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Chronic Biological Effects of Methyl Methacrylate Vapor

III. Histopathology, Blood Chemistries, and Hepatic and Ciliary Function in the Rat[1]

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Inhalation experiments were conducted by exposing rats, mice, and frogs to methyl methacrylate (MMA) monomer vapor in air. Rats were exposed for periods of up to 6 months to near threshold limit value (TLV) concentrations and for a shorter period to approximately ten times the TLV. Blood chemistries were unremarkable. Histopathological findings were essentially nil except for microscopic indications of damage to the tracheal mucosa, supported by scanning electron microscopy (SEM) observations that the cilia and microvilli characteristic of these epithelial cells were absent. A reduction in oropharyngeal transport efficiency was shown when frogs were exposed to 400 ppm of MMA vapor. The sodium pentobarbital detoxification function of mouse liver may be altered consequent to exposures to 160 hr to both 100 and 400 ppm of MMA vapor. It is concluded that, at the TLV, cellular responses may be manifested regardless of whether gross systemic effects are seen.

INTRODUCTION

Practical considerations have played an important role in setting TLV criteria for industrial exposures to toxic vapors, especially in the case of methyl methacrylate. The 100 ppm level was set mindful of the facts that an occupational exposure to this level generally precluded discomfort from irritation, and that it was well below the concentration range which was then accepted as giving rise to systemic effects.

Retrospective evaluation of this standard is fraught with the difficulty that no evidence has been presented which associates frank damage or dysfunction of a target organ or organ system to chronic exposures to this vapor at comparable concentrations. The lack of adequate dosimetry pertaining to individual occupational exposures is compounded by questionable dosimetry reported in various vapor exposure experiments of other species.

A compendium of data pertaining to the literature on MMA toxicity has recently been published by the National Institute for Occupational Safety and Health (NIOSH) and it includes results of epidemiological studies carried out by NIOSH in industrial settings (Cromer and Kronoveter, 1976). NIOSH apparently believes

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changes in pulmonary function parameters indicate chronic effects. We feel that an observation (for example) of liver damage in a rat which received a nonfatal intraperitoneal or intravenous infusion of MMA could also represent acute or chronic damage inasmuch as the critical data (which are also unanswered for inhalatory studies) of concentration at the site of action, biological half-life, and the presence and half-lives of toxic intermediates are unknown. Moreover, the large volume of experimental literature cited in the NIOSH report frequently suffers from a common qualitative handicap: unknown or very high dosages and noninhalatory routes of administration.

In the previous reports (Tansy *et al.*, 1976, 1979) we summarized the results of body and tissue weights, blood chemistries, gross metabolic performance, and intestinal transit in rats which had received relatively long-term exposures to concentrations of 116 ppm of MMA vapor in air. This report proposes to document the results of histopathological observations from this aforementioned exposure and to summarize the results of other studies which were conducted using other species and higher concentrations of MMA vapor.

MATERIALS AND METHODS

In all cases, young adult male Sprague–Dawley rats were received, evaluated, equilibrated, and randomized as previously described (Tansy *et al.*, 1976). Gas generation, gas exposure, and gas dosimetry were conducted with the same configurations and instrumentation as previously reported (Tansy *et al.*, 1976). Histopathological observations reported for 3- and 6-month exposures to 116 and 400 ppm were made from specimens collected during the course of the experiments described in that report. Coefficients of variation of reported mean exposure concentrations are within 5%.

In a more recent study we employed a group of 19 mature male Sprague–Dawley rats randomly allocated into an exposure group of ten animals and a sham group of nine. These rats received an interrupted exposure or sham exposure for 56 hr over a 7-day period. The experimental group exposure concentration was 1000 ppm. At necropsy, blood chemistries were analyzed, and appropriate visceral organs were removed and preserved for routine histopathological examination by light microscopy.

A separate study employed 60 male Swiss Webster mice weighing 20–25 g. This study proposed to determine whether it could be inferred that exposure to MMA vapor was significantly associated with changes in hepatic function (Lawrence and Autian, 1972). Two experimental groups received intermittent daily exposures to either 100 or 400 ppm of MMA vapor in air for total exposure times of 160 hr for each group. A third group of mice were sham exposed to air on an identical schedule. Twenty-four hours after the last exposure or sham exposure, each mouse received an intraperitoneal dosage of 50 mg/kg sodium pentobarbital. In each case, following injection, a stopwatch was used to measure the times of the loss and subsequent return of the righting reflex. These measurements indicate induction and sleeping times, respectively.

To test the functional significance of MMA exposure of the respiratory epithelium we used a modified version of a classical student laboratory technique which demonstrates propulsion of particulates by ciliated epithelium. Thirty-eight adult northern grass frogs were used in these studies. Twenty were sham exposed, eight were exposed to 116 ppm of MMA in air, and ten were exposed to 400 ppm of MMA in air. Interrupted MMA exposures and sham exposures were conducted for a total of 61 hr over a 7-day period. Frogs were used one day following the date of last exposure or sham exposure.

In all cases the following procedures were used. Four reference pins were inserted in a cork board and wired together to form two parallel boundaries 4 mm apart (Fig. 1). Frogs were doubly pithed, the lower jaws excised, and then placed on their dorsal surface and slid under the parallel wires on the cork board. Each frog could be positioned in the same fashion under the pair of reference boundaries. Mucosal ciliary function was estimated by measuring the transit times between boundaries of 0.25-mm-diameter microspheres with a stopwatch. At least four consecutive trials were made on each frog at intervals of 60 sec. Performance for each frog was for each trial. Mean values for each trial of each exposure group were statistically compared to those of corresponding trials to the sham group by means of a two-tailed Student's t test. The null hypothesis was rejected at the 5% level of confidence.

RESULTS

The average blood chemistries of the rats which received the 56-hr 1000 ppm MMA exposure are summarized in Table 1 along with the corresponding mean values for the sham control group. Six of the 14 observed parameter means were observed to be significantly lower than those of the sham group. These included albumin, glucose, blood urea nitrogen, serum glutamateoxaloacetate transaminase, serum glutamatepyruvate transaminase, and the albumin–glucose ratio.

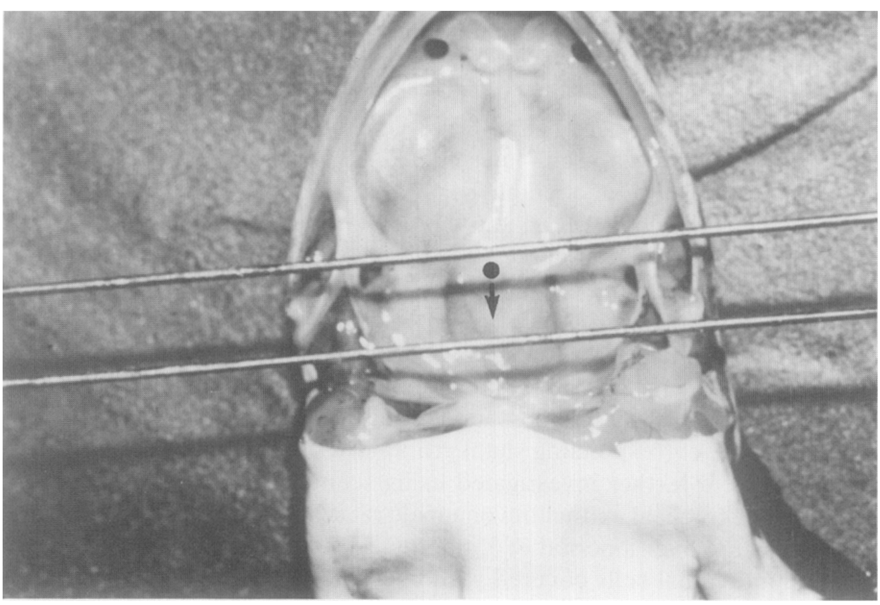


FIG. 1. Photograph depicts the dorsum of the oropharynx of a frog. Two distinct reference wires are shown bracketing the area of interest. The black dot with arrow is a schematic representation of the normal transit direction of a glass microsphere.

TABLE 1

SMA 12/60 BLOOD SERUM ANALYSES AFTER 56 hr OF INTERRUPTED EXPOSURE TO 1000 ppm METHYL METHACRYLATE VAPOR[a]

Test	Sham control group (n = 9)	Experimental group (n = 10)
T.P. (g%)	7.73 ± 0.61	7.23 ± 0.54
Alb. (g%)	2.38 ± 0.45	1.53 ± 0.54*
Ca^{2+} (meq/liter)	5.47 ± 0.36	5.28 ± 0.38
Inor. Phos. (mg%)	7.20 ± 1.25	7.20 ± 0.53
Chol. (mg%)	89.00 ± 16.43	73.80 ± 24.58
Glu. (mg%)	141.22 ± 13.42	120.90 ± 15.03*
BUN (mg%)	19.67 ± 3.39	14.10 ± 2.47*
Uric acid (mg%)	1.60 ± 0.15	1.41 ± 0.28
T. Bili. (mg%)	0.10 ± 0.21	0.03 ± 0.09
Alk. Phos. (units)	453.67 ± 94.32	326.40 ± 175.60
SGOT	302.67 ± 36.14	208.20 ± 46.52*
SGPT	103.33 ± 15.40	54.90 ± 15.62*
LDH	885.33 ± 243.38	815.90 ± 141.53
A/G ratio	0.47 ± 0.12	0.28 ± 0.10*

[a] T.P., total protein; Alb., albumin; Ca^{2+}, total calcium; Inor. Phos., inorganic phosphate; Chol., cholesterol; Glu., glucose; BUN, blood urea nitrogen; T. Bili., total bilirubin; Alk. Phos., alkaline phosphatase; SGOT, serum glutamate–oxaloacetate transaminase; SGPT, serum glutamate-pyruvate transaminase; LDH, lactate dehydrogenase; A/G ratio, albumin/glucose ratio.

* Statistically significant difference when compared with the values of sham control rats ($P \leq 0.05$).

In all three rat groups (116 ppm for 3 and 6 months and 1000 ppm for 56 hr), no animals died, no tumors, growths, or other remarkable abnormalities were observed upon gross postmortem examination, and routine hematoxylin and eosin specimens failed to reveal any remarkable appearances in sections from the heart, kidney, spleen, stomach, small intestine, and adrenals. At the highest dosage there was frank lung damage: The visceral pleura adhered to the parietal pleura; in some cases, fibrosis was seen, lung edema was present, and the parenchyma showed changes suggestive of emphysema. Similar appearances were also seen in some sham animals, albeit of qualitatively lesser degree. Occasional lung damage of the same nature but lesser degree was also seen in the 116 ppm 3- and 6-month exposure groups, but similar observations were also made for some members of the corresponding sham groups.

Histological examination of sections from the upper respiratory tracts of the rats exposed to 116 ppm of MMA for 6 months revealed evidence of damage to the tracheal mucosa in all six of the sampled rats and none in the cases of the six sampled from the corresponding sham group. These changes, small areas of focal hemorrhage, were further investigated using scanning electron microscopy. Figure 2 shows the tracheal epithelium of a male rat who had received a total of 542 hr of sham exposure over a period of 3 months. This epithelium bears a lush growth of cilia and individual cells covered with microvilli are readily apparent. Figure 3 shows the epithelium of a rat which had been chronically exposed for the same period of time to 116 ppm of MMA in air. The epithelium is denuded of cilia, and the cellular covering of microvilli is reduced. This was observable even in specimens from exposed rats which did not exhibit dramatic decreases in apparent ciliary density.

To test the functional significance of MMA exposure of the respiratory epithelium we used a modified version of a classical student laboratory technique. The results are depicted in Table 2. The exposed frogs show a progressive degradation of performance as a function of trial number. Within any trial, average

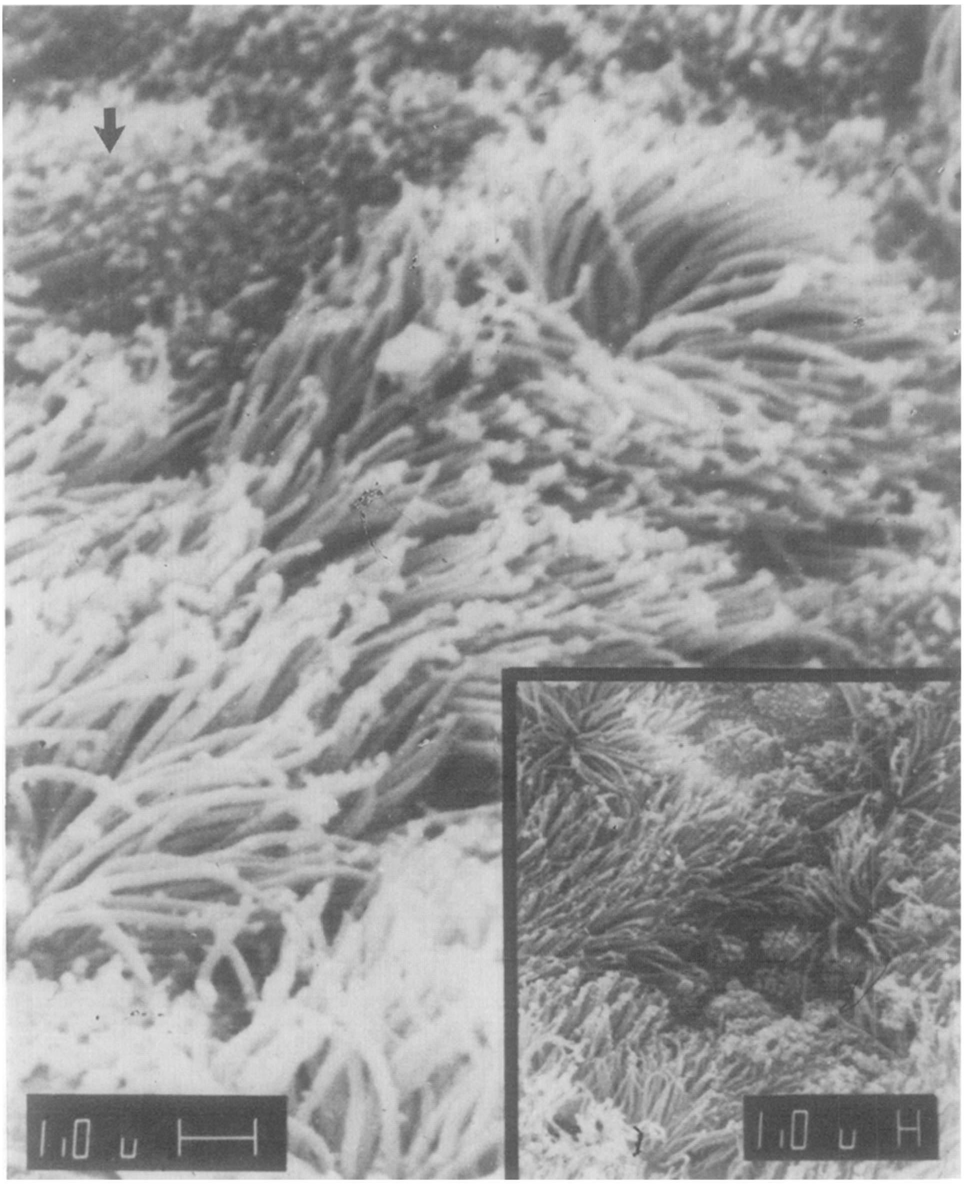


FIG. 2. Scanning electron micrograph of the tracheal epithelium of a sham-exposed rat showing a dense proliferation of cilia. The inset is the region indicated by the arrow at higher magnification showing microvilli on nonciliated cells.

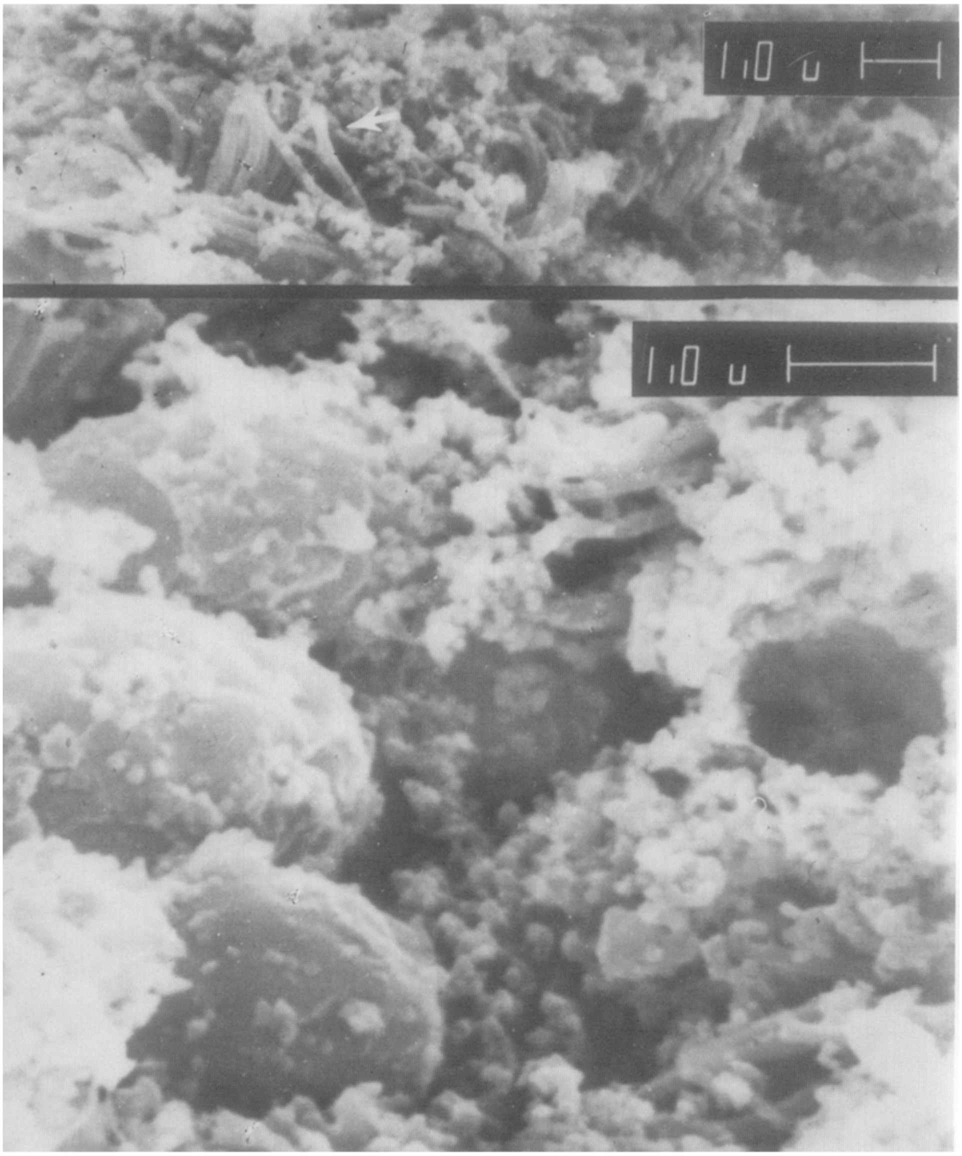
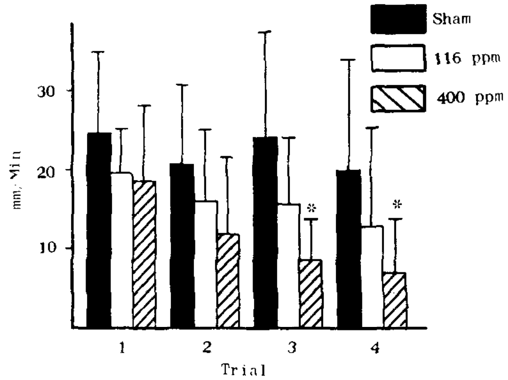


FIG. 3. Scanning electron micrograph of the tracheal epithelium of a rat exposed for 542 hr to 116 ppm of MMA vapor in air showing a general denudation of microvilli and the presence of adherent material. The top panel shows that some cilia (arrow) can be observed and that others seem to be coated with and matted together by an adherent substance.

values were significantly reduced only during the third and fourth trials of frogs from the 400-ppm exposure group. At the conclusion of these experiments, these mucosae were prepared for examination by SEM. Although not depicted, denuded areas were readily apparent in the 400-ppm exposed group. In the absence of heavy ciliary overgrowth, it became apparent that the density of cellular microvilli was also lower in the case of the group receiving the higher exposure. The

TABLE 2

GROUP MEAN TRANSIT RATE OF GLASS MICROSPHERES IN THE ORO-PHARYNGEAL CAVITY OF PITHED FROGS IN SHAM ANIMALS AND ANIMALS EXPOSED TO MMA[a]



[a] mm/min, Millimeters per minute transit of microspheres ± standard deviation.
* Indicates significant change in transit.

lack of ciliary denudation observed in frogs exposed to the 116-ppm dose agrees with our failure to observe degradation in the propulsive function tests. The SEM observations were made in the same frogs which provided the propulsion data.

The liver also showed changes suggestive of damage. Unlike the lungs, these changes appeared to be subtle and apparently unrelated to dose or time of exposure inasmuch as they were observed in liver sections from both rats and mice at all gas concentrations employed. These changes varied from occasional small focal necrosis, changes in the texture of the cytoplasm, swelling of individual cells, and loss of cord definition (Fig. 4). Infrequently rat liver surfaces presented a mottled appearance. Similar changes (including mottling) were occasionally seen in liver specimens from sham-exposed animals. Such changes were neither quantifiable nor consistently observed. We tested liver function in Swiss Webster mice to determine whether it could be inferred that mean sleeping times of mice intermittently exposed for 160 hr to 100 and 400 ppm of MMA vapor were significantly different than the mean sleeping time of a sham-exposed group.

As can be seen in Table 3, the mean induction time was significantly shorter for the mice exposed to 100 ppm of MMA and the mean sleeping time was significantly decreased for the 400-ppm exposed mice. At the present time we believe that the induction time effect may possibly be an effect of chance alone inasmuch as the average group values do not appear to be dose-related. While not statistically significant at 100 ppm, the sleeping times appear to be somewhat dose-related and thus may represent the manifestation of an effect upon the liver (such as increased synthesis of a particular enzyme) which is associated with chronic exposure to MMA vapor. Once again, occasional hematoxylin–eosin-stained mouse liver slides showed small areas of focal necrosis. Nevertheless, the literature indicates that significant increases in average sleeping times are associated with histologically apparent massive liver damage associated with various toxic agents.

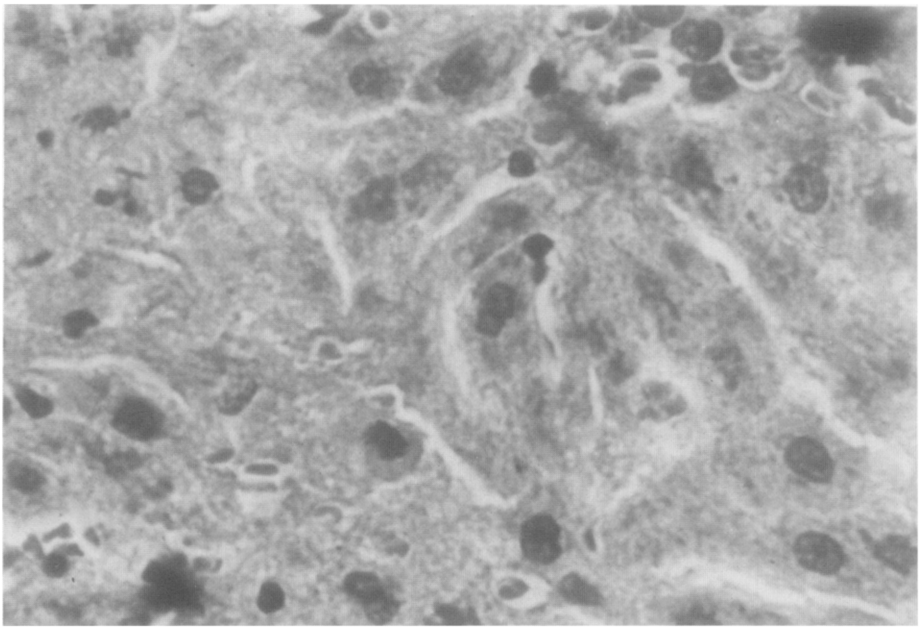


FIG. 4. Hematoxylin–eosin stained section from the liver of a rat exposed for 542 hr to 116 ppm of MMA vapor in air. Note the loss of definition of cellular structure. ×344.

DISCUSSION

The lack of any remarkable gross or microscopic pathology at all dose levels in the cases of the heart, gastrointestinal tract, spleen, and adrenals agrees with the negative findings of four other investigators who used large doses by other routes of administration (Deichmann, 1941; Spealman *et al.*, 1945; Borzelleca *et al.*, 1964; Holland *et al.*, 1973).

Our previous experiments with other stressors has indicated that in the cases of all but profound and dramatic effects, the laboratory rat is not an ideal species to use to assess subtle changes by means of histopathological studies of the lung parenchyma or by means of routine studies of the formed elements of the peripheral blood. In both cases, the biological noise is too great.

We regard the histopathological changes observed in the livers to be equivocal. They were observed by four different groups of pathologists representing govern-

TABLE 3

SLEEPING TIME IN MICE AFTER 160 hr MMA INHALATION EXPOSURE[a]

Parameter	Sham control	MMA Concentration (ppm) 100	400
Body weight (G)	31.94 ± 5.65 (17)	31.73 ± 4.26 (22)	32.65 ± 4.23 (20)
Induction time (min)	3.58 ± 0.75 (17)	3.06 ± 0.41 (22)*	3.73 ± 0.86 (20)
Sleeping time (min)	50.50 ± 14.43 (17)	45.35 ± 16.41 (22)	34.41 ± 12.07 (20)*

[a] Values are reported as means ± standard deviation.

* Indicates significant change in parameter ($P < 0.05$).

ment, private industry, and academic institutions. And they were observed in both exposed and sham-exposed animals. We regard the decreases in sleeping time to be consistent with possible altered liver function, not of a type which would be expected of that massive and indiscriminate liver damage which histopathological examination showed did not in fact occur.

We also regard the blood chemistry data to be inconclusive because most values, even when significantly different from corresponding mean values for sham groups, still fall close to or within normal ranges for the Sprague–Dawley rat.

A TLV is set on the basis of criteria above and beyond that associated with narrow adherence to a dose–response curve for "the most sensitive effect," but in most instances no one knows what the most sensitive effect is because of observational limitations. Most common observables, for instance blood chemistries, oxygen consumption rate of a liver slice, *ad infinitum*, are really average responses of multicellular organs, complex feedback-regulated metabolic pathways at the cellular level, and not infrequently controlled processes involving several organs and tissues. Average responses yield no clue as to whether 100% of an observed response was produced by 27% of the cells of a given organ or whether 100% of an unobserved response was produced by 1% of the cells.

But, if an alteration of liver function in favor of decreased sleeping time is subsequently confirmed, such confirmation will provide some reason to suspect that either MMA, a metabolite, or a substance related to them produces an effect at the level of the genome of liver cells in the mouse.

The hematoxylin and eosin observations of focal lesions of the tracheas of rats and frogs and the SEM views of the cells of both species suggest that an irritative process has occurred. We do not know whether the absence of cilia and, particularly microvilli, is necessarily a manifestation of cellular damage or whether it is an equally likely manifestation of cellular immaturity resulting from a proliferative response. Thus, if the objective is to examine the adequacy of a TLV which has been set with due regard to known pathological data and common sense, then the places to begin are those areas where cellular responses seem likely.

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