

## The effects of inhalation anaesthetics on common clinical pathology parameters in laboratory rats

K. Deckardt<sup>a</sup>, I. Weber<sup>a</sup>, U. Kaspers<sup>a</sup>, J. Hellwig<sup>a</sup>, H. Tennekes<sup>b</sup>, B. van Ravenzwaay<sup>a,\*</sup>

<sup>a</sup> BASF Aktiengesellschaft, Experimental Toxicology and Ecology, Z 470, D-67056 Ludwigshafen, Germany

<sup>b</sup> Experimental Toxicology Services (ETS) Nederland BV, Frankensteeg 4, NL-7201 KN Zutphen, The Netherlands

Received 13 February 2006; accepted 7 March 2007

### Abstract

Effects of common anaesthetics such as ether, methoxyflurane, isoflurane, carbon dioxide (at 100%, 80% or 60% admixed with O<sub>2</sub>) on toxicity and clinical pathology parameters in rats were investigated. Ether, methoxyflurane and 100% CO<sub>2</sub> induced toxicity in some animals. Erythrocyte, haemoglobin and haematocrit were reduced in females by 100% CO<sub>2</sub>, methoxyflurane and isoflurane. Glucose was increased by 60% CO<sub>2</sub>, 80% CO<sub>2</sub>, ether, isoflurane and methoxyflurane in males. Chloride was reduced by isoflurane and all CO<sub>2</sub> concentrations in females. Serum proteins were reduced by isoflurane and methoxyflurane. Sodium, inorganic phosphate, calcium and magnesium were reduced by methoxyflurane and isoflurane, but increased by all CO<sub>2</sub> concentrations. Potassium was reduced by ether, methoxyflurane or isoflurane. Triiodothyronine and thyroxine were reduced by all anaesthetics. Prolactin was reduced by methoxyflurane, but raised by ether and isoflurane.

Erythrocyte cholinesterase (E-ChE) activity is markedly reduced (20–40%) after anaesthesia with all CO<sub>2</sub> concentrations in both sexes. E-ChE was unaffected by ether, methoxyflurane, or isoflurane. Serum and brain cholinesterase activities were not affected. E-ChE inhibition correlated with decreased blood pH, suggesting that this was caused by acidosis. This is of practical relevance in the risk assessment of cholinesterase inhibitors.

**Conclusions:** Clinical pathology data were affected by all anaesthetics. CO<sub>2</sub>/O<sub>2</sub> (80%/20%) and isoflurane are the most suitable anaesthetics. If E-ChE activity is to be determined, isoflurane is the anaesthetic of choice.

© 2007 Elsevier Ltd. All rights reserved.

**Keywords:** Haematology; Clinical biochemistry; Hormones; Anaesthesia; Carbon dioxide; Ether; Methoxyflurane; Isoflurane; Erythrocyte cholinesterase

### 1. Introduction

For routine clinical pathology in laboratory rodents, blood samples are commonly collected by puncture of the orbital sinus. This procedure is potentially stressful for the animals and should therefore be conducted under anaesthesia. Inhalation anaesthesia is usually preferred over parenteral anaesthesia, because the former procedure is fast-acting and quickly reversible (Wixson and Smiler, 1997; Thompson et al., 2002). The choice of an inhalation

anaesthetic for clinical pathology sampling in small laboratory animals is driven by its ethical acceptability, safety, speed, cost, and of course its effects on blood parameters. No inhalation anaesthetic appears to be completely satisfactory. Ether is fast-acting and cheap, causes minimal physiological effects, but is irritating to the respiratory tract (Brunson, 1997) and stressful during the induction period (Van Herck et al., 2001). Moreover, if not carefully handled, ether can cause explosions (Horn, 1977). Methoxyflurane is safe and reasonably cheap, but induction is slow and the compound is extensively metabolized to nephrotoxic compounds like inorganic fluoride, oxalates and trifluoroacetic acid (Brunson, 1997; Brunson et al., 1979). Also, methoxyflurane is no longer being manufactured

\* Corresponding author. Tel.: +49 621 605 64 19; fax: +49 621 605 81 34.  
E-mail address: [bennard.ravenzwaay@basf.com](mailto:bennard.ravenzwaay@basf.com) (B. van Ravenzwaay).

and therefore not easily available. Isoflurane anaesthesia is fast and the molecule is stable and, thus, metabolic and toxic effects on liver and kidney are minimal (Brunson, 1997), but a precision vaporizer is required for dosing and the compound is expensive. Carbon dioxide is safe, fast, odourless, cheap and acceptable for rats (Kohler et al., 1999), although 100% CO<sub>2</sub> may produce unacceptable pain and distress or possibly fatal effects (Fenwick and Blackshaw, 1989), so CO<sub>2</sub>/O<sub>2</sub> mixtures are preferable (Urbanski and Kelley, 1991; Danneman et al., 1997). Despite significant information on the anaesthetic effects of the compounds very little is known about their potential effects on clinical pathology parameters. To elucidate these potential effects, essential for the selection of the most suitable anaesthetic for blood sampling procedures, we have examined the effects of various inhalation anaesthetics (ether, methoxyflurane, isoflurane, carbon dioxide 100%, or at 80% or 60% admixed with O<sub>2</sub>) on common clinical pathology parameters. While generating these baseline clinical pathology data, marked inhibitory effects of carbon dioxide anaesthesia on erythrocyte (but not on serum and brain) cholinesterase were discovered. The potential relationship of this effect with blood acidity caused by CO<sub>2</sub> inhalation was also investigated.

## 2. Materials and methods

### 2.1. Test substances

Carbon dioxide (CO<sub>2</sub>) and oxygen (O<sub>2</sub>), 100% pure, were obtained from Messer, Griesheim, Germany. Methoxyflurane (CAS No. 76-38-0), 100% pure, described as “Metofane”, was obtained from Janssen-Cilag GmbH, Neuss, Germany. Diethyl ether (CAS No. 60-29-7), 100% pure, was obtained from Asid Bonz GmbH, Böblingen, Germany. Isoflurane (CAS No. 26675-46-7), 100% pure, described as “IsoFlo<sup>®</sup>vet” was obtained from ESSEX Tierarznei, Munich, Germany.

### 2.2. Animals and maintenance conditions

Male and female Crl:WI (Glx/BRL/HAN) BR rats were supplied by Charles River Deutschland GmbH, Sulzfeld, Germany. For routine clinical pathology assays, the age of the animals was approximately 6 weeks at delivery. For hormone and brain cholinesterase assays, the age of the animals was approximately 12 weeks at delivery. The animals were singly housed in wire cages (type DK III, floor area 800 cm<sup>2</sup>), supplied by Becker & Co., Castrop-Rauxel, Germany. Waste trays containing bedding material (type 3/4 dust free embedding, supplied by Ssniff, Soest, Germany) were fixed underneath the cages. The animals were maintained in an air-conditioned room at a temperature of 20–24 °C, a relative humidity of 30–70%, and a 12 h light/12 h dark cycle. The animals were maintained on ground small rodent maintenance diet (Provimi Kliba SA, Kaiseraugst, Switzerland) and tap water ad libitum. Food was withdrawn overnight prior to blood sampling for clinical pathology assays.

### 2.3. Experimental design

The investigations were approved by the *Landesuntersuchungsamt* (State Research Bureau) of Rheinland-Pfalz, Koblenz, Germany, and conducted in compliance with the German Animal Protection Act (1998) (May 25th, 1998) and Good Laboratory Practice provisions of the German “Chemikaliengesetz” (Chemicals Act; *Bundesgesetzblatt*, 1994, Part

I, July 29, 1994; FR Germany) and the OECD Principles of Good Laboratory Practice (Paris, 1981).

### 2.4. Anaesthesia

For carbon dioxide or carbon dioxide/oxygen anaesthesia, rats were individually placed in a Plexiglas chamber connected via a pressure-reduction valve to gas cylinders containing compressed 100% carbon dioxide (CO<sub>2</sub>) and 100% oxygen (O<sub>2</sub>); the desired CO<sub>2</sub>/O<sub>2</sub> ratio and flow rate (200 L/h) was achieved by adjusting calibrated flow meters to the respective flow rates of CO<sub>2</sub> and O<sub>2</sub> independently by needle valves. The anaesthesia chamber was equilibrated with the appropriate gas mixture before introduction of the animals.

Diethyl ether or methoxyflurane anaesthesia was induced in a covered glass or stainless steel chamber. The liquid anaesthetics were volatilized by placing them on gauze squares in the bottom of the anaesthesia chamber, covered by a stainless steel wire mesh grid which prevented the animal's feet contacting the gauze. The animals were carefully placed in the equilibrated chamber through an opening in its upper wall.

For isoflurane anaesthesia, rats were individually placed in a Plexiglas chamber filled continuously with 4.5% isoflurane in air, generated by a precision vaporizer (Model “Dräger-Vapor<sup>®</sup> 19.n”, Lübeck, Germany) connected with a compressor. The fresh gas flow rate was 60 L/h.

### 2.5. Clinical observations

Overt signs and time to onset of anaesthesia (as indicated by lateral recumbency and muscular hypotonia) were recorded. During the recovery period the behaviour of the rats was observed.

### 2.6. Blood sampling procedures for haematology and clinical biochemistry

The order of anaesthetization and blood sampling on each sample day was randomized according to a computer-generated list. For routine clinical pathology, blood samples (approximately 2 mL) were collected by puncture of the retro-orbital sinus. In order to save animals and to prevent that always the same animals were stressed by anaesthesia, the following procedure of blood collection was performed: Samples were taken from one group of 10 male and 10 female rats at the age of 10 weeks (without anaesthesia), at 12 weeks (with 60% CO<sub>2</sub> anaesthesia), at 14 weeks (without anaesthesia), at 16 weeks (with ether anaesthesia), and at 18 weeks of age (without anaesthesia), and from another group of 10 male and 10 female rats at the age of 10 weeks (with 80% CO<sub>2</sub> anaesthesia), at 12 weeks (without anaesthesia), at 14 weeks (with methoxyflurane anaesthesia), at 16 weeks (without anaesthesia), and at 18 weeks of age (with 100% CO<sub>2</sub> anaesthesia). At the age of 26 weeks, samples were collected from one group of 10 male and 10 female rats without anaesthesia and from another group of 10 male and 10 female rats anaesthetized with isoflurane.

Samples for haematology were mixed with K3-EDTA. Samples for clotting analysis were mixed with sodium citrate. Remaining blood was emptied into serum separation test tubes and processed for clinical chemistry assays. Whole blood for analysis of haemoglobin derivatives was sampled in capillaries coated with heparin sodium directly from the collection site, and an aliquot of the blood was used for erythrocyte preparation to determine cholinesterase activity. All sample workups and analyses were performed in randomized order on the same day as blood collection, under internal laboratory quality control procedures with commercial reference controls.

### 2.7. Blood and brain sampling for serum hormone and brain cholinesterase assays

For hormone analyses and brain cholinesterase determination animals were killed by decapitation using a guillotine. Ten animals per group and sex were killed at the age of 17 weeks (males) and 21 weeks (females)

without anaesthesia, or after anaesthesia with 100% carbon dioxide, 80%CO<sub>2</sub>/20%O<sub>2</sub>, 60%CO<sub>2</sub>/40%O<sub>2</sub>, methoxyflurane, diethyl ether or isoflurane. Blood samples of females were taken in diestrus. Blood was collected from the trunk. Serum was prepared and stored at –80 °C until analyzed for serum hormone assays. Brains were rapidly removed from the skull, cooled on ice and dissected longitudinally. The left hemisphere of the brain was removed and stored at –80 °C until cholinesterase assay. For cholinesterase determinations, the cortex was separated and homogenised in phosphate buffer (66.7 mmol/L, pH 7.2) containing Triton-X-100 (0.75%) using a homogenizer (Ultra-Turrax, JKA-Werke, Staufen, Germany) for 30 s on ice at a ratio of 1:11 (weight/volume; e.g. 1 g of cortex homogenized in 10 mL of buffer).

### 2.8. Blood sampling for erythrocyte cholinesterase assays and blood pH determinations

Investigations on the effects of carbon dioxide concentration in the anaesthetic on erythrocyte cholinesterase activity were performed with eight male rats per group at the age of 14 weeks, with CO<sub>2</sub> concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%. Blood was collected by puncturing the retro-orbital venous plexus. Erythrocytes were isolated and haemolysed. Concurrently the pH-value of each blood sample was measured.

### 2.9. Haematology

Complete blood cell evaluations used an H\*1E Multi-Species Haematology System (Bayer Diagnostics, Tarrytown, NY, USA) to quantify total white blood cell count, differential leukocyte count, red blood cell count, platelet count, haemoglobin, haemoglobin and erythrocyte indices (mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration). Prothrombin time of sodium citrated blood was measured on a KC 10 ball coagulometer (Amelung, Lemgo, Germany). Haemoglobin derivatives, such as oxyhaemoglobin, deoxyhaemoglobin, and oxygen capacity were determined on a OSM 3 Haemoximeter (Radiometer, Copenhagen, Denmark).

### 2.10. Clinical biochemistry

Serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase,  $\gamma$ -glutamyltransferase, sodium, potassium, chloride, inorganic phosphate, calcium, magnesium, urea, creatinine, glucose, total bilirubin, total protein, albumin, triglycerides, and cholesterol were measured with an Hitachi 917 analyzer (Roche, Mannheim, Germany).

### 2.11. Serum hormone assays

Corticosterone (CORTICO), progesterone (PROGES), testosterone (TESTOS) and total thyroxine (T<sub>4</sub>) levels were measured by radioimmunoassays (RIA) kits obtained from Diagnostic Systems Laboratories (Webster, TX, USA). Serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL) and thyroid-stimulating hormone (TSH) were determined with RIA kits from Amersham pharmacia biotech (Freiburg, Germany) and total triiodothyronine (T<sub>3</sub>) levels were measured by a RIA kit of Diagnostic Products Corp. (Los Angeles, CA, USA). Radioactivity of <sup>125</sup>I was measured with a  $\gamma$ -counter (Berthold Technology, Bad Wildbad, Germany).

### 2.12. Cholinesterase assays

Cholinesterase activities were measured with a Cobas Fara II (Roche, Grenzach-Wyhlen, Germany), using a colorimetric analysis based on the method of Ellman et al. (1961) and modified according to EPA (1996).

For erythrocyte cholinesterase, aliquots of 50  $\mu$ L whole blood were washed in 2450  $\mu$ L 0.9% aqueous NaCl; after centrifuging at 1500g for 15 min at 4 °C, supernatant was discarded and 1 mL 0.1% aqueous Triton-

X-100 solution added to the sediment, which was mixed and left for 1 h in an ice bath until complete haemolysis. After spinning down, the supernatant was pipetted off and assayed for AChE activity. The assay was conducted in 0.1 M sodium phosphate buffer, pH 8.0. The substrate was acetylthiocholine iodide at a final concentration of 1.0 mM in the assay as recommended by the EPA (1996) guideline for erythrocyte cholinesterase assay. The colour reagent was 6,6'-dithiodinitrocinic acid (DTNA) (final concentration 0.25 mM); the extinction due to its reaction product (6-mercaptodinitrocinic acid) was measured at a wavelength of 343 nm 12 times per minute for an interval of 2.5 min after a preincubation time of 5 min, at 37 °C; enzyme activity, which is directly proportional to extinction, was expressed in  $\mu$ kat/L erythrocytes, based on each individual sample's haematocrit value.

For serum and brain cholinesterase, the assay was also conducted in 0.1 M sodium phosphate buffer, pH 8.0 with the same substrate, acetylthiocholine iodide, but at a higher final concentration of 2.89 mM. The colour reagent was 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) (final concentration 0.25 mM), and extinction due to its reaction product (5-thio-2-nitrobenzoic acid) was measured (12 times per minute for an interval of 2.5 min after a preincubation time of 5 min, at 37 °C), at the absorption maximum of 412 nm (very close to a haemoglobin absorbance peak at 415–420 nm, which is why DTNA, reading at 343 nm, was used in the erythrocyte assay). Extinction is directly proportional to serum and brain cholinesterase activity, which was expressed in  $\mu$ kat/L serum or  $\mu$ kat/g protein, respectively.

### 2.13. Statistics

For all variables, means and standard deviations were calculated. Treated versus control groups were compared, separately by sex, using the Wilcoxon test for equal medians (except differential blood counts, which were not compared statistically). Statistical significance is shown in tables as \* =  $p \leq 0.05$ , \*\* =  $p \leq 0.01$ .

## 3. Results

### 3.1. Clinical observations

During induction of ether anaesthesia, the animals showed signs of discomfort and were restless. One of 10 males died during anaesthesia. The duration of induction ranged from approximately 60 to 180 s and recovery of the animals was smooth and uneventful.

Two of 10 females died during anaesthesia with methoxyflurane after blood sampling. The mean duration of induction was 480 s in females and 600 s in males and the animals were restless during both induction and recovery.

Isoflurane produced rapid anaesthesia (induction time 60–120 s) as well as rapid recovery. Induction and recovery were smooth and almost free of discomfort.

By contrast, with carbon dioxide, the animals were markedly restless and hyperactive during the induction phase. The mean time to recumbency ranged from 30 s with 100% CO<sub>2</sub> to approximately 120 s with 60% CO<sub>2</sub>. The recovery period of the rats anaesthetized with a high carbon dioxide concentration (100% and 80% CO<sub>2</sub>) was quick and smooth. In contrast, the anaesthesia with a lower carbon dioxide content (60%) led to a distressed recovery of the animals. One of 10 males and one of 10 females died during anaesthesia with 100% CO<sub>2</sub>.

Table 1A  
Effect of inhalation anaesthetics on haematology parameters<sup>a</sup> in male rats

Parameter	Control	Ether	Control	MF	Control	IF	Control	CO <sub>2</sub> /O <sub>2</sub> (60/40)	Control	CO <sub>2</sub> /O <sub>2</sub> (80/20)	Control <sup>b</sup>	CO <sub>2</sub> <sup>b</sup> (100)
RBC	8.14	8.12	8.13	7.70	8.53	8.41	7.95	7.87	7.67	7.89	8.46	8.04
HGB	9.5	9.3	9.4	<b>8.9**</b>	9.5	9.3	9.4	9.1	9.1	9.3	9.6	<b>9.2**</b>
O-HGB	53.0	52.4	48.0	55.6	54.9	<b>70.9**</b>	49.6	<b>73.3**</b>	46.7	45.8	54.0	<b>16.5**</b>
DO-HGB	45.3	46.2	50.5	42.7	43.7	<b>27.3**</b>	48.7	<b>25.5**</b>	52.0	54.1	44.6	<b>83.4**</b>
O-CAP	10.0	10.1	10.2	<b>9.6**</b>	10.2	10.3	10.1	10.0	9.9	10.1	10.2	10.1
HCT	0.415	0.41	0.42	<b>0.397**</b>	0.436	0.423	0.426	<b>0.444**</b>	0.412	<b>0.444**</b>	0.422	0.421
MCV	51.0	50.5	51.7	51.8	51.1	50.4	53.6	<b>56.5**</b>	53.8	<b>56.3**</b>	49.8	<b>52.3**</b>
MCH	1.17	1.15	1.16	1.16	1.11	1.11	1.18	1.16	1.19	1.18	1.14	1.15
MCHC	22.85	22.76	22.50	22.46	21.71	22.04	22.06	<b>20.53**</b>	22.06	<b>20.88**</b>	22.86	<b>21.93**</b>
THROMBO	724	<b>817**</b>	707	702	690	725	749	677	711	<b>605*</b>	756	<b>630**</b>
WBC	4.58	4.58	4.86	4.47	4.66	4.71	5.10	<b>6.80**</b>	4.18	<b>6.46**</b>	5.05	5.95
NEUTRO	0.85	0.64	0.66	0.59	0.93	0.88	0.65	0.69	0.54	0.59	0.81	0.90
LYMPHO	3.46	3.76	3.93	3.62	3.35	3.57	4.15	5.83	3.43	5.54	3.95	4.63
MONO	0.16	0.10	0.14	0.13	0.19	0.12	0.14	0.14	0.10	0.16	0.15	0.17
EOSINO	0.08	0.06	0.09	0.10	0.12	0.09	0.12	0.08	0.07	0.10	0.11	0.15
BASO	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.02	0.01	0.04
LUC	0.03	0.02	0.04	0.02	0.06	0.05	0.04	0.05	0.03	0.06	0.03	0.05
PT	36.4	<b>33.9**</b>	35.6	35.2	30.7	30.0	39.9	39.2	36.5	38.0	36.4	37.8

*Abbreviations:* MF: methoxyflurane; IF: isoflurane; RBC: erythrocytes (tera/L); HGB: haemoglobin (mmol/L); O-HGB: oxyhaemoglobin (%); DO-HGB: deoxyhaemoglobin (%); O-CAP: oxygen capacity (mmol/L); HCT: haematocrit (L/L); MCV: mean corpuscular volume (fl); MCH: mean corpuscular haemoglobin (fmol); MCHC: mean corpuscular haemoglobin concentration (mmol/L); THROMBO: platelets (giga/L); WBC: leukocytes (giga/L); NEUTRO: polymorphonuclear neutrophils (absolute, giga/L); LYMPHO: lymphocytes (absolute, giga/L); MONO: monocytes (absolute, giga/L); EOSINO: eosinophils (absolute, giga/L); BASO: basophils (absolute, giga/L); LUC: large unstained cells (absolute, giga/L); PT: prothrombin time (s).

\*  $p < 0.05$  (Wilcoxon test versus control).

\*\*  $p \leq 0.01$  (Wilcoxon test versus control).

<sup>a</sup> Mean values ( $n = 10$  unless indicated otherwise).

<sup>b</sup>  $n = 9$ .

Table 1B  
Effect of inhalation anaesthetics on haematology parameters<sup>a</sup> in female rats

Parameter	Control <sup>b</sup>	Ether	Control	MF	Control	IF	Control	CO <sub>2</sub> /O <sub>2</sub> (60/40)	Control	CO <sub>2</sub> /O <sub>2</sub> (80/20)	Control	CO <sub>2</sub> <sup>c</sup> (100)
RBC	7.59	7.32	7.57	<b>7.19*</b>	7.91	<b>7.38**</b>	7.60	7.50	7.52	7.65	7.71	<b>7.19**</b>
HGB	9.3	9.1	9.4	<b>8.8**</b>	9.5	<b>8.9**</b>	9.3	9.1	8.9	9.1	9.4	<b>8.9**</b>
O-HGB	52.7	49.9	49.7	<b>43.1**</b>	50.5	<b>68.3**</b>	50.9	<b>67.1**</b>	50.1	<b>38.1**</b>	52.9	<b>26.8**</b>
DO-HGB	46.0	48.7	48.9	<b>55.5*</b>	48.4	<b>29.8**</b>	47.3	<b>31.6**</b>	48.8	<b>61.3**</b>	45.7	<b>72.9**</b>
O-CAP	9.9	9.9	10.2	<b>9.7**</b>	10.3	<b>9.8*</b>	10.0	10.1	9.7	9.9	10.2	9.9
HCT	0.402	0.396	0.416	<b>0.387**</b>	0.436	<b>0.404**</b>	0.417	0.433	0.400	<b>0.424*</b>	0.411	<b>0.395**</b>
MCV	53.0	54.1	54.9	53.8	55.1	54.7	54.9	<b>57.8**</b>	53.1	<b>55.4*</b>	53.3	<b>55.0**</b>
MCH	1.22	1.24	1.24	1.22	1.20	1.21	1.22	1.21	1.18	1.18	1.22	1.23
MCHC	23.03	22.92	22.56	22.69	21.73	22.07	22.24	<b>21.01**</b>	22.21	<b>21.4**</b>	22.94	<b>22.43**</b>
THROMBO	720	784	714	658	756	777	807	<b>685**</b>	624	642	804	<b>572**</b>
WBC	2.66	2.40	2.84	2.64	2.77	2.57	3.14	3.94	2.48	<b>4.12**</b>	2.96	3.40
NEUTRO	0.43	0.31	0.48	0.37	0.51	0.48	0.54	0.62	0.45	0.52	0.45	0.53
LYMPHO	2.05	1.97	2.17	2.08	2.07	1.95	2.37	3.10	1.87	3.33	2.33	2.65
MONO	0.08	0.04	0.07	0.07	0.08	0.06	0.08	0.09	0.05	0.10	0.06	0.09
EOSINO	0.08	0.07	0.11	0.11	0.08	0.05	0.12	0.09	0.08	0.12	0.09	0.09
BASO	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.02	0.00	0.01
LUC	0.01	0.01	0.01	0.01	0.03	0.02	0.03	0.03	0.02	0.03	0.01	0.03
PT	31.8	31.6	33.3	31.4	26.2	26.4	36.3	35.5	35.8	34.6	32.1	32.0

*Abbreviations:* MF: methoxyflurane; IF: isoflurane; RBC: erythrocytes (tera/L); HGB: haemoglobin (mmol/L); O-HGB: oxyhaemoglobin (%); DO-HGB: deoxyhaemoglobin (%); O-CAP: oxygen capacity (mmol/L); HCT: haematocrit (L/L); MCV: mean corpuscular volume (fl); MCH: mean corpuscular haemoglobin (fmol); MCHC: mean corpuscular haemoglobin concentration (mmol/L); THROMBO: platelets (giga/L); WBC: leukocytes (giga/L); NEUTRO: polymorphonuclear neutrophils (absolute, giga/L); LYMPHO: lymphocytes (absolute, giga/L); MONO: monocytes (absolute, giga/L); EOSINO: eosinophils (absolute, giga/L); BASO: basophils (absolute, giga/L); LUC: large unstained cells (absolute, giga/L); PT: prothrombin time (s).

\*  $p < 0.05$  (Wilcoxon test versus control).

\*\*  $p \leq 0.01$  (Wilcoxon test versus control).

<sup>a</sup> Mean values ( $n = 10$  unless indicated otherwise).

<sup>b</sup>  $n = 8$ .

<sup>c</sup>  $n = 7$ .

## 3.2. Haematology (Tables 1A and 1B)

Ether anaesthesia caused no notable changes in haematology parameters. Methoxyflurane produced a slight decrease in red blood cell counts, haemoglobin, haematocrit and oxygen capacity in both males and females. Females exposed to isoflurane displayed slight decreases in red blood cell counts, haemoglobin, haematocrit and deoxyhaemoglobin was reduced in both sexes; increased oxyhaemoglobin values were observed in both sexes. In the carbon dioxide-anaesthetized groups, mean corpuscular volume (MCV) was increased and mean corpuscular haemoglobin concentration (MCHC) was reduced in both sexes. Lower red blood cell counts, haematocrit and haemoglobin were noted in females at 100% CO<sub>2</sub> and reduced platelets were observed at all CO<sub>2</sub> levels except in females at 80% CO<sub>2</sub> (possibly due to an unusually low control mean in this group). Higher leukocyte and lymphocyte counts were recorded in males at 60% CO<sub>2</sub> and both sexes at 80% CO<sub>2</sub>. Lower oxyhaemoglobin and higher deoxyhaemoglobin levels were observed in males and females exposed to 100% carbon dioxide, but higher oxyhaemoglobin and lower deoxyhaemoglobin levels were seen in animals exposed to 60% carbon dioxide plus 40% oxygen. Inhalation of 80% carbon dioxide plus 20% oxygen caused lower oxyhaemoglobin and higher deoxyhaemoglobin levels in females, only.

## 3.3. Clinical biochemistry (Tables 2A and 2B)

Ether anaesthesia produced increased alkaline phosphatase and glucose levels in males, and decreased potassium, inorganic phosphate and calcium levels in both sexes. Methoxyflurane produced higher glucose levels and lower alkaline phosphatase, sodium and potassium, glucose, inorganic phosphate, calcium, creatinine, total protein, albumin and globulins levels in both sexes. Isoflurane produced lower sodium, potassium, inorganic phosphate, calcium, magnesium, creatinine, total protein, albumin and globulins levels in males and females.

For males and females in carbon dioxide-anaesthetized groups markedly lower erythrocyte cholinesterase activities, lower chloride levels, and higher inorganic phosphorus, calcium and magnesium levels were noted. Lower aspartate aminotransferase activities were recorded in males at 60% and 100% CO<sub>2</sub> and in females at 80% and 100% CO<sub>2</sub>. Higher glucose levels were noted in both sexes at 60% CO<sub>2</sub> and in males at 80% CO<sub>2</sub>. Decreased total protein, albumin and globulin levels were recorded in males and females at 60% CO<sub>2</sub>.

## 3.4. Serum hormones (Table 3)

Ether anaesthesia produced higher testosterone and LH levels in males and higher PRL as well as lower T<sub>3</sub> and T<sub>4</sub>

Table 2A  
Effects of inhalation anaesthetics on clinical biochemistry parameters<sup>a</sup> in male rats

Parameter	Control	Ether	Control	MF	Control	IF	Control	CO <sub>2</sub> /O <sub>2</sub> (60/40)	Control	CO <sub>2</sub> /O <sub>2</sub> (80/20)	Control <sup>b</sup>	CO <sub>2</sub> <sup>b</sup> (100)
ALAT	0.64	0.7	0.72	<b>0.58**</b>	0.68	0.67	0.66	0.72	0.65	0.63	0.73	0.62
ASAT	1.64	1.61	1.93	1.56	2.14	<b>1.69*</b>	1.86	<b>1.62**</b>	1.91	1.79	1.70	<b>1.42**</b>
ALP	4.19	<b>4.85*</b>	6.32	<b>4.64**</b>	3.29	3.22	5.80	<b>7.08**</b>	9.33	<b>7.42**</b>	4.48	<b>3.86*</b>
GGT	9	8	19	20	2	1	4	3	3	1	13	5
S-ChE	11.28	10.67	11.26	10.36	16.84	13.59	11.67	10.83	10.67	11.47	12.05	11.72
E-ChE	40.71	38.73	29.22	28.66	34.04	33.57	37.2	<b>8.12**</b>	32.22	<b>9.29**</b>	36.43	<b>9.57**</b>
Na	146.0	146.1	145.6	<b>144.0*</b>	146.1	<b>144.0*</b>	147.3	148.9	146.7	<b>150.6**</b>	146.0	<b>148.9**</b>
K	5.96	<b>4.55**</b>	6.33	<b>4.32**</b>	6.65	<b>4.58**</b>	6.50	6.01	6.15	6.35	5.93	6.22
Cl	107.1	107	105.5	104.7	108.8	108.2	101.7	100.6	99.7	<b>96.5**</b>	106.1	<b>103.6**</b>
PHOS	2.21	<b>1.94**</b>	2.53	<b>2.23**</b>	2.16	<b>1.82**</b>	2.67	<b>3.32**</b>	3.03	3.19	2.14	<b>2.47**</b>
Ca	2.78	<b>2.68*</b>	2.79	<b>2.61**</b>	2.89	<b>2.74**</b>	2.71	<b>2.90**</b>	2.72	<b>2.83**</b>	2.81	<b>2.90*</b>
UREA	5.88	5.79	6.12	6.05	7.56	7.43	6.08	6.25	6.30	6.44	5.68	6.01
CREAT	52.2	50.4	55.3	<b>46.5**</b>	66.7	<b>59.3**</b>	53.4	51.0	49.8	50.2	56.6	55.2
GLUC	5.41	<b>7.44**</b>	5.30	<b>6.44**</b>	5.46	<b>6.06*</b>	5.29	<b>7.65**</b>	5.14	<b>5.76*</b>	5.31	5.52
BILI	1.77	1.48	2.14	2.10	2.50	2.38	1.74	1.69	1.52	1.82	1.67	1.78
TP	65.93	65.13	68.87	<b>62.08**</b>	68.70	<b>65.58**</b>	65.59	<b>62.10**</b>	62.96	64.63	71.49	69.38
ALB	37.65	37.04	33.44	<b>30.93**</b>	37.58	<b>36.26*</b>	34.05	<b>32.15**</b>	32.92	33.88	38.66	37.84
GLOB	28.27	28.08	35.43	<b>31.14**</b>	31.12	<b>29.31*</b>	31.54	29.94	30.04	30.75	32.83	31.54
TRIGL	0.69	0.56	0.67	0.48	1.08	<b>0.73*</b>	0.67	0.61	0.60	0.61	0.78	0.76
CHOL	1.57	1.67	1.73	<b>1.40**</b>	2.11	1.86	1.62	1.69	1.73	1.61	1.84	<b>1.54*</b>
Mg	0.95	0.95	0.99	0.94	1.03	<b>0.91**</b>	1.00	<b>1.22**</b>	0.94	<b>1.12**</b>	0.95	<b>1.14**</b>

Abbreviations: MF: methoxyflurane; IF: isoflurane; ALAT: alanine aminotransferase (μkat/L); ASAT: aspartate aminotransferase (μkat/L); ALP: alkaline phosphatase (μkat/L); GGT: γ-glutamyltransferase (nkat/L); S-ChE: serum cholinesterase (μkat/L); E-ChE: erythrocyte cholinesterase (nkat/L erythrocytes); Na: sodium (mmol/L); K: potassium (mmol/L); Cl: chloride (mmol/L); PHOS: inorganic phosphate (mmol/L); Ca: calcium (mmol/L); UREA: urea (mmol/L); CREAT: creatinine (μmol/L); GLUC: glucose (mmol/L); BILI: total bilirubin (μmol/L); TP: total protein (g/L); ALB: albumin (g/L); GLOB: globulins (g/L); TRIGL: triglycerides (mmol/L); CHOL: cholesterol (mmol/L); Mg: magnesium (mmol/L).

\*  $p < 0.05$  (Wilcoxon test versus control).

\*\*  $p \leq 0.01$  (Wilcoxon test versus control).

<sup>a</sup> Mean values ( $n = 10$  unless indicated otherwise).

<sup>b</sup>  $n = 9$ .

Table 2B  
Effect of inhalation anaesthetics on clinical biochemistry parameters<sup>a</sup> in female rats

Parameter	Control <sup>b</sup>	Ether	Control	MF	Control	IF	Control	CO <sub>2</sub> /O <sub>2</sub> (60/40)	Control	CO <sub>2</sub> /O <sub>2</sub> (80/20)	Control	CO <sub>2</sub> <sup>c</sup> (100)
ALAT	0.54	0.58	0.59	0.59	0.70	0.92	0.58	0.59	0.66	0.56	0.6	0.6
ASAT	1.56	1.70	1.69	1.79	1.99	2.33	1.78	1.69	2.37	<b>1.48**</b>	1.80	<b>1.67*</b>
ALP	2.46	2.99	4.21	3.27	1.10	1.16	4.01	4.73	5.64	4.93	2.59	1.98
GGT	7	9	27	21	0	3	7	6	4	7	10	16
S-ChE	48.4	46.1	48.05	44.33	67.53	62.29	45.17	42.41	35.11	38.3	54.11	55.34
E-ChE	37.32	37.63	28.61	25.81	32.45	<b>36.74*</b>	37.69	<b>9.29**</b>	31.87	<b>11.54**</b>	36.93	<b>12.69**</b>
Na	143.5	144.4	145.1	<b>142.5*</b>	146.4	<b>142.2**</b>	146.5	147.1	146.8	<b>149.1*</b>	145.3	146.9
K	5.79	<b>4.46**</b>	6.18	<b>4.31**</b>	6.18	<b>4.31**</b>	6.31	6.05	6.29	6.65	6.07	6.57
Cl	107.2	108.1	106.7	105.1	109.4	<b>107.7**</b>	103.6	<b>102.2*</b>	102.6	<b>99.0**</b>	107.6	<b>104.9**</b>
PHOS	1.99	<b>1.63*</b>	2.06	1.96	1.76	<b>1.28**</b>	2.34	<b>3.03**</b>	2.57	<b>3.36**</b>	1.74	<b>2.31**</b>
Ca	2.77	<b>2.63**</b>	2.73	<b>2.59**</b>	2.97	<b>2.72**</b>	2.73	<b>2.86**</b>	2.63	<b>2.83**</b>	2.79	<b>2.90**</b>
UREA	7.10	7.41	7.73	7.18	7.96	7.76	7.98	7.59	7.73	7.15	7.32	6.98
CREAT	54.7	54.0	59.3	<b>51.3**</b>	74.5	<b>67.5*</b>	58.6	<b>53.0**</b>	50.9	50.1	61.6	59.3
GLUC	5.22	5.53	5.08	<b>5.67**</b>	5.14	5.68	5.06	6.08	5.64	5.12	5.16	5.12
BILI	2.28	1.96	2.52	2.27	4.07	3.25	2.08	2.04	2.07	1.95	2.17	2.03
TP	67.28	65.94	71.26	<b>64.88**</b>	76.58	<b>69.80**</b>	67.36	<b>63.49**</b>	61.78	64.00	73.74	70.60
ALB	38.75	38.80	36.20	<b>33.22**</b>	43.44	<b>40.19**</b>	35.95	<b>34.16**</b>	33.80	34.45	41.23	39.65
GLOB	28.53	27.14	35.06	<b>31.65**</b>	33.14	<b>29.61**</b>	31.41	<b>29.33**</b>	27.98	29.55	32.51	30.95
TRIGL	0.35	<b>0.27**</b>	0.31	<b>0.23**</b>	0.54	0.46	0.35	0.32	0.28	0.31	0.34	0.39
CHOL	1.25	1.09	1.09	1.12	1.93	1.73	1.28	1.13	1.03	1.08	1.17	1.18
Mg	0.99	1.04	1.07	1.03	1.09	<b>0.94**</b>	1.07	<b>1.28**</b>	1.01	<b>1.17**</b>	1.02	<b>1.18**</b>

Abbreviations: MF: methoxyflurane; IF: isoflurane; ALAT: alanine aminotransferase (μkat/L); ASAT: aspartate aminotransferase (μkat/L); ALP: alkaline phosphatase (μkat/L); GGT: γ-glutamyltransferase (nkat/L); S-ChE: serum cholinesterase (μkat/L); E-ChE: erythrocyte cholinesterase (nkat/L erythrocytes); Na: sodium (mmol/L); K: potassium (mmol/L); Cl: chloride (mmol/L); PHOS: inorganic phosphate (mmol/L); Ca: calcium (mmol/L); UREA: urea (mmol/L); CREAT: creatinine (μmol/L); GLUC: glucose (mmol/L); BILI: total bilirubin (μmol/L); TP: total protein (g/L); ALB: albumin (g/L); GLOB: globulins (g/L); TRIGL: triglycerides (mmol/L); CHOL: cholesterol (mmol/L); Mg: magnesium (mmol/L).

\*  $p < 0.05$ .

\*\*  $p \leq 0.01$  (Wilcoxon test versus control).

<sup>a</sup> Mean values ( $n = 10$  unless indicated otherwise).

<sup>b</sup>  $n = 8$ .

<sup>c</sup>  $n = 7$ .

levels in both sexes. Methoxyflurane produced lower T<sub>3</sub>, T<sub>4</sub>, TSH and PRL levels in both sexes. Isoflurane produced lower T<sub>4</sub> and T<sub>3</sub> and higher PRL levels in males and females. Lower T<sub>4</sub> and T<sub>3</sub> levels in males and higher LH levels in both sexes were noted at all CO<sub>2</sub> concentrations.

### 3.5. Cholinesterase (Tables 2A, 2B, 4 and 5)

Erythrocyte cholinesterase activities were about the same in males and females, but serum cholinesterase activities were much higher in females than males. Moreover, serum cholinesterase activity in females was directly correlated with their age (means (μkat/L), see Tables 2A and 2B) were 35–38 at 10 weeks of age, 42–45 at 12, 44–48 at 14, 46–48 at 16, 54–55 at 18, and 62–68 at 26 weeks. Age-related changes and sex differences on serum cholinesterase activity of the rat are well known phenomena (Schmidt and Schmidt, 1978; Biró et al., 1990), and are estrogen-dependent.

At all CO<sub>2</sub> concentrations, there was a marked decrease in red blood cell cholinesterase activity by about 60–80% (Table 4), compared to no anaesthesia and to all other anaesthetics. Mean AChE activity in males and females was reduced to 22–33% of non-anaesthetized control values after 60% CO<sub>2</sub>, to 28–30% of control values after 80% CO<sub>2</sub>, and 27–41% of control values after 100% CO<sub>2</sub> anaesthesia.

Inhalation of CO<sub>2</sub> did not affect serum and brain cholinesterase activities. Table 5 shows the dependency of erythrocyte cholinesterase inhibition and blood pH on CO<sub>2</sub> concentrations. Significant decreases in red blood cell cholinesterase activity and blood pH occurred at a concentration of 20% CO<sub>2</sub> and beyond. At 20% CO<sub>2</sub> erythrocyte cholinesterase activity was reduced by about 10% and blood pH by about 1%. The maximum decrease in red blood cell cholinesterase activity was observed at 90% CO<sub>2</sub> (73% below the control) and maximum fall in blood pH was noted at 80% CO<sub>2</sub> (9% below the control).

## 4. Discussion

CO<sub>2</sub>, methoxyflurane and isoflurane affected red blood cell parameters. Mean corpuscular volume (MCV) was consistently higher and mean corpuscular haemoglobin concentration (MCHC) was consistently lower in both males and females at all carbon dioxide concentrations. In addition, lower erythrocyte counts, haemoglobin concentrations and haematocrit values were found in female rats at 100% CO<sub>2</sub>. Similar results were previously reported by Walter (1999). Lower erythrocyte counts, haemoglobin concentrations and haematocrit values were also recorded in female rats exposed to methoxyflurane or isoflurane. Deoxyhaemoglobin levels were inversely proportional and

Table 3  
Effect of inhalation anaesthetics on serum hormones<sup>a</sup> in rats

Hormone	Males					Females								
	Control	Ether	MF	IF	CO <sub>2</sub> /O <sub>2</sub> (60/40)	CO <sub>2</sub> /O <sub>2</sub> (80/20)	CO <sub>2</sub> (100)	Control	Ether	MF	IF	CO <sub>2</sub> /O <sub>2</sub> (60/40)	CO <sub>2</sub> /O <sub>2</sub> (80/20)	CO <sub>2</sub> (100)
CORTICO	1403	1595	1304	1565	1573	1322	1468	1957	1509	1950	828	1061	831	890
PROGES	18.40	30.95	25.30	<b>36.41*</b>	32.37	34.94	22.93	89.97	93.18	102.74	82.41	61.12	52.14	43.82
TESTOS	6.05	<b>15.65*</b>	6.73	5.99	4.87	6.45	6.64	—	—	—	—	—	—	—
T <sub>4</sub>	62.23	<b>51.64*</b>	<b>51.86*</b>	<b>47.40*</b>	<b>45.33*</b>	<b>48.34*</b>	<b>48.51*</b>	41.36	36.35	<b>30.10**</b>	34.74	38.86	34.70	35.47
T <sub>3</sub>	1.54	<b>1.11**</b>	<b>1.09**</b>	<b>0.98*</b>	1.27	<b>1.07**</b>	<b>1.12**</b>	1.62	1.39	<b>1.18**</b>	<b>1.30*</b>	1.54	1.55	1.53
LH	0.82	<b>2.45**</b>	0.90	0.84	<b>2.29**</b>	<b>2.01**</b>	<b>1.55*</b>	1.03	0.81	0.92	1.11	<b>1.54*</b>	<b>1.53*</b>	1.43
FSH	6.86	6.23	5.54	5.33	9.10	7.74	8.27	2.26	1.58	0.65	2.12	1.05	2.72	2.30
TSH	9.73	9.72	8.04	8.64	<b>12.09**</b>	9.41	<b>11.41*</b>	7.05	5.94	<b>4.19*</b>	6.18	6.79	7.15	6.89
PRL	41.54	<b>113.0*</b>	<b>8.17*</b>	<b>110.59**</b>	28.37	<b>76.64*</b>	37.68	18.77	80.53	<b>5.10*</b>	40.95	20.58	95.14	63.60

Abbreviations: MF: methoxyflurane; IF: isoflurane; CORTICO: corticosterone (nmol/L); PROGES: progesterone (nmol/L); TESTOS: testosterone (nmol/L); T<sub>4</sub>: thyroxine (nmol/L); T<sub>3</sub>: triiodothyronine (nmol/L); LH: luteinizing hormone (µg/L); FSH: follicle-stimulating hormone (µg/L); TSH: thyroid-stimulating hormone (µg/L); PRL: prolactin (µg/L).

\*  $p < 0.05$  (Wilcoxon test versus control).

\*\*  $p \leq 0.01$  (Wilcoxon test versus control).

<sup>a</sup> Mean values ( $n = 10$ ).

oxyhaemoglobin levels proportional to the O<sub>2</sub> content in the CO<sub>2</sub> mixtures, but oxygen capacity was not affected by CO<sub>2</sub> anaesthesia. These results are in agreement with those reported by Petty and Sulkowski (1971) who examined effects of carbon dioxide on the respiratory tract. Lower deoxyhaemoglobin and higher oxyhaemoglobin levels were recorded in male and female rats exposed to isoflurane.

Higher leukocyte and lymphocyte counts were seen after CO<sub>2</sub> anaesthesia, particularly in males at lower concentrations of 60% or 80%, as previously reported by Walter (1999). Serum glucose levels were increased in male rats exposed to 60% and 80% CO<sub>2</sub>, ether, isoflurane, or methoxyflurane. Higher serum glucose levels were also found in female rats exposed to methoxyflurane. The hyperglycemia and leukocytosis most probably reflect a stress response and can be attributed to catecholamine release secondary to excitement or fear.

Reduced chloride was noted in females anaesthetized with isoflurane and CO<sub>2</sub>. Decreased total protein, albumin and globulins levels were detected after 60% CO<sub>2</sub>, isoflurane or methoxyflurane anaesthesia in both sexes. Urea and total bilirubin levels were not affected by any anaesthetic.

No relevant effect on serum enzymes was apparent for any anaesthetic. In contrast to all other inhalation anaesthetics, CO<sub>2</sub> markedly reduced erythrocyte cholinesterase activity in both sexes, while serum and brain cholinesterase activity were not affected, consistent with observations in rat brain previously reported by Berger-Sweeney et al. (1994). The inhibition of erythrocyte cholinesterase activity was shown to correlate with acidity of the blood (acidosis) and it is therefore conceivable that the effect was caused by respiratory acidosis. Erythrocyte cholinesterase consists almost entirely of acetylcholinesterase (AChE) (EC 3.1.1.7) which has the same genetic sequence as brain AChE, but is transcribed and post-translationally modified to “erythrocytic” AChE-E, a dimer form which is anchored to the red blood cell membrane by a glycosylphosphatidyl inositol tail on the C-terminal, as compared to “synaptic” AChE-S, the multimer form found in synapses and muscle endplates (Grisaru et al., 1999). Serum cholinesterase in rats consists of about equal parts of AChE and butyrylcholinesterase (BChE; EC 3.1.1.8), a close genetic homologue of AChE which catalyses the degradation of both acetylcholine and butyrylcholine (Boeck et al., 2002). In the risk assessment of organophosphorous and carbamate pesticides, erythrocyte AChE is preferred to plasma cholinesterase as a surrogate marker in the peripheral nervous system by the US Environmental Protection Agency (EPA), because erythrocyte data are considered to provide a better representation of the inhibition of the neural target enzyme, i.e. AChE (EPA, 2000). For this reason, major efforts have been made to standardize the assay, but variability remains high, for reasons including sample preparation and storage, assay pH, temperature, and substrate (Wilson et al., 2002). Erythrocyte cholinesterase

Table 4  
Effect of inhalation anaesthetics on cholinesterase activity<sup>a</sup> in serum, erythrocytes and brain in rats

Parameter	Males							Females						
	Control	Ether	MF	IF	CO <sub>2</sub> /O <sub>2</sub> (60/40)	CO <sub>2</sub> /O <sub>2</sub> (80/20)	CO <sub>2</sub> (100)	Control	Ether	MF	IF	CO <sub>2</sub> /O <sub>2</sub> (60/40)	CO <sub>2</sub> /O <sub>2</sub> (80/20)	CO <sub>2</sub> (100)
S-ChE	12.83	11.30	11.00	<b>9.39**</b>	11.84	11.77	<b>10.33*</b>	76.20	69.58	65.18	72.10	80.48	83.24	85.22
E-ChE	32.52	33.46	33.61	34.18	<b>7.25**</b>	<b>9.26**</b>	<b>13.29**</b>	29.36	29.56	35.60	<b>34.73*</b>	<b>9.72**</b>	<b>8.67**</b>	<b>7.81**</b>
B-ChE	3.43	<b>2.56*</b>	2.83	3.12	3.16	<b>2.39*</b>	3.00	1.55	1.55	1.47	1.50	1.76	1.46	1.41

Abbreviations: S-ChE: serum cholinesterase (μkat/L); E-ChE: erythrocyte cholinesterase (μkat/L erythrocytes); B-ChE: brain cholinesterase (μkat/G protein).

\*  $p < 0.05$  (Wilcoxon test versus control).

\*\*  $p \leq 0.01$  (Wilcoxon test versus control).

<sup>a</sup> Mean values ( $n = 8$ ).

Table 5  
Effects of carbon dioxide concentration in the anaesthetic on erythrocyte cholinesterase activity<sup>a</sup> and blood pH<sup>a</sup> in male rats

Parameter	Control	CO <sub>2</sub> /O <sub>2</sub> (10/90)	CO <sub>2</sub> /O <sub>2</sub> (20/80)	CO <sub>2</sub> /O <sub>2</sub> (30/70)	CO <sub>2</sub> /O <sub>2</sub> (40/60)	CO <sub>2</sub> /O <sub>2</sub> (50/50)	CO <sub>2</sub> /O <sub>2</sub> (60/40)	CO <sub>2</sub> /O <sub>2</sub> (70/30)	CO <sub>2</sub> /O <sub>2</sub> (80/20)	CO <sub>2</sub> /O <sub>2</sub> (90/10)	CO <sub>2</sub> (100)
E-ChE	35.07	32.44	<b>31.12*</b>	<b>23.25**</b>	<b>19.24**</b>	<b>14.35**</b>	<b>10.82**</b>	<b>10.28**</b>	<b>9.96**</b>	<b>9.53**</b>	<b>14.32**</b>
pH	7.49	7.47	<b>7.40*</b>	<b>7.26**</b>	<b>7.14**</b>	<b>6.94**</b>	<b>6.97**</b>	<b>6.82**</b>	<b>6.79**</b>	<b>6.91**</b>	<b>7.01**</b>

Abbreviation: E-ChE: erythrocyte cholinesterase (μkat/L erythrocytes).

\*  $p < 0.05$  (Wilcoxon test versus control).

\*\*  $p \leq 0.01$  (Wilcoxon test versus control).

<sup>a</sup> Mean values ( $n = 8$ ).

assay data display unpredictable variability between laboratories (Sramek and Cutler, 2000; Wilson et al., 2002) and it has been suggested that erythrocyte cholinesterase is unreliable as a surrogate marker in research on AChE inhibitors for the treatment of Alzheimer's disease (Sramek and Cutler, 2000). The present data indicate that carbon dioxide anaesthesia is a further source of potential variability in the outcome of erythrocyte cholinesterase assays in rats.

Sodium, inorganic phosphate, calcium and magnesium levels were reduced by methoxyflurane and isoflurane anaesthesia, but increased by CO<sub>2</sub> anaesthesia in both sexes, in line with previous observations by Nahas and Provost (2002) and Walter (1999). It is interesting to note, in this context, that Dawson and Crone (1973) reported about a 10-fold increase in the  $K_m$  of bovine erythrocyte cholinesterase (from 16–20 to 118–158 μM) when the concentration of Ca<sup>2+</sup> or Mg<sup>2+</sup> was increased from 2 mM (control) to 6–7 mM (the lowest concentration tested).

Potassium levels were markedly reduced by ether, methoxyflurane or isoflurane anaesthesia, but were not affected by CO<sub>2</sub> anaesthesia. Hypokalemia induced by isoflurane in monkeys has previously been reported by Hotchkiss et al. (1998). Hypokalaemia induced by ether and isoflurane in rats was previously reported by Chassagne et al. (2000).

T<sub>3</sub> and T<sub>4</sub> levels were reduced by all inhalation anaesthetics, particularly in males. Testosterone levels were increased by ether. Luteinizing hormone levels were increased by ether in males, and by CO<sub>2</sub> (at all concentrations) in males and females. Prolactin levels were reduced in males and females by methoxyflurane, but significantly

raised by ether as well as isoflurane. It is known, that the secretion of prolactin in rats differs significantly during the administration of various anaesthetics (Nazian, 1988). The regulation of the prolactin secretion from the anterior lobe of the pituitary gland is influenced by numerous hormones and paracrin factors (Horseman and Gregerson, 2006). It is published that the dopaminergic and the opioid system seem to play a major role in the prolactin secretion during ether anaesthesia in rats (Yogev et al., 1994; Reis et al., 1994). Subramanian et al. (1975) assumed that methoxyflurane and ether induce different prolactin response mechanisms due to the different hormone secretion course in a two hours anaesthesia period as well as due to the different prolactin secretion during inhibition by dopaminergic receptor stimulators.

It is known that handling of the rats prior to the venipuncture and the blood sampling itself may elevate some clinical pathology parameters, which were assessed in this study (corticosterone, prolactin, TSH, T<sub>3</sub>, packed cell volume, white blood cell count, haemoglobin, glucose, protein, albumin; Balcombe et al., 2004).

The blood sampling in this study was standardised and was performed on each time by the same skilled technicians, so that a variability of the values for this reason can be excluded. However, it has to be considered that values of the mentioned parameters might be at a higher level when assessed by blood taken from the retro-orbital sinus compared to samples taken by other venipuncture techniques or by decapitation (Mahl et al., 2000; Doehler et al., 1977). It may be assumed, that the different values assessed in this study were based on the different anaesthetics. Based on the results of these investigations, we

conclude that baseline clinical pathology data are affected by the choice of the anaesthetic. Based on practical considerations and the observed clinical toxicity, CO<sub>2</sub>/O<sub>2</sub> (80%/20%) and isoflurane are considered to be the most suitable inhalation anaesthetics for retro-orbital blood sampling. Although isoflurane is expensive and requires a precision vaporizer for dosing, induction and recovery are rapid and smooth and almost free of discomfort. A similar rapid induction as well as a smooth recovery period is also achieved with a CO<sub>2</sub>/O<sub>2</sub> mixture of 80–20%. Two of 20 rats in the present study did not survive a short anaesthesia with 100% carbon dioxide, although the surviving animals recovered quickly. On the other hand, a lower carbon dioxide concentration (60%) led to a distressed induction time as well as a restless recovery period of the animals, which is confirmed in other publications (Wixson and Smiler, 1997). However, if erythrocyte cholinesterase activity is to be determined, isoflurane is the anaesthetic of choice.

### Acknowledgements

The expert technical assistance of Mr. Bohrer and the staff of BASF's Clinical Pathology Laboratory in the study procedures and data collection is gratefully acknowledged. The authors thank the Landesuntersuchungsamt Rheinland-Pfalz for supporting this study. The initial manuscript was prepared by Rex FitzGerald.

### References

- Balcombe, J.P., Barnard, N.D., Sandusky, C., 2004. Laboratory routines cause animal stress. *Contemporary Topics* 43, 42–51.
- Berger-Sweeney, J., Berger, U.V., Sharma, M., Paul, C.A., 1994. Effects of carbon dioxide-induced anesthesia on cholinergic parameters in rat brain. *Laboratory Animal Science* 44, 369–371.
- Biró, L., Wachnik, A., Velösy, G.A., Rodics, K., Antal, M., 1990. Sex dependence of naphthyl butyrate esterase activity in rat serum. *Journal of Clinical Chemistry and Clinical Biochemistry* 28, 119–120.
- Boeck, A.T., Schopfer, L.M., Lockridge, O., 2002. DNA sequence of butyrylcholinesterase from the rat: expression of the protein and characterization of the properties of rat butyrylcholinesterase. *Biochemical Pharmacology* 63, 2101–2110.
- Brunson, D.B., 1997. Pharmacology of inhalation anesthesia. In: Kohn, D.F., Wixson, S.K., White, W.J., Benson, G.J. (Eds.), *Anesthesia and Analgesia for Laboratory Animals*. Academic Press, San Diego, California, pp. 29–41.
- Brunson, D.B., Stowe, C.M., McGrath, C.J., 1979. Serum and urine inorganic fluoride concentrations following methoxyflurane anesthesia in the dog. *American Journal of Veterinary Research* 40, 197–203.
- Chassagne, M.C., Descotes, J., Heritier-Pingeon, B., Forichon, A., Garnier, F., Burnett, R., 2000. A comparison of the effects of repeated anaesthesia with ether or isoflurane in rats. *Comparative Hematology International* 10, 126–131.
- Chemikaliengesetz (Chemicals Act); Bundesgesetzblatt 1994, Part I, July 29, 1994; FR Germany.
- Danneman, P.J., Stein, S., Walshaw, S.O., 1997. Humane and practical implications of using carbon dioxide mixed with oxygen for anesthesia or euthanasia of rats. *Laboratory Animal Science* 47, 376–385.
- Dawson, R.M., Crone, H.D., 1973. Inorganic ion effects on the kinetic parameters of acetylcholinesterase. *Journal of Neurochemistry* 21, 247–249.
- Doehler, K.-D., von zur Muehlen, A., Gaertner, K., Doehler, U., 1977. Effect of various blood sampling techniques on serum levels of pituitary and thyroid hormones in the rat. *Journal of Endocrinology* 74, 341–342.
- Ellman, G.L., Courtney, K.D., Andres Jr., V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology* 7, 88–95.
- EPA Environmental Protection Agency [OPP-00432; FRL-5364-5], 1996. Standard Operating Procedure For Measuring Cholinesterases. In: *Laboratory Rats And Dogs Exposed to Non-Reversible Cholinesterase Inhibitors*. Federal Register, April 26, 1996, vol. 61(82), pp. 18593.
- EPA Environmental Protection Agency, 2000. Office of Pesticide Programs Science Policy on The Use of Data on Cholinesterase Inhibition for Risk Assessments of Organophosphorous and Carbamate Pesticides. Office of Pesticide Programs, US Environmental Protection Agency, Washington DC 20460, August 18, 2000. (URL given in Federal Register vol. 65(175), pp. 54521–54523 (September 8, 2000)).
- Fenwick, D.C., Blackshaw, J.K., 1989. Carbon dioxide as a short-term restraint anaesthetic in rats with subclinical respiratory disease. *Laboratory Animals* 23, 220–228.
- German Animal Protection Act, 1998. Tierschutzgesetz in der Fassung der Bekanntmachung vom 25. Mai 1998 (BGLB IS. 1105–1120).
- Grisaru, D., Sternfeld, M., Eldor, A., Glick, D., Soreq, H., 1999. Structural roles of acetylcholinesterase variants in biology and pathology. *European Journal of Biochemistry* 264, 672–686.
- Horn, B., 1977. Explosiveness of ether. *JAMA* 238, 1631.
- Horseman, N.D., Gregerson, K.A., 2006. Regulation of Pituitary Prolactin Synthesis and Secretion. In: De Groot, L.J., Jameson, J.L., de Kretser, D., Grossman, A.B., Marshall, J.C., Melmed, S., Potts, J.T., Weir, G.C. (Eds.), fifth ed., . In: *Endocrinology*, vol. 1 Elsevier Saunders, Philadelphia, PA, pp. 311–313.
- Hotchkiss, C.E., Brommage, R., Du, M., Jerome, C.P., 1998. The anesthetic isoflurane decreases ionized calcium and increases parathyroid hormone and osteocalcin in cynomolgus monkeys. *Bone* 23 (5), 479–484.
- Kohler, I., Meier, R., Busato, A., Neiger-Aeschbacher, G., Schatzmann, U., 1999. Is carbon dioxide (CO<sub>2</sub>) a useful short acting anaesthetic for small laboratory animals? *Laboratory Animals* 33, 155–161.
- Mahl, A., Heining, P., Ulrich, P., Jakubowski, J., Bobadilla, M., Zeller, W., Bergmann, R., Singer, T., Meister, L., 2000. Comparison of clinical pathology parameters with two different blood sampling techniques in rats: retrobulbar plexus versus sublingual vein. *Lab Animal* 34, 351–361.
- Nahas, K., Provost, J.-P., 2002. Blood sampling in the rat: current practices and limitations. *Comparative Clinical Pathology* 11, 14–37.
- Nazian, S.J., 1988. Serum concentrations of reproductive hormones after administration of various anesthetics to immature and young adult male rats (42692). In: *Proceedings of the Society for Experimental Biology and Medicine*, vol. 187, pp. 482–487.
- OECD, 1981. *OECD Principles of Good Laboratory Practice*. Paris, Organisation for Economic Cooperation and Development.
- Petty, W.C., Sulkowski, T.S., 1971. CO<sub>2</sub> narcosis in the rat: I. Effects on respiration and blood parameters. *Aerospace Medicine* 42, 547–552.
- Reis, F.M., Santos, M.A.R., Reis, A.M., Coimbra, C.C., 1994. Effects of hyperprolactinemia on plasma glucose and prolactin in rats exposed to ether stress. *Physiology and Behavior* 56, 495–499.
- Schmidt, E., Schmidt, F.W., 1978. Sex differences on plasma cholinesterase in the rat. *Enzyme* 23, 52–55.
- Sramek, J.J., Cutler, N.R., 2000. RBC cholinesterase inhibition: a useful surrogate marker for cholinesterase inhibitor activity in Alzheimer disease therapy? *Alzheimer Disease and Associated Disorders* 14, 216–227.
- Subramanian, M.G., Lawson, D.M., Gala, R.R., 1975. The effects of methoxyflurane and ether alone or in combination with apomorphine

- or 2 Br- $\alpha$ -ergocryptine (CB-154) on prolactin release in ovariectomized, estrogen-treated rats. *Life Sciences* 18, 305–310.
- Thompson, J.S., Brown, S.A., Khurdayan, V., Zeynalzadedan, A., Sullivan, P.G., Scheff, S.W., 2002. Early effects of tribromoethanol, ketamine/xylazine, pentobarbital, and isoflurane anesthesia on hepatic and lymphoid tissue in ICR mice. *Comparative Medicine* 52, 63–67.
- Urbanski, H.F., Kelley, S.T., 1991. Sedation by exposure to a gaseous carbon dioxide-oxygen mixture: application to studies involving small laboratory animal species. *Laboratory Animal Science* 41, 80–82.
- Van Herck, H., Baumans, V., Brandt, C.J.W.M., Boere, H.A.G., Hesp, A.P.M., van Lith, H.A., Schurink, M., Beynen, A.C., 2001. Blood sampling from the retro-orbital plexus, the saphenous vein and tail vein in rats: comparative effects on selected behavioural and blood variables. *Laboratory Animals* 35, 131–139.
- Walter, G.L., 1999. Effects of carbon dioxide inhalation on hematology, coagulation, and serum clinical chemistry values in rats. *Toxicologic Pathology* 27, 217–225.
- Wilson, B.W., Henderson, J.D., Ramirez, A., O'Malley, M.A., 2002. Standardization of clinical cholinesterase measurements. *International Journal of Toxicology* 21, 385–388.
- Wixson, S.K., Smiler, K.L., 1997. Anesthesia and analgesia in rodents. In: Kohn, D.F., Wixson, S.K., White, W.J., Benson, G.J. (Eds.), *Anesthesia and Analgesia for Laboratory Animals*. Academic Press, San Diego, California, pp. 165–203.
- Yogev, L., Yavetz, H., Gottreich, A., Oppenheim, D., Homonnai, Z.T., Paz, G., 1994. Serum prolactin response to ether stress in diabetic rats: opiate system contribution. *PSEBM* 205, 248–252.