		15	32	1.1
	EMS	600	108	26.5 ***

^a EMS = ethyl methanesulfonate.

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

points to the possibility also of a carcinogenic action of welding fumes, and, as mentioned in the introduction, there is some preliminary epidemiologic support

for such an assumption. The elevated incidence of throat cancer observed among workers in a Danish factory (11) may be connected with MMA welding of stainless

steel. One of the welding processes used in that factory was the same as the one employed for sample 4 in the present investigation; it is evidently particularly active as a mutagen.

Since MMA welding seems to be more hazardous than MIG welding from a mutagenicity point of view, the same might be expected concerning general toxicity and might be predicted by studies of fume chemistry. Such toxic effects are dependent on two properties of the fume particles — size, which determines retention in the respiratory system, and biological activity, primarily due to their chemical composition. The welding techniques differ in both these respects (12). MMA welding with coated electrodes produces larger particles with a shorter retention time than MIG welding. Furthermore, some MMA processes (rutile) produce particles encapsulated in glass. Therefore with MMA welding both the availability of the particles in the respiratory system and the biological activity of the particles can be expected to be different than with MIG welding.

It is evident that stainless steel welding produces one or several genetically active components. The identification of the mutagenic agent(s) is of course important in order to eliminate, or at least diminish, the exposure of the welders to the agent(s) through an appropriate technological change. The bacterial test system should be of great value to this identification also. Systematic mutagenicity tests could be performed in combination with chemical fractionations and analyses of the welding fumes. At present it can only be a matter of speculation which component(s) in the fumes from stainless steel welding is responsible for the mutagenic activity. A few characteristics, however, are indicated by the mutagenicity tests. The effect when using water as compared to ethanol as the solvent shows that at least the main mutagenic agent is soluble in water but not in ethanol. This information, in combination with the fact that stainless steel is involved, focuses the attention on soluble chromium compounds, which are known to be carcinogenic. Recently reports have appeared also on the mutagenicity of hexavalent chromium compounds in tests

with *Bacillus subtilis* (Rec⁻/Rec⁺) and *E. coli* (Trp⁻) (6), as well as *Salmonella typhimurium* (4,7). In *Salmonella* tests base substitutions, as well as frame shift mutations, were indicated with hexavalent chromium, while no effect was found with trivalent chromium (4). The magnitude of the mutagenic effects observed by us appear to follow the degree of water solubility of the chromium compounds in the fumes. Preliminary studies show significantly more hexavalent chromium (at least by a factor 10—1,000) in fumes from MMA than in fumes from MIG welding.

The liver microsomal fraction detoxifies the agent(s) and causes base substitutions, at least in the in vitro system used in the present experiments. This finding agrees with investigations on the mutagenicity of hexavalent chromium in *Salmonella* (G. Löfroth, personal communication). It should be emphasized, however, that welding fumes can act directly on the tissues of the respiratory system, where the detoxification may be different qualitatively and quantitatively when compared to the liver.

The mutagenic effects of the MIG stainless steel sample showed, as pointed out, characteristics which point to the presence of a mutagenic agent other than the one found in the case of MMA stainless steel welding.

ACKNOWLEDGMENTS

The authors are indebted to Prof. C. A. Wachtmeister for his helpful discussions and criticism.

This investigation was supported by grants from the Department of Occupational Health, Stockholm, the National Swedish Environment Protection Board, the Swedish Board for Technical Development, and the Scandinavian Association for the Replacement of Animals in Medical Experiments.

REFERENCES

1. AMES, B. N., MC CANN, J. and YAMASAKI, E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutation res.* 31 (1975) 347—364.
2. FISHER, R. A. *Statistical methods for*

- research workers (eleventh ed.). F. A. E. Crew, Edinburgh 1950.
3. KIER, L. D., YAMASAKI, E. and AMES, B. N. Detection of mutagenic activity in cigarette smoke condensates. *Proc. nat. acad. sci. u.s.a.* 71 (1974) 4159—4163.
 4. LÖFROTH, G. and AMES, B. N. Mutagenicity of inorganic compounds in *Salmonella typhimurium*: Arsenic, chromium and selenium. Presented at the Eighth annual meeting of the Environmental Mutagen Society, Colorado Springs, Co. Feb. 13—17, 1977.
 5. MC CANN, J., SPINGARN, N. E., KOBBERI, J. and AMES, B. N. Detection of carcinogens as mutagens: Bacterial tester strains with R factor plasmids. *Proc. nat. acad. sci. u.s.a.* 72 (1975) 979—983.
 6. NISHIOKA, H. Mutagenic activities of metal compounds in bacteria. *Mutation res.* 31 (1975) 185—189.
 7. PETRILLI, F. L. and DE FLORA, S. Toxicity and mutagenicity of hexavalent chromium on *Salmonella typhimurium*. *Appl. environ. microbiol.* 33 (1977) 805—809.
 8. RANNUG, U. and RAMEL, C. Mutagenicity of waste products from vinyl chloride industries. *J. toxicol. environ. health* 2 (1977) 1019—1029.
 9. SCHNEIDER, E. L., STANBRIDGE, E. J. and EPSTEIN, C. J. Incorporation of ^3H -uridin and ^3H -uracil into RNA: A simple technique for the detection of mycoplasma contamination of cultured cells. *Exp. cell. res.* 84 (1974) 311—318.
 10. SLATER, E. E., ANDERSON, M. D. and ROSENKRANZ, H. S. Rapid detection of mutagens and carcinogens. *Cancer res.* 31 (1971) 970—973.
 11. SOERENSEN, H. H. and SIDENIUS, K. Forekommelse af strubekraft på en Københavns maskin fabrik. (Occurrence of throat cancer in a machine factory in Copenhagen). *Ugeskr. laeg.* (To be submitted for publication)
 12. STERN, R. M. *Production and characterization of a reference standard welding fume*. The Danish Welding Institute and Physics Laboratory, Royal Danish College of Pharmacy, Copenhagen. [1. Preliminary report, (1976) 103 p. 2. Final report. Part 1. The factors affecting the production of welding fume. No SS 7605, (1977) 110 p. Part 2. The chemistry of welding smoke and its variation with welding process and process parameter, (1977) 142 p.]
 13. VAN ZEELAND, A. A. and SIMONS, J. W. I. M. Linear dose-response relationships after prolonged expression times in V 79 Chinese hamster cells. *Mutation res.* 35 (1976) 129—138.

Received for publication: 1977-07-26