

# Experimental study of toxic effect of neutral and acid Anolyte on the body of a warm-blooded animal

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*Neutral Anolyte (AN, ANK) and acid Anolyte (A) do not cause death of laboratory animals when they are introduced orally in daily doses of 30ml for 3 days. High (A) Anolyte doses accounted for changes of behavioural reactions in laboratory warm-blooded animals, as well as for marked vegetative responses. After prolonged percutaneous application of Anolyte with high and medium concentration of active chlorine all three types of invested Anolytes caused dermatitis. In case of AN and ANK application it was manifested as skin dryness and desquamation, and in case of A Anolyte – as developing dermatosis on the background of punctuate haemorrhages. Low concentrations of AN, ANK and A Anolytes (active chlorine concentration not higher than 150mg/l) do not produce skin-irritating effect. All three Anolytes types possess no sensitising ability and cause no allergic reactions if their active chlorine concentration is 150mg/l. Intravenous injection of Neutral Anolytes to laboratory animals with active chlorine concentration over 150-200mg/l resulted in their death, apparently due to haemolytic effect and development of organ and tissue hypoxia. A tolerant dose of active chlorine compounds when AN is injected intravenously is 60mg/kg; for ANK it is 80 mg/kg.*

This study was aimed at studying the peculiarities of biological action of Anolytes used as disinfectants. The Anolytes were analysed according to their physical-chemical parameters: pH and ORP, which were measured with the help of a portable pH-meter of the type pH-150. Active chlorine concentration was determined by iodometry [3.].

Toxicological analyses included:

- Evaluation of AN, ANK and A Anolyte toxicity parameters in an acute experiment when the above Anolytes were infused intragastrically;
- Assessment of clinical picture (using no special investigation methods) when AN and ANK were injected intravenously;
- Estimating skin-resorptive action of all Anolyte types
- Investigation of allergenic activity of An, ANK and A Anolyte when they were applied percutaneously.

As test animals, white mongrel rats, rabbits and light-colour guinea pigs were used. The animals with light colour skin were chosen for easier registration of their pathologic changes in case of dermatitis and skin allergic reaction [1]. Toxicological studies were carried out in conformity with recommendations and methods presented in [2,6,7,8]. Sensitisation development was investigated in reactions of specific leukocytolysis (RSLL) [4], reaction of specific micro-precipitation of humoral antibodies, circulating in blood (RSMP) [5], and reaction of specific leukocyte agglutination (RSLA) [1]. Before experiment, working hapten dilutions of Anolyte doses causing no positive RSLL, RSMP and RSLA reactions in intact animals were determined.

The results of reactions proving sensitisation development were estimated on days 7 and 14 of experiment. At the first stage, acute AN, ANK and A Anolyte toxicity was studied in conditions of intragastrical infusion. 90 white mongrel male rats were used for the experiment, each weighing 200g on the average. All animals were streamed into 3 group of 30 animals. Anolyte solutions were injected once, intragastrically, by a syringe supplied with a probe. The doses were 10ml,

i.e. maximal tolerable single injections to laboratory animals of the given species and age (body weight).

The first group of animals received AN Anolyte. The animals were divided into 5 subgroups each containing 6 of them. The first subgroup received An with an active chlorine content of 728mg/l and pH 5.75. The following groups were given Anolyte of 609mg/l, 497mg/l, 420mg/l and 310mg/l. Anolyte was always given to fasting rats, and after priming the animals were not fed for 8 hours. In all test groups the behavioural reactions of the animals were not different from those of intact rats. Cognitive reflex of test animals was not effected, they were active throughout the whole period of observation.

Two days after the first primer, the animals of the first subgroup receiving Anolyte with the highest content of active chlorine were given 10 ml portions of Anolyte with initial 755mg/l and pH 6.0 every 4 hours, the whole amount of Anolyte given being 30ml. The rats were observed for 72 hours. There were no changes in behavioural reactions, animal death was not registered either.

Estimation of doses, calculated for mg/kg of body weight by active chlorine, given to the animals was as follows: 146 for the first subgroup, 122 for the second, 99 for the third, 84 for the fourth, and 62 for the fifth. As result of portion Anolyte intake, each animal of the first subgroup received a total dose of 453mg per 1 kg of the body weight.

The animals of the second group were given ANK Anolyte. The rats were also divided into 5 subgroups, and each of them in the same order received ANK with active chlorine concentration of about 100mg/l less than the proceeding one. Thus the first subgroup was given ANK of 820mg/l, the second 711mg/l, the third 591mg/l, the fourth 407mg/l, and the fifth 300mg/l. The test animals were observed for 72 hours. No death of rats was registered. No deviations from the standard in their behavioural reactions were noted.

In the same way as in the first part of the experiment, 24 hours after the experiment's beginning the animals of the first group were given 30ml of ANK, in 10ml portions. The initial Anolyte concentration was 806mg/l and pH 7.95. During the following three days of observation, there were no pathological symptoms in the primed animals' behaviour.

Estimated in mg/kg of the body weight, ANK concentration were as follows: 164mg/kg for the first subgroup, 142 for the second, 118 for the third, 81 for the fourth, and 60 for the fifth. The animals of the first subgroup in the course of fragmentally intake received about 483mg/kg by active chlorine.

The animals of the third group received A Anolyte intragastrically. They were also streamed into 5 subgroups, and the plan of Anolyte introduction was similar. A concentration was 548mg/l, 402,329,200,117; the pH varied between 3.59 and 4.21. Several minutes after infusion the animals of the first two subgroups manifested obvious anxiety, swiftly moved about the cage sometimes falling on the floor with their stomachs. After 30-40 minutes the animals became quiet, rolled themselves up into a ball, and practically did not move for 2-3 hours. Later their behaviour was no different from that of the control group. In other test groups there was no deviations in the behavioural reactions of the animals. There were no rat deaths either during the experiment, or after it.

Similarly to the above described experiment plans, the animals of the first subgroup were fragmentally given A Anolyte with an active chlorine doses of 560mg/l and pH4, 30 ml altogether

(in 10ml portions). After each intake the rats manifested deep anxiety. The above indicated symptoms were more pronounced. After the third intake the animals bunched in a cage corner and were inactive for a long time. Their pain and pupillary reflexes were checked. The reflexes were not decreased in comparison with the control group.

Taking into account flaccidity of behaviour and cold extremities, rectal temperature was taken with a medical contact electric thermometer TPEM-1. The average rectal temperature of the control animals was  $37.4 \pm 0.59$  °C. 20 minutes after the third fragmental dose of A Anolyte was given to the test animals, their temperature was observed to fall, and the average temperature was  $35.87 \pm 0.74$ °C. Two hours after A Anolyte infusion the temperature continued to be low compared to the control group animals and was  $36.2 \pm 0.39$ °C. The body temperature became normal 24 hours after priming.

Analysis of the obtained results indicates that high acid A Anolyte concentrations cause rather strong vegetative reactions expressed in decreased body temperature. Behavioural reactions of the rats of the first two subgroups returned to normal 24 hours after the end of intragastrically priming. There were no animal deaths in any of the experimental rat subgroups.

Summing up the results of the acute toxicological experiment on infusing the Anolytes to warm blooded laboratory animals, it should be stated that all the studied disinfectant solutions are practically non-toxic. Vegetative reactions observed in animals primed with acid A Anolyte were caused, in all probably, by its rather acid reaction and acidosis development, but not by active chlorine concentration.

A second stage of the experiment was studying skin-irritating, skin-resorptive and skin-allergic reaction of the Anolytes.

Skin-irritating and skin-resorptive effects of AN, ANK and A Anolyte were assessed in the course of experiments on white mongrel rats, whose tails by 2/3 of their length were immersed into examined solutions for 4 hours a day during 10 days.

During the entire period of investigation visual assessment of the test-animals skin condition was performed, and to detect possible resorption, the content of SH and SS-groups as well as ATPase activity and the state of peroxide lipid oxidation (PLO) in the blood of the test-rats was estimated. The findings of the biochemical research are given in Table 1.

Anolyte	Active chlorine concentration, mg/l	Biochemical blood indicators			
		SH-group (microMol/ml)	SS-Group (microMol/ml)	ATPase (microMol/ml)	PLO (microMol/ml)
AN	710	8.67±0.64	6.5±0.32	1.62±0.05	7.53±0.44
	300	8.16±0.48	5.8±0.48	1.67±0.06	7.35±0.51
	150	8.83±0.32	6.0±0.48	1.63±0.07	8.03±0.62
ANK	625	8.00±0.81	6.5±0.48	1.63±0.07	8.28±0.73
	335	8.33±0.32	6.7±0.32	1.60±0.07	7.75±0.51
	120	8.83±0.64	6.3±0.32	1.63±0.09	7.57±0.60
A	500	10.00±0.48*	5.7±0.64	1.50±0.06*	7.07±0.39
	230	8.83±0.32	6.7±0.32	1.57±0.07	8.38±0.58
	100	9.00±0.48	6.8±0.32	1.67±0.08	7.63±0.58
Control		9.00±0.32	6.5±0.48	1.64±0.05	8.03±0.5

\* = statistically significant tendency ( $t > 1.5$ )

As can be seen from the resented data, the studied solutions did not produce resorptive action on intact skin. Only acid A Anolyte increased SH-groups, reduced ATPase activity and the PLO content in the blood of the test animals. However these changes were not significant and could be characterised as a statistically significant tendency ( $t > 1.5$ ).

It should be noted that high-concentrated An, ANK and A caused skin dermatitis symptoms. Thus AN and ANK produced tail skin desquamation and dryness starting from the third day of the experiment, and these manifestations were most pronounced at the end of the experiment. When A Anolyte was used four out of six rats demonstrated tiny haemorrhages, which appeared two days after the beginning of the experiment. Three days later skin reactions were registered in all test animals of this group. Towards the end of this experiment erythema was bright, and on its background symptoms dermatitis – skin desquamation- appeared.

Average AN and ANK concentrations accounted for slight white rats' tail skin desquamation on day 6 of the experiment. A Anolyte of 230mg/l concentration triggered erythemous reactions on day 5 of the experiment. Towards day 8, these effects aggravated accompanied by skin desquamation. The lowest concentrations of all studied Anolytes caused no skin effects.

Allergenic properties of Anolytes were investigated in the course of a special experiment with the aim to assess their sensitising effect if applied percutaneously. 24 light-colour guinea pigs weighing 280-350g were used for the experiment. The animals made up three test groups and one control one. Anolyte with active chlorine of 150mg/l were used in the experiment. The studied solutions were daily applied on preliminarily depilated skin segment of 1x1cm in size ,for 14 days. The results of the experiments are given in Table 2.

Observation period, days	RSLL (%)			
	Control	AN	ANK	A
7	9.1	8.3	9.3	9.0
14	8.7	8.8	8.8	8.1
Observation period, days	RSLA (in points $X \pm m$ )			
	Control	AN	ANK	A
7	0.37±0.27	0.31±0.15	0.41±0.15	0.35±0.2
14	0.44±0.30	0.38±0.20	0.52±0.22	0.47±0.15
Observation period, days	RSMP ( $\log_2$ of antibody titer)			
	Control	AN	ANK	A
7	-	-	-	-
14	0	0	0	0

As can be seen from the experimental results, none of the studied Anolytes demonstrated allergenic properties if applied percutaneously

At the final stage of the experiments the effect of intravenously injected Anolytes on warm-blooded animals was assessed. The objects of the given type of Anolyte exposure were rabbits weighing on the average 3.1 kg. Altogether 10 animals were subjected to AN and ANK injections. 20ml portions of the studied solutions were injected to each animal intravenously with the help of a syringe. The solutions were injected slowly, the total volume of the Anolytes was injected in 3-4 minutes. Since the solutions themselves were disinfectants, they were not sterilised. The animals were observed during the first three days from the moment of injection. Special clinical or laboratory diagnostic tests were not performed. General behavioural reaction and mortality of the animals was controlled. The following active chlorine concentrations of Anolytes were tested: AN- 600,500,400,300,150mg/l ; ANK- 500,400,300,200,100mg/l.

The results of the investigations show that after injecting a 600mg/l dose of AN, the animals death was registered in 1.5 hours after the moment of injection. 500mg/l dose caused death after 2 hours 25 minutes, 400mg/l after 15 hours, and 300mg/l one day after the moment of exposure. In the process the dose of substance the animals received, as counted in active chlorine quantity, was as followed: 240mg/kg of body weight, 200mg/kg, 160mg/kg, and 120mg/kg respectively. When 150mg/l of AN was injected (in active chlorine 60mg/kg of body weight) the animals remained alive, there were no deviations of behavioural reactions as compared to the control group. In several minutes after the moment of injecting the dying animals became flaccid, demonstrated decreased behavioural activity; in the following periods of observation they rejected food, and were in a comatose state when death occurred.

Similar picture was observed when ANK was injected. The animals receiving Anolyte with active chlorine dose of 500mg/l (200mg/kg of body weight), 400mg/l (160mg/kg) and 300mg/l (120mg/kg) died. The clinical picture was similar to that observed during AN injection. The animals receiving Anolyte with the chlorine concentration of 200mg/l (80mg/kg) and 100mg/l (40mg/kg) remained alive, and did not manifest any changes of behavioural reactions.

Thus, parental injection of large doses of neutral Anolyte leads to death of warm-blooded animals. Taking into account high oxidising ability of Anolyte we can assume that the rabbits' death resulted from hemolysis of erythrocytes and further hypoxia of the body's organs and tissues.

Doses of AN Anolytes 150mg/l (60mg/kg), ANK 200mg/l and 100mg/l (80mg/kg and 40mg/kg) did not lead to death of laboratory animals, so they can be considered inactive in this parameter.

#### Conclusions:

1. Neutral Anolyte (AN ,ANK) and acid Anolyte (A), taken per Os, do not cause death of laboratory animals.
2. Only high doses of A Anolyte led to changed behavioural reactions in warm-blooded laboratory animals. Apparently, that is associated with acid pH of infused solutions and acidosis development, and not with the action of chlorine contained in the solution.
3. Prolonged percutaneous application of all three types of the examined Anolytes with high concentration of active chlorine generates dermatitis, which manifest itself by skin dryness and desquamation when AN and ANK are applied, and dermatosis development on the background of punctate haemorrhages when A Anolyte was applied.
4. Average concentrations of all three Anolytes cause similar skin reaction
5. Low concentrations of all three Anolytes do not produce skin-irritating action.
6. All three types of Anolytes possess no sensitising ability and cause no allergic reactions in a dose to 150mg/dm<sup>3</sup> by active chlorine.
7. Intravenous injection of AN and ANK to laboratory animals in doses exceeding 150-200mg/dm<sup>3</sup> by active chlorine results in lethal outcome associated in all probability with the haemolytic action of the studied solutions and development of organ and tissue hypoxia.