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Metabolic Interactions in Rats Treated with Acetylsalicylic Acid and Trichloroethylene

B. Zielińska-Psuja, J. Orłowski, A. Plewka*, M. Kamiński*, J. Kowalówka-Zawieja, B. Zięba-Proc

Department of Toxicology, Karol Marcinkowski University of Medical Sciences, Poznan, Poland
*Department of Histology & Embriology, Silesian School of Medicine,
40-752 Katowice-Ligota, Medyków 20, Poland

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Abstract

The influence of acetylsalicylic acid (ASA) on the elimination of trichloroacetic acid and trichloroethanol in the urine, liver and kidney of cytochrome P-450-dependent monooxygenase systems in rats exposed to trichloroethylene (TRI) vapours were examined. The obtained results show that acetylsalicylic acid decreased the elimination of both trichloroethylene metabolites and induced cytochrome P-450 concentration in the liver, whereas slightly inhibited cytochrome b_5 and activites of NADPH-cytochrome P-450 reductase and NADH-cytochrome b_5 reductase. After mixed exposures, acetylsalicylic acid elevated cytochrome P-450 concentration and NADPH-cytochrome P-450 reductase activity to values observed in the control group. The concentration of cytochrome b_5 was much lower than after ASA or trichloroethylene alone. The activity of NADPH-cytochrome P-450 reductase was strongly inhibited by TRI, whereas in ASA the presence was elevated, but was still lower than in control. Acetylsalicylic acid stimulates cytochrome P-450; however, other compounds of the MFO system do not react positively on this ester.

Keywords: acetylsalicylic acid, trichloroethylene, trichloroethanol, trichloroacetic acid, cytochrome P-450 dependent monooxygenases

Introduction

The use of salicylate has been established for over 100 years. Today salicylates are available in hundreds of different forms and annual consumption of tablets can be measured in millions. For many years they were used as drugs of first choice for the treatment of rheumatic diseases. Now, according to their properties, they are widely used for modifying inflammation, in the prophylaxis of cerebrovascular disease, coronary artery disease, and arterial trombosis [1]. Acetylsalicylic acid (the salicylate ester of acetic acid) is still the most commonly used salicylate in Poland (Polpiryna, Polopiryna S, Polopiryna C, Asprocol, Calcipiryna, Aspirin etc.). After oral administration their absorption follows first-order kinetics with an absorption time ranging from 5 to 16 minutes. One route of metabolism of acetylsalicylic acid is the way of its rapid hydrolisis to salicylic acid (2-hydroxybenzoic acid) in reaction catalized by a nonspecific enzyme that has been referred to as aspirinase. Unlike most esterases (which are predominantly bound to the microsomal fraction) the enzymes hydrolyzing acetylsalicylic acid are present in equal amounts in the cytosol [2]. Enzymatic hydrolysis of ASA has been observed in intestinal mucosa, in liver cytosol, and in serum, each being an effective site for detoxication of xenobiotics, so only about 68% of the dose reaches the systemic circulation as an acetylsalicylic acid. The serum half time duration of acetylsalicylic acid is approximately 20 minutes. Substantial evidence indicates that mitochondria contain a cytochrome P-450 like enzyme system which is involved in the biotransformation of salicylate to reactive intermediates, capable of protein binding. Renal mitochondria were 4 times more active than liver ones in converting salicylate to 2,3-dihydroxybenzoic acid, the catechol analogue of salicylate [3]. Isolated renal mitochondria converted salicylate to its ortho hydroxylation products, 2,3-dihydro-

xybenzoate (2,3-DHBA), but produced very little gentisinate (2,5-DHBA). The formation of salicylate derived reactive intermediates can also occur via hydroxyl radials generation, but this mechanism of salicylate oxidation is still unknown. Hepatic and renal metabolism of salicylic acid produce the glicyne conjugate, salicyluric acid (49% - 72%), and the glucuronic conjugates, salicyl phenolic and acyl glucuronide as the major urinary metabolites of acetylsalicylic acid in man after therapeutic doses. The ring products of salicylic acid, gentisic acid (2,5-DHBA) and 2,3-dihydroxybenzoic acid, together with gentisuric acid and salicyluric acid phenolic glucuronide are all formed in minor amounts [4,5]. At low salicylic acid concentrations, the maximum formation of salicylic phenolic glucuronide and salicyluric acid is easy to obtain and their formation is described by Michaelis-Menten kinetics. The other metabolic products follow first-order kinetics [1]. Salicylic acid (small amounts of unchanged) and its metabolites are renally excreted. The rate of elimination is influenced by urinary pH, the presence of organic acids, and the urinary flow rate.

Metabolic pathways for trichloroethylene (TRI) appear to be qualititavely similar in mice, rats, and humans. TRI metabolism is quantitaly different as metabolic capacity is considerably lower in rats compared to mice. The amount of metabolized TRI in mice is more proportional to body surface than to body weight. It would be expected that the same would be true for humans. The main TRI metabolites are trichloroacetic acid (TCA) and trichloroethanol (TCE). Both are accepted as biological markers in the assessment of risk from TRI exposure.

Occupational exposure to high atmospheric concentrations of trichloroethylene can cause headaches and nausea, which may lead to taking analgetics. Acetylsalicylic acid and other drugs containing this substance are non-prescription medications commonly taken by people, both accidentally and intentionally, so there is the possibility that this drug might influence liver biotransformation of trichloroethylene, and finally may change the urine excretion of its main metabolites which are accepted as biological markers in the assessment of risk from exposure.

The aim of this study was to evaluate the influence of acetylsalicylic acid on the liver and kidney system of cytochrome P-450-dependent monooxygenases and the urine elimination of trichloroacetic acid and trichloroethanol in rats exposed to trichloroethylene.

Materials and Methods

Materials

All chemicals and reagents used were of analitycal grade. Trichloroethylene was purchased from Ubichem (Hampshire, England). Carbon disulfide and acetylsalicylic acid were purchased from Merck (Darmstadt, Germany).

The rats were obtained from Animal Husbandry, Department of Toxicology, Karol Marcinkowski University of Medical Sciences of Poznan, Poland.

Experimental Procedures

The experiments were performed on adult male Wistar rats weighing approximately 270 g (± 10%). The rats were housed in a humidity (60%) and temperature (22 ± 2°C) controlled room with 12/12 hours light/dark cycle. The animals were fed the standard Murigram chow and allowed tap water ad libitum.

The rats were treated in a dynamic exposure chamber (126 dm^3) for five consecutive days, six hours daily in spring (from 8 a.m. till 2 p.m.).

Two concentrations of trichloroethylene in the air were estimated: 1.5 $mmol/m^3$ and 4.5 $mmol/m^3$. Vapour trichloroethylene was generated by heating liquid solvent in a washer. The designed concentration of vapour was obtained by diluting them in the air. Trichloroethylene concentration in the chamber was monitored by gas chromato-

Table 1. Changes in urine elimination of trichloroacetic acid (TCA) and trichloroethanol (TCE) in rats exposed to trichloroethylene at the concentration of 1.5 or 4.5 $mmol/m^3$ of air (6h/day, 5 days), separately or in combination with acetylsalicylic acid (ASA) at doses of 150 or 300 mg per kg of body weight (x ± SD, n = 6).

Type of exposure	Metabolite exretion [mg]					
	TCA			TCE		
	Time of urine collection [h]					
	8-24	25-48	0-48	0-24	25-48	0-48
Trichloroethylene [1.5 $mmol/m^3$]	1.94 ± 0.21	0.88 ± 017	2.82 ± 0.16	3.52 ± 0.35	1.22 ± 0.03	4.74 ± 0.35
Trichloroethylene [1.5 $mmol/m^3$] and ASA [150 mg/kg b.w.]	1.53 ± 0.15*	0.77 ± 0.14*	2.30 ± 0.26*	3.04 ± 0.13*	0.20 ± 0.03*	3.24 ± 0.10*
Trichloroethylene [1.5 $mmol/m^3$] and ASA [300 mg/kg b.w.]	1.21 ± 0.17*	0.53 ± 0.13*	1.74 ± 0.20*	2.59 ± 0.37*	0.21 ± 0.04*	2.80 ± 0.39*
Trichloroethylene [4.5 $mmol/m^3$]	8.20 ± 0.33	1.40 ± 0.09	9.60 ± 0.38	16.48 ± 1.07	3.08 ± 0.29	19.55 ± 1.01
Trichloroethylene [4.5 $mmol/m^3$] and ASA [150 mg/kg b.w.]	7.74 ± 0.38*	1.08 ± 0.13*	8.83 ± 0.38*	14.98 ± 0.62*	2.51 ± 0.33*	17.49 ± 0.45*
Trichloroethylene [4.5 $mmol/m^3$] and ASA [300 mg/kg b.w.]	6.37 ± 0.32*	0.91 ± 0.06*	7.28 ± 0.31	13.26 ± 0.37*	1.88 ± 0.29*	15.14 ± 0.32*

* significantly different from control group exposed only to trichloroethylene, $p < 0.05$.

graphic analysis of air samples that were withdrawn from the chamber at 30 min intervals. The chromatograph Chrom-5 was used. The separation was performed with 4 m glass column packed with bentone (5%), squalene (5%), dinonyl phthalate (2%) on Chromosorb W. Nitrogen was used as a carrier gas. The column temperature was 80°C, while that of the injection port and detector was set at 100°C.

Before the start of inhalation, rats were pretreated with acetylsalicylic acid, administered intragastrically, at doses of 150 mg or 300 mg per kg of body weight (1/10 or 1/5 LD_{50} for rat). In control groups, the rats received only distilled water.

After termination of exposure, rats were placed in Simax glass metabolic cages (Czech Republic), and the urine samples were collected for the two following days. The amounts of trichloroethanol and trichloroacetic acid excreted in the urine were determined according to the Ogata et al. method [6].

72 hours after exposure termination, rats were decapitated between 8.30 and 9.30 to eliminate circadian fluctuations in the activity of the MFO system [7-10]. Liver and kidney microsomes were isolated by the method of Dallner [11]. In the microsomal fractions cytochromes P-450 and b_5 contents were determind by the method of Estabrook and Werringloer [12]. NADPH-cytochrome P-450 reductase and NADH- cytochrome b_5 reductase activities were determined by Hodges and Leonard [13].

Cytochromes levels were expressed in nanomoles of a cytochrome per 1 milligram of microsomal protein, whereas both the reductase activites were expressed in micromoles of reduced cytochrome c per 1 min/mg protein. The protein concentration was determined by the method of Lowry et al. [14] with bovine albumin as a standard.

Statistical Analysis

The results of biochemical analysis were expressed as the arithmetic mean of six or three independent measurements for liver and kidney, respectively. To compare different groups we used Student's t-test at $p = 0.05$.

The levels of metabolites in urine were presented as the arithmetic mean of six independent measurements. Analysis of variance and the Tuckey test were used for statistical evaluation of the results [15, 16].

Results

Elimination of Trichloroacetic Acid and Trichloroethanol with Urine

The results of urine elimination of trichloroacetic acid (TCA) and trichloroethanol (TCE) in rats exposed to trichloroethylen (TRI) at the concentration of 1.5 mmol/m^3 or 4.5 mmol/m^3 of air, separately or together with acetylsalicylic acid (ASA) at doses of 150 mg or 300 mg per kg of body weight are presented in Table 1.

Our results show that ASA, in dependently of dose, diminished excretion of trichloroacetic acid and trichloroethanol in both experimental inhalations of trichloroethylene at the concentration of 1.5 mmol/m^3 and 4,5 mmol/m^3. At the lower concentration of TRI (1.5 mmol/m^3) we observed

Table 2. Liver and kidney systems of cytochrome P-450-dependent monooxygenases in rats exposed to TRI (trichloroethylene) at concentrations of 1.5 mmol/m^3 or 4.5 mmol/m^3 of air, separately or together with ASA (acetylsalicylic acid) *per os* at doses of 150 or 300 mg per kg of body weight (6h/day, 5days, $x \pm SD$, $n = 6$). For more information see Materials and Methods. Abbreviations used: P-450, cytochrome P-450; NADPH, NADPH-cytochrome P-450 reductase; cyt. b_5, cytochrome b_5; NADH, NADH-cytochrome b_5 reductase.

Type of exposure	Liver system				Kidney system			
	P-450	NADPH	Cyt. b_5	NADH	P-450	NADPH	Cyt. b_5	NADH
Control group	0.829 ± 0.058	0.131 ± 0.011	0.807 ± 0.109	0.606 ± 0.019	0.181 ± 0.007	0.022 ± 0.001	0.144 ± 0.004	0.168 ± 0.008
ASA [150 mg/kg b.w.]	0.897 ± 0.103	0.109 ± 0.010*	0.705 ± 0.038	0.451 ± 0.031*	0.196 ± 0.004	0.214 ± 0.001	0.151 ± 0.001	0.125 ± 0.006*
ASA [300 mg/kg b.w.]	1.068 ± 0.140*	0.125 ± 0.009	0.720 ± 0.058	0.665 ± 0.076	0.205 ± 0.007*	0.023 ± 0.001	0.160 ± 0.008	0.144 ± 0.005*
TRI [1.5 mmol/m^3]	0.649 ± 0.141*	0.100 ± 0.009	0.586 ± 0.015*	0.461 ± 0.010*	0.144 ± 0.006*	0.018 ± 0.001*	0.123 ± 0.004*	0.125 ± 0.006*
TRI [1.5 mmol/m^3] and ASA [150 mg/kg b.w.]	0.696 ± 0.015*	0.105 ± 0.011	0.503 ± 0.017*	0.573 ± 0.022	0.142 ± 0.004*	0.022 ± 0.001	0.129 ± 0.003*	0.137 ± 0.004*
TRI [1.5 mmol/m^3] and ASA [300 mg/kg b.w.]	0.792 ± 0.021*	0.103 ± 0.009	0.516 ± 0.015*	0.521 ± 0.014*	0.152 ± 0.008*	0.026 ± 0.001	0.129 ± 0.002*	0.139 ± 0.005*
TRI [4.5 mmol/m^3]	0.648 ± 0.109*	0.093 ± 0.006*	0.600 ± 0.065*	0.349 ± 0.028	0.113 ± 0.003*	0.016 ± 0.001*	0.112 ± 0.001*	0.097 ± 0.003*
TRI [4.5 mmol/m^3] and ASA [150 mg/kg b.w.]	0.648 ± 0.079*	0.145 ± 0.012	0.553 ± 0.054*	0.494 ± 0.041*	0.119 ± 0.003*	0.021 ± 0.001	0.118 ± 0.004*	0.142 ± 0.001*
TRI [4.5 mmol/m^3] and ASA [300 mg/kg b.w.]	0.833 ± 0.057	0.153 ± 0.016*	0.584 ± 0.052*	0.525 ± 0.041*	0.139 ± 0.004*	0.022 ± 0.001	0.118 ± 0.004*	0.150 ± 0.001*

* significantly different from control group, $p < 0.05$.

a stronger inhibitory effect of ASA than in others. The elimination of TCA in the first 24 hours after exposure was diminished by ASA at dose of 150 mg/kg of b.w. by about 21%, whereas a dose of 300 mg/kg was about 38%. The excretion of TCE was weakly inhibited by about 14% and 26%, respectively. In rats exposed to trichloroethylene at a concentration of 4.5 mmol/m^3, ASA at the dose of 150 mg inhibited TCA and TCE excretion by 6% and 9%, respectively, whereas in dose of 300 mg, the elimination of both trichloroethylene metabolites was reduced by about 22% and 20%.

Liver Cytochrome P-450-dependent Monooxygenase System

In Table 2 the results of contents of cytochrome P-450 and cytochrome b_5 as well as activities of reductases of NADPH-cytochrome P-450 and NADH-cytochrome b_5 in liver and kidney microsomal fraction are presented.

After the lower ASA dose liver cytochrome P-450 content tended to increase, while the 1/5 DL_{50} dose of ASA significantly induced this protein (about 130% of the control value; Table 2). NADPH-cytochrome P-450 reductase activity markedly decreased after the lower ASA dose (about 85% of control value, Table 2) and did not change after the higher dose. After both ASA doses, cytochrome b_5 tended to decrease, but the decrease was not significant ($p < 0.05$). The 1/10 DL_{50} dose of ASA inhibited enzyme activity, while after the 1/5 DL_{50} dose the activity tended to increase (Table 2).

Inhalation of TRI at 1.5 mmol/m^3 caused a decrease in cytochrome P-450 content (to 85% of control value) but did not alter NADPH-cytochrome P-450 reductase activity. TRI also decreased cytochrome b_5 content (95% of control value) and NADH-cytochrome b_5 reductase activity (80% of control value; Table 2).

The lower ASA dose combined with TRI decreased the extent of inhibition of cytochrome P-450 (90% of control value). The higher dose slightly induced this hemoprotein (Table 2). None of the ASA doses used in combination with TRI altered the activity of NADPH-cytochrome P-450 reductase as compared with the control.

The decrease in cytochrome b_5 content caused by TRI combined with ASA was greater than after TRI alone. In contrast, the inhibitory effect of TRI on NADH-cytochrome b_5 reductase activity was reduced by the lower ASA dose, but the higher ASA dose combined with TRI decreased this enzyme activity (about 90% of control value). Inhalation with TRI at 4.5 mmol/m^3 still more decreased cytochrome P-450 content (about 80% of control value) and produced a comparable decrease in NADPH-cytochrome P-450 reductase activity. Cytochrome b_5 content and NADH-cytochrome b_5 reductase activity were also decreased, 75% and 55% of control value, respectively (Table 2). TRI at 4.5 mmol/m^3 combined with 150 mg/kg ASA decreased cytochrome P-450 content, but the decrease was no longer observed after the higher dose of ASA. Acetylsali-cylic acid reversed the inhibiting effect of TRI on NADPH-cytochrome P-450 reductase activity, and the higher ASA dose even increased this activity (160% of the value found in TRI-treated rats). ASA still more decreased cytochrome *b$* content compared with the content decreased by TRI alone, and reduced the inhibiting effect of TRI on NADH-cytochrome b_5 reductase activity.

Kidney Cytochrome P-450-dependent Monooxygenase System

The lower ASA dose (150 mg/kg) had no effect on kidney cytochrome P-450 content, while the doubled dose increased it (Table 2). Both ASA doses had no effect on kidney NADPH-cytochrome P-450 reductase activity. In contrast, NADH-cytochrome b_5 reductase activity markedly decreased after ASA, while cytochrome b_5 content remained unchanged.

TRI at the lower dose decreased the levels of cytochromes P-450 and b_5 to 90% and 75% of control values, respectively. The activities of their reductases also decreased (Table 2).

TRI combined with ASA had no significant effect of cytochrome P-450 content as compared with the effect of TRI alone. However, ASA reduced the inhibiting effect of TRI on NADPH-cytochrome P-450 reductase activity; even the lower ASA dose restored the reductase activity to the control level.

Cytochrome b_5 content was not changed by the combined action of ASA and TRI when compared with that after TRI alone. The higher ASA dose reduced the inhibiting effect of TRI on NADH-cytochrome b_5 reductase activity, although enzyme activity was still 80% of control value.

TRI inhalation (Table 2) inhibited all components of both microsome electron transport chains. The weakest inhibiting effect was found for NADPH-cytochrome P-450 reductase (about 75% of control value), while the other reductases activity decreased almost 2-fold. Both cytochromes decreased to about 60% of control values.

ASA did not neutralize the inhibiting effect of TRI on cytochrome P-450 content, but only slightly modified it. In contrast, the inhibition of NADPH-cytochrome P-450 reductase activity by TRI was reduced even by the lower ASA dose. Compared with TRI alone, both ASA-TRI combinations did not affect cytochrome b_5 content but reduced the inhibiting effect of TRI on NADH-cytochrome b_5 reductase activity. This activity, however, was still lower compared with control (about 85% of control value).

Discussion

Trichloroethylene is an industrial solvent that has been extensively used for the degreasing of metals and a variety of other purposes. TRI is regarded as a weak hepatotoxicant. Trichloroethylene is metabolized by the liver microsomal cytochrome P-450 system to reactive intermediates, which are considered responsible for its hepatotoxicity. The izoenzymes of cytochrome P-450 involved in trichloroethylene metabolism have been identified as CYP 2E1, CYP 2B1/2, CYP 2C11, and others [17]. Phenobarbital and ethanol are inducers of cytochrome P-450, which accelerates the TRI metabolism and potentiates its hepatotoxicity [18, 19].

In rats metabolism of orally administered acetylsalicylic acid resulted in excretion of 81-91% dose in urine in the first 24 h. Salicylic acid is the major urinary metabolite (43%-51%). The excretion of salicyluric acid decreased with increasing dosages, whereas the excretion of gentisic acid and salicyl phenolic and acyl glucuronides increased [5]. The profile of acetylsalicylic acid metabolites is quali-

tatively similar in man and rat. But there are quantitative differences. The rat, like man, has a limited capacity to form salicyluric acid but relies on this pathway to a lesser extent; saturation was compensated for by increased utilization of other routes. Human dependence on salicyluric acid formation was high and in overdose, compensation by other routes was incomplete [4, 5, 20].

TRI and ASA show differences in their toxicokinetics, mainly with respect to metabolism. TRI is transformed by oxidation to trichloroethylene oxide, which is subsequently converted to chloralhydrate. Chloralhydrate is partialy reduced to TCE and partialy oxidized to TCA. The first step of the metabolic conversion of TRI to epoxides is catalyzed by an appropriate cytochromes P-450 [17]. ASA is rapidly hydrolyzed by nonspecific enzymes to salicylic acid. Hepatic and renal biotransformation of salicylic acid produce the glicyne and glucuronic conjugates as the main metabolites. Gentisic acid as well as 2,3-dihydroxybenoic acid together with gentisuric acid and salicyluric acid phenolic glucuronide are formed in minor amounts [5].

The mechanism of inhibitory effects of acetylacetic acid upon TRI transformation to TCA and TCE is not clear.

Certain administrated chemicals may alter the metabolism of both substances. The inhibition of acetyl salicylic acid metabolism has been demonstrated following treatment with benzoic acid, salicylamid and m-xylene. In contrast, the elimination of salicylic acid is enchanced in oral contraceptive steroid users and during corticosteroid treatment [21]. The biotransformation of trichloroethylene may be changed, for example by ethanol [22-24], organic solvents [25-28] and drugs [29].

The decrease in urine TCE excretion with a concomitant increase in the ASA dose can be explained by a higher rate of oxidation TCE to either chloral hydrate (CH) [30] or TCA **[31,** 32]. The major metabolic pathway of TCE is oxidation to CH by cytochrome P-450 [33]. Cytochrome P-450 content slightly increased after ASA treatment, suggesting a higher rate of conversion of TCE into CH than into TCA. The concomitant decrease in urine TCA excretion indicates that TCE was not converted into TCA.

We found that the amount of TRI metabolites in rat urine increased rapidly with increasing TRI concentrations to which the rats were exposed. Surprisingly, concomitant administration of ASA and TRI did not increase the levels of metabolites excreted in urine. ASA is a weak inducer of cytochrome P-450 [34, 35], and this cytochrome is directly involved in the metabolism of TRI. To explain this finding, one should study changes in the levels of P-450 isoforms, but it seems that CYP2E1 and CYP2B1/2 play key roles [17, 36, 37].

Low TRI concentrations also affect other liver parameters. Firstly, relative rat liver weight increases [38], and secondly, prolonged TRI treatment results in the proliferation of peroxisomes [39]. The latter may change the metabolic pathway of TRI in hepatocytes, and perhaps this is the cause of our findings.

TRI treatment slightly decreased cytochrome P-450 content, which disagrees with some reports [19] and is consistent with other authors [40, 41]. It decreased the rate of its metabolism indicating that TRI damages target enzymatic systems [42]. Literature data show that especially phenobarbital-induced P-450 isoforms are damaged [17, **18],** which definitely confirms the hepatotoxicity of TRI [42], and which impairs the conversion of TRI into TCE and TCA.

The decreasing percentages of TCE and TCA excreted in urine indicate the more effective excretion of TRI through the lungs [43]. However, one should bear in mind that the non-metabolized TRI is also excreted in urine. The amount of non-metabolized TRI may reach 20% of the whole dose [40] or more [44] when the dose is high. Another explanation is that ASA, which was shown to be a P-450 inducer, enhances other enzyme activities, including UDP-glucuronic acid transferase. An increased activity of the transferase accelerate the binding of TCE and TCA to glucuronic acid, thus increasing the elimination of this metabolite in the form of glucuronate.

The changes observed in the MFO system are in agreement with other reports [17, 32, 39]. We have shown that TRI inhibits both microsome electron-transport systems, which convert TRI into TCA and TCE, the compounds having no effect on MFO system activity [45]. One can therefore state that TRI impairs the function of the MFO system by triggering lipid peroxidation [31]. However, the question still remains whether ASA reverses the effect of TRI by inhibiting lipid peroxidation or in another way [46]. This issue requires further study.

Interestingly, both the hepatic and renal MFO systems showed comparable alterations. It is widely known that the renal MFO system is more sensitive [47]. It is difficult to find an explanation for this similarity. The reason may be the experimental model in which TRI inhaled by rats was metabolized mainly in the lungs, while the renal MFO system was spared.

In conclusion, ASA stimulates cytochrome P-450 but other components of MFO system. Obtained results showed protect the MFO system from the negative effect of TRI vapors, especially at high doses of trichloroethylene.

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