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# SUBCHRONIC INHALATION TOXICITY OF 1,2,3-TRIMETHYLBENZENE (HEMIMELLITENE) IN RATS

ZOFIA KORSAK[1], JAN STETKIEWICZ[2], WANDA MAJCHEREK[1], IRENA STETKIEWICZ[2], JOLANTA JAJTE[3] and KONRAD RYDZYŃSKI[1]

[1] Department of Toxicology and Carcinogenesis
[2] Department of Pathomorphology
[3] Department of Physical Hazards
The Nofer Institute of Occupational Medicine
Łódź, Poland

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**Abstract.** Toxic effects of exposure to 1,2,3-trimethylbenzene (hemimellitene) in the condition of subchronic inhalation experiment were examined. Rats were exposed to vapours of hemimellitene at concentrations of 123 mg/m$^3$, 492 mg/m$^3$ and 1230 mg/m$^3$, 6 h/day, 5 days/week for 3 months. After termination of a 3-month inhalation, animals were necropsied. Blood samples were obtained and selected organs were weighed and prepared for histological examinations.
Subchronic inhalation exposure to hemimellitene resulted in an overall, low systemic toxicity. There were no changes in body weight gain and food consumption. At a concentration of 1230 mg/m$^3$, the increase in relative liver weight was observed in male rats. It was accompanied by slight increase in sorbitol dehydrogenase activity. The increase in alkaline phosphatase activity was found in females only. Some disturbances in haematological parameters, characterised by the decrease in red blood cells and slight increase in white blood cells, segmented neutrophils and limphocytes were observed in rats at high exposure concentration of 1230 mg/m$^3$. The pulmonary lesions as well as the increased number of goblet cells and interstitial lung parenchyma infiltration were noted in male and female rats from the highest exposure groups.

## INTRODUCTION

Hemimellitene (1,2,3-trimethylbenzene, CAS No. 526-73-6) one of trimethylbenzene isomers is produced mostly during catalytic reforming of petroleum. It also enters the composition of many commonly used commercial solvent mixtures like Solvesso 100 (Exxon Chemical, Belgium), Shellsol A (Shell Netherland Chemie B.V.), Jolasol (J.L.C. Chemie, Austria) and Farbasol (Polifarb-Cieszyn S.A., Poland).

Address reprint requests to dr Z. Korsak, Department of Toxicology and Carcinogenesis, The Nofer Institute of Occupational Medicine, P.O. Box 199, 90-950 Łódź, Poland.

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For example, in Farbasol trimethylbenzene isomers (pseudocumene, mesitylene, hemimellitene) constitute 44% of the whole mixture (21). They are present in gasoline and white spirit, and are used in paint and varnish formulations, paint thinners, printing inks and pesticide formulations. They also serve as intermediates in the dye and plastic industries.

A large number of studies on the neurotoxic and irritating effects of the exposure to different methyl (toluene) and dimethylbenzene (xylenes) derivatives have been reported (8,9,13). However, toxicological data on trimethylbenzenes are scarce. The ACGIH TLV-TWA concentration of 25 ppm (123 mg/m$^3$) (1), and the Polish MAC value of 100 mg/m$^3$ (ca. 20 ppm) (17) for trimethylbenzene isomers have been established using incomplete, unreliable and obsolete data (4,5).

The impairment symptoms and signs of the respiratory and nervous systems were observed in 27 industrial workers exposed for a number of years to a paint thinner containing alkylbenzenes (pseudocumene – 50%, mesitylene – 30%; hemimellitene, 1-methyl-2-ethylbenzene and 1-methyl-4-ethylbenzene: percentage not specified). The hematopoietic effects were also observed, but they were probably due to benzene contaminations of the thinner. The levels of hydrocarbon vapours in the atmosphere ranged between 49 mg/m$^3$ and 295 mg/m$^3$ (10-60 ppm) (4,5).

In acute inhalation exposure, hemimellitene caused concentration-dependent irritative effects in mice, and neurotoxic effects in rats stronger than those of pseudocumene and mesitylene (15,16). The established hemimellitene concentration of 2662 mg/m$^3$ (16), depressing the respiratory rate in mice to 50% ($RD_{50}$) may indicate the MAC value lower than that binding at present (17).

Bearing in mind three very active methyl groups in trimethylbenzene isomer molecules, the extensive use of hemimellitene, its stronger neurotoxic and irritative effects in condition of acute inhalation exposure, and limited data on its toxicity, the studies were undertaken to evaluate the subchronic toxic effects of hemimellitene in rats.

## MATERIALS AND METHODS

### Chemicals

Hemimellitene (1,2,3-trimethylbenzene) purum > 97% was supplied by Fluka.

### Animals

Male and female outbred Imp:WISTAR rats from the breeding colony of The Nofer Institute of Occupational Medicine were used.

They were identified by the ear number and allowed to acclimatise to laboratory conditions for two weeks prior to initiation of the study. Test animals were 2.5–3 months old at the start of inhalation. The mean initial body weight was 290±25 g and 215±13 g, for male and female rats, respectively. They were housed in polypropylene cages with wire-mesh covers, five animals in each, and maintained under 12 h light/12 h dark cycle, lighting on from 6 a.m. to 6 p.m. Food (Murigran pelleted rodent chow, Fodder Plant, Motycz, Poland) and water were available *ad libitum* in their home cages. Relative humidity and temperature were maintained at 50–65% and 20.5–22.5°C, respectively.

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Animals were randomised and assigned to the experimental groups. Each of the four experimental groups was composed of 10 male and 10 female rats except for the one group with high exposure concentration (20 males and 20 females). The control group was sham exposed, and experimental animals were exposed to hemimellitene at concentrations of 123 mg/m$^3$ = 25 ppm (low concentration), 492 mg/m$^3$ = 100 ppm (mid concentration), and 1230 mg/m$^3$ = 250 ppm (high concentration). Ten male and female rats exposed to hemimellitene at a concentration of 1230 mg/m$^3$ were observed and examined during one additional month after termination of exposure. All rats were deprived of food and water during 6 h exposure periods. Animals were exposed 6 h/day, 5 days/week for 3 months, and observed twice a day for overt signs of toxicity. Body weight was recorded prior to the first inhalation exposure and weekly thereafter during the test period. Food consumption was measured weekly.

**Inhalation exposure.** Animals were exposed to vapours of hemimellitene in a dynamic inhalation chamber (1.3 m$^3$ volume); 16 air changes per hour. Vapours of hemimellitene were generated by heating liquid solvent in washers. The desired concentrations of vapours were obtained by diluting them in the air. Concentrations of solvent vapours in the exposure chamber were measured every 30 min by a Hewlett-Packard gas chromatograph with a flame ionisation detector, using 40 m capilary HP-5 column (0.53 mm · 0.33 $\mu$m) at 100°C column temperature.

**Haematological** parameters in the tail blood were evaluated prior to the beginning of the study and 1 week before termination of the experiment. Erythrocyte, leucocyte and platelet counts, as well as hemoglobin concentration, hematocrit and clotting time were determined in the animal control and exposure groups.

**Clinical pathology determinations.** Clinical laboratory studies were conducted 18h after termination of a 3-month inhalation exposure. Animals were deprived of food 24 h prior to the experiment, then anaesthetised in light ether anaesthesia, exsanguined from the femoral artery and vein, and subjected to gross necropsy. Blood samples were collected for serum chemistry determinations. These were made for aspartate aminotransferase (AspAT), alanine aminotransferase (ALAT), alkaline phosphatase (ALP), sorbitol dehydrogenase (SDH), gamma glutamyltransferase (GGT), bilirubin, total cholesterol, glucose, total protein, albumin, creatinine, urea and electrolytes, calcium, phosphorous, sodium, potassium, chloride.

Beckman Clinical System 700 autoanalyser, Ciba-Corning 614 analyser and standard test combination kits were used for determinations.

**Histopathology.** The necropsy was performed in all animals. Organ weights were obtained for the lungs, liver, spleen, kidneys, adrenals, heart and gonads (ovaries, testes). For the histopathological investigation the organs were preserved in neutral formalin. The lungs were perfused with 10% neutral buffered formalin via trachea at a pressure of 20 cm $H_2O$. The tissue were embedded in paraffin-wax, sectioned at 5 $\mu$m, and stained with hematoxylin and eosin.

The following organs were examined histopathologically: brain, nose, larynx, trachea, thymus, lungs, heart, liver, spleen, kidney, adrenals, thyroid gland, pancreas, gonads, urinary bladder, stomach, duodenum, small and large intestines, and salivary glands. The changes in the lungs reflecting the degree of proliferation of peribronchial lymphatic tissue, lymphoepitelium in bronchi mucosa, interstitial lymphocytic infiltration, macrophage infiltration and inflamatory processes, were graded using an arbitrary scale of 0–3 or 0–4 (0 – normal status, 1 – minimal, 2 – mild, 3 – moderate, and 4 – marked) (19).

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### Statistical analysis

Except for the body weight data, other parameters were compared statistically with those of the control group using Bartlett's test of homogeneity of variance to find out whether the groups had equivalent variances at $p < 0.01$ (22). If the variances were not significantly different, the groups were compared using a standard one-way analysis of variance (ANOVA) (23). In the case of significant differences, Dunnett's test was used to determine which of the groups differed fom controls (23). If the groups had no equivalent variances at $p < 0.01$, then the Kruskal-Wallis test was used to assess differences in group means. If the means were different, Dunn's summed rank test was used to determine which of the groups differed significantly from controls (11). Body weight data for each sex were analysed by one-way classification analysis of covariance (ANCOVA), using pre-exposure (day 0) weights as the covariate. Graduated morphological changes in the lungs were analysed by the Kruskal-Wallis test with Dunn's modification (7). The trend analysis was used to evaluate the relation between the degree of changes in haematological, clinical chemistry and morphological parameters in the lungs, and the exposure level (14).

## RESULTS

During a 3-month exposure, mean concentrations of hemimellitene vapours were: $128 \pm 10$ mg/m$^3$, $523 \pm 38$ mg/m$^3$, $1269 \pm 36$ mg/m$^3$. There were no deaths during the course of the study. Clinical observations were of no toxicological relevance.

When compared with controls, no significant differences in body weight gain were observed in rats exposed to hemimellitene (data not shown). A pattern of statistically significant differences in food consumption throughout the study was not apparent for either sex (data not shown).

After 3 months of exposure to hemimellitene at a concentration of 1230 mg/m$^3$, significant increase in relative liver weight was found in male rats. As to other organs no difference between exposed and control animals was revealed (Table 1).

There were some statistically significant differences in mean haematological values between the exposed and control groups (Table 2). They included a decrease in red blood cell counts in male rats of highly exposed group, a decrease of segmented neutrophil counts, an increase in lymphocyte counts in rats of both sexes in highly exposed groups, and an increase in reticulocyte counts in highly exposed males and in females of all exposure groups. In animals of parallel high exposure groups, one month after termination of exposure, the haematological parameters were comparable to those of controls.

Among various clinical chemistry measurements, only statistically significant increase in sorbitol dehydrogenase in males from highly exposed group, and an increase in alkaline phosphatase in females exposed to mild and high concentrations were observed (Table 3).

Microscopic examinations of the upper respiratory (mucosa of nose and trachea) and urinary tracts, the digestive system, spleen, endocrine glands, and gonads of rats did not reveal significant changes related to hemimellitene exposure at concentrations of 123, 492, 1230 mg/m$^3$.

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**Table 1.** Effect of a 3-month exposure to hemimellitene on organs and terminal body weight

| Parametrs | Control (n = 10) | Hemimellitene (mg/m³) 123 (n = 10) | 492 (n = 10) | 1230 (n = 10) | Jonckheere's trend test |
|---|---|---|---|---|---|
| **Males** | | | | | |
| Terminal body weight (g) | 390±35 | 408±50 | 404±33 | 413±46 | |
| Absolute organ weight (g) | | | | | |
| Lungs | 1.90±0.22 | 1.86±0.26 | 1.99±0.37 | 1.88±0.34 | p = 0.4327 |
| Liver | 8.28±0.97 | 8.83±1.40 | 9.05±0.99 | 9.54±1.50 | p = 0.0284 |
| Spleen | 0.71±0.06 | 0.72±0.10 | 0.82±0.11 | 0.79±0.20 | p = 0.0318 |
| Kidney | 2.34±0.27 | 2.29±0.23 | 2.48±0.25 | 2.50±0.25 | p = 0.0616 |
| Adrenals | 0.059±0.012 | 0.061±0.016 | 0.061±0.013 | 0.061±0.012 | p = 0.3336 |
| Testes | 3.78±0.44 | 3.69±0.24 | 3.71±0.36 | 3.91±0.12 | p = 0.1398 |
| Heart | 1.04±0.13 | 0.98±0.11 | 1.08±0.13 | 1.15±0.19 | p = 0.0231 |
| Relative organ weight (g) | | | | | |
| Lungs | 0.510±0.071 | 0.479±0.026 | 0.504±0.082 | 0.468±0.073 | p = 0.1257 |
| Liver | 2.208±0.163 | 2.271±0.129 | 2.287±0.115 | 2.414*±0.214 | p = 0.0117 |
| Spleen | 0.190±0.019 | 0.187±0.015 | 0.207±0.021 | 0.203±0.058 | p = 0.3402 |
| Kidney | 0.623±0.049 | 0.594±0.029 | 0.629±0.033 | 0.637±0.060 | p = 0.1801 |
| Adrenals | 0.016±0.003 | 0.016±0.003 | 0.015±0.003 | 0.016±0.003 | p = 0.4174 |
| Testes | 1.014±0.087 | 0.961±0.091 | 0.941±0.063 | 1.002±0.106 | p = 0.3103 |
| Heart | 0.277±0.027 | 0.252±0.018 | 0.274±0.032 | 0.284±0.026 | p = 0.1707 |
| **Females** | | | | | |
| Terminal body weight (g) | 268±18 | 262±21 | 263±14 | 259±23 | |
| Absolute organ weight (g) | | | | | |
| Lungs | 1.62±0.15 | 1.55±0.33 | 1.47±0.18 | 1.51±0.16 | p = 0.1378 |
| Liver | 6.05±0.42 | 5.85±0.47 | 5.94±0.51 | 6.05±0.44 | p = 0.4205 |
| Spleen | 0.63±0.05 | 0.61±0.10 | 0.57*±0.05 | 0.56*±0.06 | p = 0.0042 |
| Kidney | 1.58±0.16 | 1.53±0.12 | 1.54±0.10 | 1.62±0.16 | p = 0.3628 |
| Adrenals | 0.080±0.014 | 0.082±0.010 | 0.083±0.011 | 0.075±0.015 | p = 0.3125 |
| Ovaries | 0.12±0.03 | 0.12±0.03 | 0.13±0.02 | 0.14±0.04 | p = 0.4205 |
| Heart | 0.74±0.05 | 0.71±0.50 | 0.75±0.06 | 0.73±0.08 | p = 0.4402 |
| Relative organ weight (g) | | | | | |
| Lungs | 0.651±0.053 | 0.637±0.122 | 0.604±0.049 | 0.639±0.076 | p = 0.2533 |
| Liver | 2.434±0.143 | 2.400±0.088 | 2.448±0.190 | 2.555±0.214 | p = 0.0530 |
| Spleen | 0.257±0.027 | 0.249±0.032 | 0.234±0.019 | 0.237±0.022 | p = 0.4506 |
| Kidney | 0.639±0.076 | 0.628±0.024 | 0.638±0.032 | 0.686±0.058 | p = 0.0490 |
| Adrenals | 0.032±0.005 | 0.034±0.004 | 0.034±0.005 | 0.032±0.008 | p = 0.4850 |
| Ovaries | 0.051±0.014 | 0.050±0.014 | 0.056±0.006 | 0.060±0.018 | p = 0.1169 |
| Heart | 0.298±0.016 | 0.291±0.012 | 0.309±0.024 | 0.307±0.026 | p = 0.1736 |

* Mean±SD.
* Significantly different from controls ($p < 0.05$).

In comparison with controls some changes were noted in the respiratory system of rats exposed to hemimellitene (Table 4). In the bronchi of female rats exposed to mid and high concentrations of hemimellitene an increased number of goblet cells was noted (Table 4). The trend analysis revealed that the number of goblet cells in female rats was related to the concentration of hemimellitene ($p = 0.001$). The significant increase in the intensity of lung perivascular and interstitial

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Table 2. Effect of a 3-month exposure to hemimellitene on haematological parameters

| Parametrs | Control | Hemimellitene (g/m³) | | | | Jonckheere's trend test |
|---|---|---|---|---|---|---|
| | | 123 | 492 | 1230 | 1230[a] | |
| **Hematocrit** (%) | | | | | | |
| Male | 46.4±1.6 | 45.8±2.6 | 45.7±1.3 | 45.5±2.1 | 43.5±2.6 | P = 0.1615 |
| Female | 42.7±2.2 | 45.0±2.4 | 41.8±1.6 | 41.5±2.4 | 41.7±2.0 | P = 0.0198 |
| **Hemoglobin** (g/dl) | | | | | | |
| Male | 16.4±1.0 | 17.6±1.6 | 17.6±0.8 | 15.0±1.2 | ND | P = 0.0688 |
| Female | 13.9±0.7 | 15.1±1.0* | 14.6±0.6 | 14.7±0.9 | ND | P = 0.0748 |
| **Red blood cells** ($\times 10^6$/mm³) | | | | | | |
| Male | 9.49±2.03 | 10.25±1.29 | 10.11±1.27 | 8.05±1.38* | 8.6±1.5 | P = 0.0011 |
| Female | 8.03±1.11 | 8.73±1.24 | 7.79±1.57 | 7.27±1.32 | 6.6±1.8 | P = 0.0185 |
| **White blood cells** ($\times 10^3$/mm³) | | | | | | |
| Male | 10.09±2.23 | 9.38±3.29 | 7.71±3.45 | 9.03±2.75 | 6.3±4.6 | P = 0.1661 |
| Female | 10.71±4.28 | 9.54±2.37 | 13.02±3.07 | 13.01±4.53 | 6.2±2.5 | P = 0.0189 |
| **Rod neutrophil** (%) | | | | | | |
| Male | 0.8±1.0 | 1.0±1.1 | 0.4±0.5 | 0.5±0.6 | 5.2±3.0 | P = 0.1878 |
| Female | 0.4±0.8 | 0.6±0.6 | 1.1±1.4 | 0.4±0.8 | 1.8±2.2 | P = 0.4711 |
| **Segmented neutrophil** (%) | | | | | | |
| Male | 24.8±4.5 | 25.4±5.8 | 20.7±5.8 | 17.7±8.3* | 27.5±9.2 | P = 0.0032 |
| Female | 23.1±6.1 | 19.7±3.4 | 16.4±4.2* | 11.9±7.1** | 19.6±8.3 | P = 0.0000 |
| **Eosinophil** (%) | | | | | | |
| Male | 1.3±1.4 | 0.8±1.0 | 0.8±1.1 | 0.6±0.8 | 0.6±0.6 | P = 0.1439 |
| Female | 1.4±1.0 | 0.6±0.6 | 0.7±0.8 | 0.8±0.9 | 0.7±0.8 | P = 0.2778 |
| **Lymphocyte** (%) | | | | | | |
| Male | 71.2±5.0 | 71.6±6.8 | 75.4±4.7 | 79.3±78.0** | 63.7±11.3 | P = 0.0015 |
| Female | 73.2±7.9 | 77.5±4.9 | 80.4±5.1 | 84.0±78.0** | 75.7±9.9 | P = 0.0003 |
| **Monocyte** (%) | | | | | | |
| Male | 1.9±1.6 | 1.3±1.4 | 2.3±2.0 | 1.6±2.2 | 3.1±3.7 | P = 0.3014 |
| Female | 2.0±2.0 | 1.6±1.6 | 1.1±1.3 | 2.1±1.7 | 1.3±1.8 | P = 0.2426 |
| **Lymphoblast** (%) | | | | | | |
| Male | 0.0±0.0 | 0.0±0.0 | 0.2±0.6 | 0.2±0.6 | 0.0±0.0 | P = 0.2911 |
| Female | 0.0±0.0 | 0.0±0.0 | 0.1±0.3 | 0.3±0.7 | 0.0±0.0 | P = 0.1403 |
| **Myelocyte** (%) | | | | | | |
| Male | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | P = 0.5000 |
| Female | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.5±0.2 | 0.0±0.0 | P = 0.3963 |
| **Erytroblast** (%) | | | | | | |
| Male | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | P = 0.5000 |
| Female | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.1±0.3 | 0.0±0.0 | P = 0.2995 |
| **Reticulocyte** (%) | | | | | | |
| Male | 2.8±1.3 | 2.1±1.7 | 3.8±2.1 | 4.5±1.8* | 6.9±3.1** | P = 0.0017 |
| Female | 2.6±0.9 | 4.6±2.5* | 5.2±.5** | 4.4±3.0 | 6.8±3.5** | P = 0.0459 |
| **Blood platelet** ($\times 10^3$/mm³) | | | | | | |
| Male | 262±51 | 266±70 | 257±81 | 242±76 | 277±80 | P = 0.1708 |
| Female | 224±68 | 290±70 | 249±53 | 204±44 | 258±45 | P = 0.0329 |
| **Clotting time** (s) | | | | | | |
| Male | 29.7±8.6 | 23.0±10.0 | 37.9±9.9 | 29.2±15.6 | 21.7±5.4 | P = 0.4650 |
| Female | 27.2±2.8 | 25.0±9.4 | 23.8±9.5 | 25.1±12.1 | 25.9±8.0 | P = 0.3479 |

[a] Parallel group, 14 days after termination of exposure. Mean values±SD for 10 rats.
Statistically significant difference as compared to controls * $p < 0.05$, ** $p < 0.01$.
ND – not determined.

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Table 3. Effect of a 3-month exposure to hemimellitene on clinical chemistry values

| Parametrs | Control | Hemimellitene (mg/m³) | | | Jonckheere's trend test |
|---|---|---|---|---|---|
| | | 123 | 492 | 1230 | |
| **Aspartate aminotransferase (U/dl)** | | | | | |
| Male | 107.8±14.2 | 102.9±15.1 | 103.6±14.5 | 119.6±27.3 | P = 0.2223 |
| Female | 96.1±9.4 | 96.9±9.9 | 117.1±23.9 | 104.6±15.7 | P = 0.2118 |
| **Alanine aminotransferase (U/dl)** | | | | | |
| Male | 41.3±2.0 | 40.7±3.1 | 41.5±5.5 | 45.5±5.6 | P = 0.0637 |
| Female | 39.7±3.5 | 39.5±6.4 | 36.2±3.3 | 30.5±9.9* | P = 0.1844 |
| **Alkaline phosphatase (U/dl)** | | | | | |
| Male | 70.5±15.2 | 70.6±11.7 | 66.5±10.8 | 63.7±15.7 | P = 0.1518 |
| Female | 21.5±2.7 | 25.8±8.4 | 31.1±8.6* | 30.5±9.9* | P = 0.1740 |
| **Sorbitol dehydrogenase (U/dl)** | | | | | |
| Male | 1.6±0.7 | 2.3±1.3 | 2.5±0.9 | 2.7±0.7* | P = 0.0083 |
| Female | 1.7±0.7 | 1.9±0.9 | 1.5±0.7 | 1.8±1.0 | P = 0.0637 |
| **γ-glutamyltransferase (U/ml)** | | | | | |
| Male | 0.77±0.66 | 0.77±0.97 | 0.40±0.51 | 0.50±0.75 | P = 0.4700 |
| Female | 0.55±0.72 | 0.44±1.01 | 0.66±1.11 | 0.30±0.48 | P = 0.2821 |
| **Bilirubin (mg/dl)** | | | | | |
| Male | 0.600±0.516 | 0.600±0.516 | 0.800±0.422 | 0.625±0.518 | P = 0.2594 |
| Female | 0.911±0.348 | 1.161±0.469 | 0.930±0.463 | 0.976±0.421 | P = 0.3092 |
| **Total cholesterol (mg/dl)** | | | | | |
| Male | 63.1±10.1 | 62.2±11.6 | 64.5±16.2 | 65.0±9.1 | P = 0.0920 |
| Female | 60.1±12.2 | 62.4±15.3 | 62.3±7.7 | 64.4±14.1 | P = 0.4775 |
| **Glucose (mg/dl)** | | | | | |
| Male | 95.5±13.1 | 110.8±14.7 | 100.2±15.2 | 114.5±20.6 | P = 0.0876 |
| Female | 115.9±8.5 | 121.0±17.5 | 109.2±5.8 | 109.8±10.8 | P = 0.4838 |
| **Total protein (g)** | | | | | |
| Male | 7.84±0.13 | 8.02±0.50 | 7.76±0.27 | 8.04±0.59 | P = 0.3242 |
| Female | 8.24±1.24 | 8.36±1.14 | 8.65±0.84 | 8.62±0.96 | P = 0.4036 |
| **Albumin (g)** | | | | | |
| Male | 3.15±0.73 | 3.15±1.33 | 3.08±1.30 | 2.95±1.12 | P = 0.2279 |
| Female | 3.22±1.28 | 3.17±1.03 | 2.58±1.28 | 3.60±1.17 | P = 0.2408 |
| **Creatinine (mg/dl)** | | | | | |
| Male | 41.24±8.94 | 41.35±11.28 | 40.79±9.30 | 43.61±13.10 | P = 0.3982 |
| Female | 62.54±10.66 | 61.60±7.07 | 67.11±10.86 | 59.71±7.51 | P = 0.1641 |
| **Urea (mg/dl)** | | | | | |
| Male | 38.7±4.5 | 38.1±9.1 | 36.9±4.1 | 41.7±7.5 | P = 0.1145 |
| Female | 42.0±5.5 | 43.5±4.4 | 40.0±4.3 | 39.0±2.9 | P = 0.4718 |
| **Calcium (mg/dl)** | | | | | |
| Male | 10.6±0.6 | 10.7±0.8 | 10.8±0.7 | 10.9±0.5 | P = 0.2449 |
| Female | 11.1±0.8 | 11.7±0.3 | 11.8±0.2 | 11.8±0.7 | P = 0.3011 |
| **Phosphorous (mg/dl)** | | | | | |
| Male | 8.60±0.95 | 8.26±0.60 | 9.19±0.88 | 9.41±0.55 | P = 0.1580 |
| Female | 6.56±0.70 | 6.25±1.17 | 6.41±1.02 | 7.18±1.09 | P = 0.4050 |
| **Sodium (mmol/l)** | | | | | |
| Male | 143.9±2.1 | 144.1±1.5 | 143.9±2.5 | 144.8±2.4 | P = 0.4950 |
| Female | 144.0±1.5 | 143.8±1.3 | 142.7±1.3 | 143.8±1.4 | P = 0.3628 |
| **Potassium (mmol/l)** | | | | | |
| Male | 4.70±0.35 | 4.45±0.28 | 4.75±0.37 | 4.97±0.56 | P = 0.2907 |
| Female | 4.52±0.41 | 4.51±0.43 | 4.28±0.41 | 4.37±0.34 | P = 0.4108 |
| **Chloride (mmol/l)** | | | | | |
| Male | 107.3±2.3 | 107.7±4.3 | 106.8±1.8 | 106.5±1.9 | P = 0.4353 |
| Female | 108.1±3.2 | 108.1±1.5 | 107.1±1.3 | 107.2±2.3 | P = 0.0601 |

Statistically significant difference as compared to controls * $p < 0.05$, ** $p < 0.01$.

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**Table 4.** Pulmonary lesions observed in male and female rats exposed to hemimellitene at concentrations of 0, 123, 490, 1230 mg/m$^3$ for 3 months

| Changes | | Hemimellitene (mg/m$^3$) | | | | Statistical analysis | |
|---|---|---|---|---|---|---|---|
| | | 0 | 123 | 490 | 1230 | Comparison with control | Jonckheere's trend test |
| | | 1 | 2 | 3 | 4 | | |
| Proliferation of peribronchial Lymphatic tissue (0-3)[a] | M | 2.0[b] (23.4)[c] | 1.2 (11.5) | 1.8 (22.0) | 2.0 (23.5) | 1-2* | J = 0.6; p = 0.2 |
| | F | 2.4 (22.8) | 1.3 (12.1) | 1.5 (16.4) | 1.3 (22.3) | 1-2**; 1-3* | J = –0.7; p = 0.2 |
| Formation of lymphoepithelium in bronchi (0-3) | M | 1.5 (23.9) | 0.9 (14.9) | 1.0 (16.0) | 1.5 (25.7) | 1-3*; 1-4** | J = 0.3; p = 0.3 |
| | F | 1.8 (27.9) | 0.7 (11.1) | 1.1 (16.9) | 1.5 (23.8) | | J = –0.3; p = 0.3 |
| Goblet cells (0-3) | M | 1.8 (18.6) | 1.5 (14.5) | 2.5 (28.5) | 1.8 (18.2) | | J = 0.9; p = 0.18 |
| | F | 1.3 (11.9) | 1.6 (16.9) | 2.0 (23.1) | 2.4 (28.4) | 1-3*; 1-4** | J = 3.7; p = 0.001 |
| Interstitial lymphocytic Infiltrations (0-3) | M | 0.4 (18.0) | 0.1 (14.1) | 0.4 (18.0) | 1.5 (31.0) | 1-4* | J = 2.4; p = 0.006 |
| | F | 1.2 (23.7) | 0.6 (15.3) | 0.8 (17.9) | 1.1 (22.9) | | J = 0.12; p = 0.4 |
| Alveolar macrophages (0-3) | M | 0.9 (17.9) | 0.9 (17.9) | 1.2 (22.6) | 1.2 (21.7) | | J = 1.0; p = 0.15 |
| | F | 1.5 (26.1) | 1.1 (21.1) | 0.5 (17.8) | 0.7 (14.8) | | J = –2.3; p = 0.01 |
| Bronchitis and bronchopneumonia (0-4) | M | 0.5 (20.1) | 0.2 (16.6) | 0.8 (23.8) | 0.7 (19.5) | | J = 0.3; p = 0.3 |
| | F | 0.2 (17.6) | 0.4 (22.5) | 0.2 (17.5) | 0.6 (21.8) | | J = 0.5; p = 0.3 |
| Cumulative score of all Individuals | M | 7.1 (19.8) | 4.8 (11.2) | 7.7 (24.2) | 8.7 (25.8) | | J = 2.13; p = 0.01 |
| | F | 8.4 (24.9) | 5.7 (13.5) | 6.5 (16.8) | 8.2 (24.6) | 1-2* | J = 0.17; p = 0.4 |

[a] Grading system (0-4, 0-3), see the Materials and Methods section.
[b] Mean.
[c] Results were presented as ranges of the Kruskal-Wallis test.
* Significant at $p < 0.05$.
** Significant at $p < 0.01$.

infiltration in male rats exposed to hemimellitene at a concentration of 1230 mg/m$^3$ ($p < 0.01$) was observed. The intensity of interstitial lung infiltration was related to hemimellitene concentrations (trend significance at $p = 0.006$).

In comparison with controls, a decreased amount of peribronchial lymphatic tissue was noted in female rats exposed to hemimellitene at concentrations of 123 and 490 mg/m$^3$. In some rats, the bronchial epithelium with proliferation of peribronchial lymphatic tissue focally lost its cuboidal character forming lymhoepithelium. Lymphoepithelium formation was observed only in male groups exposed to low and mid concentrations of hemimellitene.

Based on the observed increase in the number of goblet cells in bronchi, and interstitial lung parenchyma infiltration, it may be concluded that hemimellitene exerts relatively low toxic effects on the respiratory system at a concentration of 1230 mg/m$^3$.

## DISCUSSION

Subchronic inhalation exposure to hemimellitene resulted in an overall low degree of systemic toxicity. For some parameters measured (body weight and food consumption) the high concentration of 1230 mg/m$^3$ was found to be at the no-observed-effect-level (NOEL) in both sexes.

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In male rats exposed to hemimellitene at a concentration of 1230 mg/m$^3$, a significantly higher relative weight of the liver was observed, whereas in female rats the relative weight of the liver and other organs showed no changes. For the liver relative weight increase, the concentration of 1230 mg/m$^3$ was the lowest-observed-effect-level (LOEL) in male, and NOEL in female rats.

Among the various clinical chemistry parameters measured, a statistically significant increase in sorbitol dehydrogenase activity in male at high concentration, and in alkaline phosphatase activity in female rats at mid and high concentrations were observed.

Sorbitol dehydrogenase activity proved to be the most sensitive biochemical marker of the liver damage also in the case of other hepatotoxic chemical compounds (18), whereas alkaline phosphatase activity is considered to be associated with the non-specific inflammation in the lungs (3,10).

For clinical chemistry parameters like sorbitol dehydrogenase activity, the hemimellitene concentration of 1230 mg/m$^3$ was LOEL in males and NOEL in females, whereas for alkaline phosphatase activity the concentration of 492 mg/m$^3$ was LOEL in females and of 1230 mg/m$^3$ was NOEL in male rats.

The decrease in red blood cell counts observed in male and female rats (at a concentration of 1230 mg/m$^3$, the change was statistically significant in male rats), and the increase in white blood cell counts in females were similar to those observed in mice as a result of exposure to other solvents (6,12). Macrocytic anaemia may be caused by several factors, including deficiences of vitamin $B_{12}$, folic acid, protein, as well as by hepatic diseases or chronic inflamation (20). Thus, the macrocytic anaemia and increased sorbitol dehydrogenase activity in male rats might be due to hepatotoxic hemimellitene effects. However, microscopic examinations did not show any changes in the liver that could be related to hemimellitene exposure.

It is likely that the erythrocyte counts decrease or increase in white blood cells may result from inflammation caused by the exposure to hemimellitene which indicates the microscopical pulmonary lesions, increased alkaline phosphatase activity, and quite strong acute respiratory irritative effect of hemimellitene described earlier (16). Inflammation seems to be a reasonable cause since the increased susceptibility to respiratory bacterial infections in mice exposed to toluene has already been reported (2).

As to haematological parameters, like red blood cells, segmented neutrophils and lymphocytes, the LOEL and NOEL were found for concentrations of 1230 mg/m$^3$ and 492 mg/m$^3$, respectively.

The pathological changes in the lower respiratory system observed microscopically indicated a relatively low toxic activity of hemimellitene at concentrations of 492 mg/m$^3$ and 1230 mg/m$^3$. The hemimellitene concentration of 123 mg/m$^3$ was established as NOEL in male and female rats.

On the basis of our sub-chronic studies performed on rats, it may be concluded that hemimellitene, like other solvents, may affect blood and liver, however they are not primary target tissues of hemimellitene exposure. Similarly to most other solvents, hemimellitene exerts mainly neurotoxic (15) and irritative effects on the respiratory system (16).

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