MEDICAL AND BIOLOGICAL ASPECTS RELATED TO ASSESSMENT OF IMPACTS EXERTED BY RISK FACTORS

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SUBSTANTIATING INDICATORS FOR ASSESSING POPULATION HEALTH RISKS BASED ON EXPERIMENTAL INVESTIGATION APPLYING BIOMARKERS OF EFFECT PRODUCED BY N-NITROSAMINES

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N-Nitrosodimethylamine (NDMA) is a nitrosamine with established hepatotoxicity. However, unlike its well-studied carcinogenic effects in model organisms, quantitative indicators for assessing non-carcinogenic risks under chronic exposure remain undetermined. In the context of hepatotoxicity, key biomarkers include aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT). Analyzing changes in activity of these enzymes in controlled experimental conditions allows for identifying dose-response relationships even for non-specific liver injury markers.

The study aimed to establish health risk assessment indicators based on experimental research using biomarkers of effect established for N-nitrosamines.

A 6-month toxicological experiment was conducted on Wistar rats randomly divided into five groups that were administered different oral doses of NDMA to interpolate the dose-response curve.

Dose-effect relationships were assessed using nonlinear regression analysis. Safe exposure levels for NDMA were determined using the Benchmark Dose Lower Limit (BMDL) approach with the sliding window method. Statistical analysis was performed using the Mann-Whitney U-test. Uncertainty factors were applied when converting BMDL to a reference dose (RfD).

Among the analyzed hepatotoxicity biomarkers, GGT demonstrated the highest diagnostic sensitivity and a pronounced dose-dependent response ($p \leq 0.05$). Mathematical modeling of the GGT-NDMA dose relationship yielded statistically significant indicators characterizing hepatotoxic effects. Based on significant biomarker excess, a BMDL of 0.0055 mg/kg body weight was established, and an RfD was substantiated for chronic NDMA exposure equal to $5.73 \cdot 10^{-6}$ mg/kg body weight. Dose – response indicators were established (b0 = -2.94, b1 = 35.96) for assessing non-carcinogenic risks associated with liver dysfunction. Thus, this study provides quantitative indicators for evaluating health risks under chronic NDMA exposure.

Keywords: *risk assessment, NDMA, hepatotoxicity, toxicological experiment, effect biomarkers, dose-effect, chronic exposure, BMDL, reference dose.*

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N-nitrosodimethylamine (NDMA) is a nitrosamine, a chemical with pronounced hepatotoxic and carcinogenic effects [1–4]. Although carcinogenic effects produced by NDMA have been examined quite sufficiently on animal models, issues associated with assessing its non-carcinogenic effects upon repeated exposures have not been given as much attention. The liver is the primary target organ for NDMA upon oral administration even at low doses [5, 6] due to peculiarities of its metabolism. After it is absorbed in the gastrointestinal tract, the chemical undergoes biotransformation in hepatocytes involving formation of reactive metabolites able to induce oxidative stress and damage to liver cells evidenced by biomarkers of toxic effects occurring in peripheral blood [7–9]. Therefore, assessment of hepatotoxic effects produced by NDMA becomes especially significant in studies focusing on its non-carcinogenic effects.

Use of biomarkers makes it possible to develop indicators for assessing non-carcinogenic health risks including threshold exposure levels and safe exposure ranges. Current studies pay special attention to searching and validating effect biomarkers, which can be used as indicators of toxic effects at various biological levels in the body [8]. Within assessment of NDMA hepatotoxicity, biochemical liver function indicators are of considerable interest, in particular, aspartate transaminase (AST), alanine transaminase (ALT) and gamma-glutamyl transferase (GGT). These indicators are non-specific hepatic damage indicators; nevertheless, they can reflect intensity of toxic effects in a controlled experiment [9, 10].

Toxicological studies performed on animal models have established a significant relationship between NDMA exposure and changes in the activity of liver enzymes [11]. Analysis of changes in the activity of these enzymes in a controlled experiment makes it possible to establish a dose – response relationship even for non-specific hepatic damage indicators. Use of up-to-date statistical methods for analyzing data obtained by toxicological studies on animal models makes it possible to establish quantitative indicators for risk assessment including calculation of acceptable daily intake (ADI). The established values turned out to be even stricter than those previously accepted by the Environmental Protection Agency of the United States (US EPA), which emphasizes the necessity to revise the existing approaches to assessing risks of NDMA exposures.

Therefore, **the aim of this study** was to substantiate health risk assessment indicators based on experimental research using effect biomarkers established for N-nitrosamines.

Materials and methods. The study was accomplished within implementation of the Sanitary Shield Federal Project in international cooperation between the Russian Federation and the Socialist Republic of Vietnam[1].

An experiment was conducted on Wistar rats, which were randomly divided into five groups, 13 animals in each and with approximately even male-to-female ratios (7:6 or 6:7 depending on a group). The groups differed per NDMA doses administered *per os* for interpolating the dose – response curve and the dose – NDMA level in blood curve. Group I was administered a dose of 0.04 mg/kg of body weight (b.w.) (0.001 × LD50); Group II, 0.002 mg/kg b.w. (LOAEL) [7]); Group III, 0.0004 mg/kg b.w. (0.00001 × LD50); Group IV, 0.00002 mg/kg b.w. (the dose analog to the maximum one consumed by people with meat products); Group V (control) was given distilled water. The chronic experiment lasted 180 days. NDMA was administered into the experimental animals daily on empty stomach

[1] O realizatsii federal'nogo proekta «Sanitarnyi shchit strany – bezopasnost' dlya zdorov'ya (preduprezhdenie, vyyavlenie, reagirovanie)»: Postanovlenie Soveta Federatsii Federal'nogo Sobraniya Rossiiskoi Federatsii № 673-SF ot 22 noyabrya 2023 goda [On implementation of the Federal Project 'Sanitary Shield as Health Safety (Prevention, Detection, Reaction)': The Order by the Federal Council of the Federal Assembly of the Russian Federation No. 673-SF dated November 22, 2023]. *SF*. Available at: http://council.gov.ru/activity/documents/150478/ (June 20, 2025) (in Russian).

via the tube and the doses were adjusted per body weight. The ranges of individual doses were as follows: Group I, $6.16 \cdot 10^{-3}$–$1.54 \cdot 10^{-2}$ mg/kg b.w.; Group II, $3.20 \cdot 10^{-4}$–$7.16 \cdot 10^{-4}$ mg/kg b.w.; Group III, $7.03 \cdot 10^{-5}$–$1.54 \cdot 10^{-4}$ mg/kg b.w.; Group IV, $3.43 \cdot 10^{-6}$–$7.25 \cdot 10^{-6}$ mg/kg b.w. Blood samples for analysis were taken monthly; potential effect biomarkers (AST, ALT, and GGT) were measured with a kinetic (UV-spectrophotometry) method in a certified laboratory located in Vietnam. The study was approved by the local ethics committee of the Rospotrebnadzor's Federal Scientific Center for Medical and Preventive Health Risk Management Technologies (the Meeting Report No. 12 dated March 21, 2023) and conducted in conformity with the conventional principles for scientific research stipulated in the WMA Declaration of Helsinki (as edited in 2013).

Statistical analysis was performed in Statistica 13.0. Intergroup comparisons were accomplished using the non-parametric Mann – Whitney U-test. Intergroup differences were considered significant at $p \leq 0.05$, as shown in the relevant graphs. The dose – response relationship was examined by using non-linear regression analysis. Quality of the models was estimated using the determination coefficient (R^2), which reflected the proportion of explained dispersion in the dependent variable. The models were checked for adequacy using the following statistical indicators: the likelihood ratio test (Fisher's test (F-test)), statistical significance (p-value) and Odds Ratio (OR). Interpretation of the model parameters involved analyzing the absolute term (B_0), which represented the baseline blood levels of the selected biomarkers when the predictor values were equal to zero, as well as the regression coefficient (B_1) for NDMA, which reflected a change in a level of a biomarker per a one-unit change in a predictor.

The experimental data were visualized using both graphs and box plots (box-and-whiskers), which showed the median (the central line), 25th and 75th percentiles (the boundaries of the box) and the lowest and highest values within 1.5 interquartile ranges (whiskers). Connecting lines with the stated significance level ($p \leq 0.05$) were employed to visualize significant intergroup differences.

Safe exposure levels for NDMA were determined using the Benchmark Dose Lower Limit (BMDL) approach with the sliding window method[2]. The mathematical model parameters were determined using the formula 1:

$$p = \frac{1}{1 + e^{-(b_0 + b_1 x_1)}}, \quad (1)$$

where p is probability of the response;

x_1 is the dose, mg/kg b.w. a day;

b_0, b_1 are the model parameters.

The reference dose / acceptable daily intake (RfD / ADI) was established in conformity with the Guide R 2.1.10.3968-23[3].

Several uncertainty factors were used in transfer from BMDL to the reference dose including interspecies extrapolation, extrapolation of experimental conditions, BMDL use and repeated exposure taken into account.

Results and discussion. The 6-month experiment involved assessing effects produced by different NDMA doses on the liver function indicators by estimating changes in the activity of liver enzymes (AST, ALT, and GGT) in peripheral blood. The results showed dose-dependence of changes in the analyzed indicators (Figure 1).

[2] Metodicheskie ukazaniya po ustanovleniyu i obosnovaniyu gigienicheskikh normativov soderzhaniya khimicheskikh primesei, biologicheskikh agentov v pishchevoi produktsii po kriteriyam riska dlya zdorov'ya cheloveka [Methodical guidelines on establishing and substantiating safe standards for contents of chemicals and biological agents in food products per health risk indicators]. *EEC*. Available at: https://eec.eaeunion.org/upload/medialibrary/3ae/MU-po-ustanovleniyu-i-obosnovaniyu-gigienicheskikh-normativov.pdf (June 11, 2025) (in Russian).

[3] Guide R 2.1.10.3968-23. Rukovodstvo po otsenke riska zdorov'yu naseleniya pri vozdeistvii khimicheskikh veshchestv, zagryaznyayushchikh sredu obitaniya [Health Risk Assessment upon Exposure to Chemical Pollutants in the Environment]. Moscow, Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, 2023, 221 p. (in Russian).

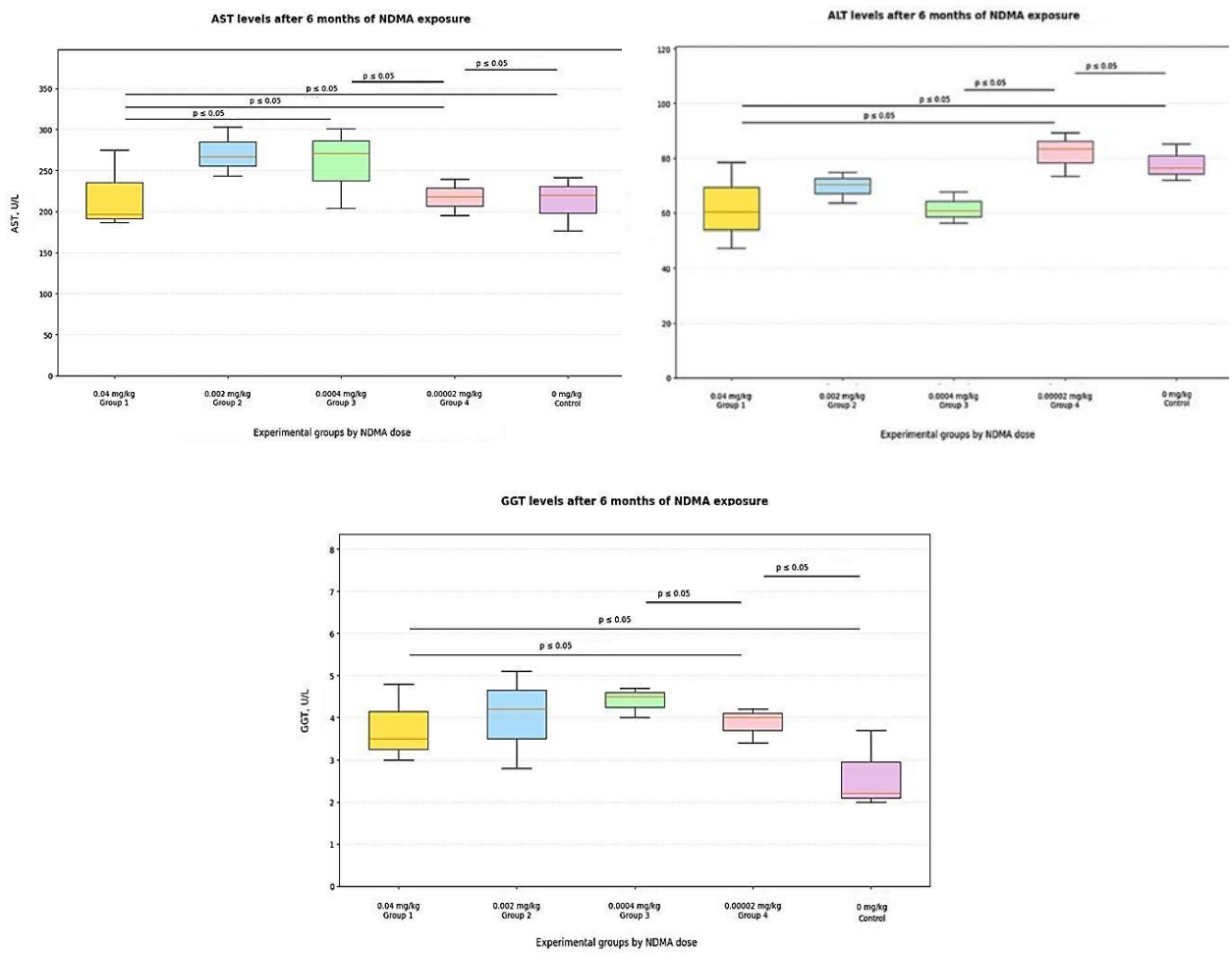

Figure 1. Box plots showing distribution of AST, ALT, and GGT levels in blood depending on a NDMA dose

Analysis of biochemical blood tests accomplished on the experimental animals after 6-month NDMA exposure in different doses established considerable changes in the activity of the key liver enzymes. Significant intergroup differences were found in distribution of the analyzed biomarkers by using the non-parametric Mann – Whitney test.

AST activity was established to have changed most pronouncedly. The highest NDMA dose (0.04 mg/kg, Group I) induced a significant increase in AST levels (*Me* = 196.4 [186.6; 274.6] U/l) against the control (U_{emp} = 35 at U_{crit} = 42, $p \leq 0.05$). Interestingly, the highest AST levels were established in Group II (0.002 mg/kg) (*Me* = 267.1 [243.7; 302.7] U/l); however, significant differences from the control were absent (U_{emp} = 52 at U_{crit} = 55, $p > 0.05$), which indicates that the dose-dependence was not linear. The lowest experimental dose (0.00002 mg/kg, Group IV) also induced significant changes in AST activity (U_{emp} = 22 at U_{crit} = 40, $p \leq 0.05$), which indicates the biomarker was sensitive to NDMA exposure.

Analysis of ALT activity found a less pronounced but still significant response to this toxic exposure. The highest NDMA dose (Group I) led to an authentic increase in ALT levels against the control (U_{emp} = 38 at U_{crit} = 45, $p \leq 0.05$), although the absolute values of the indicator (*Me* = 60.3 [47.3; 78.6] U/l) were lower than in the control group (*Me* = 76.4 [72.0; 85.3] U/l). The highest ALT levels were found in Group IV (*Me* = 83.3 [73.5; 89.2] U/l) and these differences were significant against the control (U_{emp} = 19 at U_{crit} = 30, $p \leq 0.05$).

Analysis of GGT activity established a pronounced response to NDMA exposure. The highest dose (Group I) induced a significant rise in GGT levels (*Me* = 3.5 [3.0; 4.8] U/l) against the control (*Me* = 2.2 [2.0; 3.7] U/l; U_{emp} = 40 at U_{crit} = 47, $p \leq 0.05$). Notably, the lowest NDMA dose (Group IV) also induced significant changes in the enzyme activity (U_{emp} = 22 at U_{crit} = 40, $p \leq 0.05$) evidencing the biomarker was highly sensitive to hepatotoxic effects.

GGT is not a specific biomarker of liver dysfunction; despite that, the association between the enzyme and NDMA exposure is confirmed by the accomplished experimental study, which revealed a significant relationship ($p \leq 0.05$) with an exposure dose of N-nitrosodimethylamine. This makes it possible to use GGT as a biomarker for substantiating ADI within assessment of toxic effects.

A regression model was built using the sliding window method; it reflects influence exerted by an exposure level on odds ratio (*OR*), which describes how strong the relationship is between exposure levels (NDMA) and a response (an increase in GGT levels). Presence of the relationship is evidenced by the condition $OR \geq 1$ (Figure 2).

The reference level (BMDL) was established based on analyzing the dose – response relationship; it was equal to 5.5 µg/kg of body weight corresponding to the lower confidence limit of the dose, exposure to which results in a significant ($p < 0.05$) increase in GGT activity.

The established BMDL (5.5 µg/kg b.w.) was used as a point of departure in calculating the reference dose (RfD / ADI) for NDMA. Several uncertainty factors were employed to the BMDL value including interspecies extrapolation (×10), extrapolation of experimental conditions (×6), using BMDL instead of NOAEL (×4) and repeated exposure taken into account (×4); in total, the multiplier was equal to 960. The calculations gave grounds for identifying the reference dose (RfD / ADI) for NDMA equal to 5.73 ng/kg b.w. ($5.73 \cdot 10^{-6}$ mg/kg b.w.).

To estimate effects produced by NDMA dose higher than ADI on intensity of hepatotoxic effects, we identified specific parameters of the dose – response relationship, which described likelihood of a response depending on an exposure level (Figure 3).

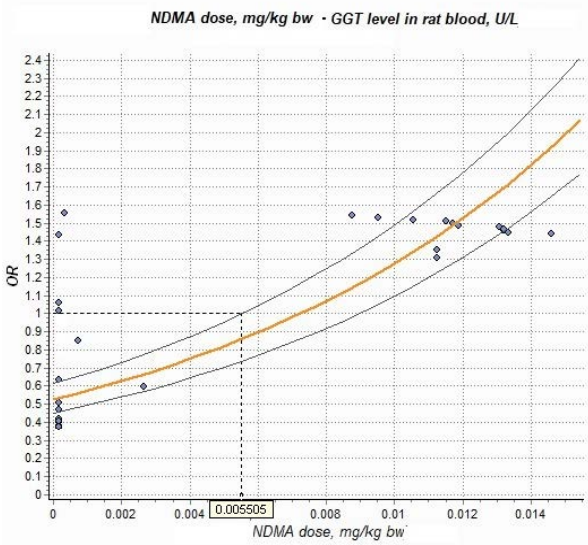

Figure 2. Visualized changes in odds ratio of elevated GGT activity depending on NDMA exposure levels based on mathematical modeling

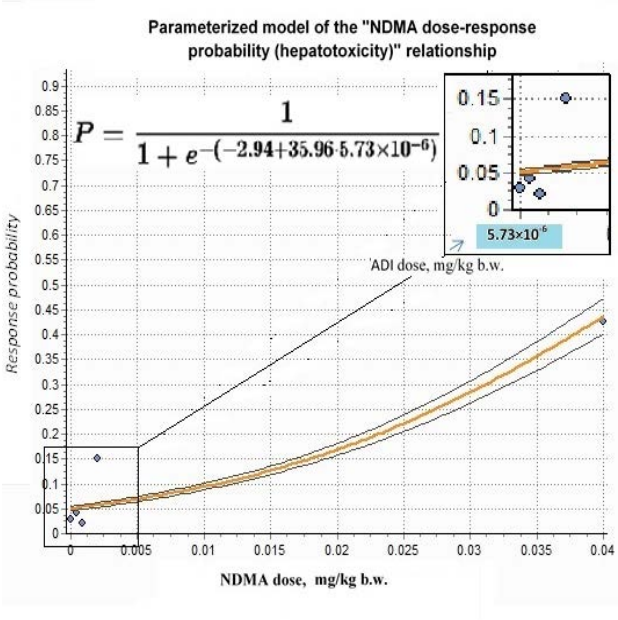

Figure 3. The results obtained by parameterizing the relationship between likelihood of a response (hepatotoxicity) and NDMA exposure levels

Table 1

The model parameters to describe the relationship between likelihood of response (hepatotoxicity) and administered NDMA dose

Model parameters		Model description			BMDL, μg/kg b.w.
Absolute term (Intercept) (b_0)	Regression coefficient for N-nitrosamines (b_1)	Adequacy (F-test)	Authenticity ($p < 0.05$)	Determination coefficient (R^2)	
-2.94	35.96	26.127	0.00001	0.73	0.55

The created model shows that the obtained parameters of the dose – response relationship (Table 1) can be used within the range between the calculated ADI value (0.0057 μg/kg b.w.) and the upper limit of the exposure dose equal to 0.04 mg/kg of body weight (40 μg/kg b.w.).

Therefore, the identified dose – response relationships made it possible to calculate indicators (BMDL, RfD) eligible for quantifying likelihood of hepatotoxic effects under various NDMA exposure levels.

The results of the present study show that GGT can be used as a sensitive effect biomarker for estimating hepatotoxic effects produced by NDMA upon repeated oral exposure. The dose – response relationship established for GGT ($p < 0.01$) is consistent with findings reported in other studies about this enzyme being highly sensitive to oxidative stress induced by N-nitrosamines[4] [12–14]. It is noteworthy that in contrast to conventional histopathological methods [15], including those used in research accomplished by the U.S. EPA[5] to identify the reference dose [16], the present study is based on dynamic monitoring of biochemical indicators, which allowed us to reveal earlier changes in the liver function.

The lower confidence limit for the minimal exposure level was calculated and the reference dose was subsequently identified by using up-to-date mathematical modeling methods that considered non-linear relationships typical for biological responses. The resulting RfD value turned out to be lower than the reference dose proposed by the U.S. EPA ($8 \cdot 10^{-6}$ mg/kg/day)[6], which is due to certain advantages of the employed methodical approaches: (1) use of a more sensitive biomarker (GGT), (2) data obtained in a long (180-day) experiment and (3) use of multifactorial statistical analysis that minimized influence exerted by random variations. These results confirm the conclusions made by the European Food Safety Authority (EFSA) about the necessity to revise conventional approaches to risk assessment by including advanced biomarker technologies in the process[7] [17–20].

Therefore, our findings make a substantial contribution to developing the methodology for assessing non-carcinogenic risks through establishing the points of departure (BMDL) for identifying safe levels of repeated oral NDMA exposure. The established indicator values can be recommended for analyzing and

[4] Annunciato I. Evaluation of acute hepatic toxicity and inflammation induced by nitrosamines in Mus musculus and Danio rerio: Dissertação de mestrado. São Vicente, University of Porto Publ., 2024, 36 p.

[5] Reference Dose (RfD): Description and Use in Health Risk Assessments. Background Document 1A, March 15, 1993 (updated on May 5, 2025). *U.S. Environmental Protection Agency*. Available at: https://www.epa.gov/iris/reference-dose-rfd-description-and-use-health-risk-assessments (June 16, 25).

[6] Provisional Peer Reviewed Toxicity Values for N-Nitrosodimethylamine (CASRN 62-75-9). USA, Cincinnati, U.S. Environmental Protection Agency, 2007, 27 p.

[7] Environmental Health Criteria 222. Biomarkers in risk assessment: Validity and validation. *INCHEM*. Geneva, World Health Organization, 2001. Available at: https://www.inchem.org/documents/ehc/ehc/ehc222.htm (June 11, 2025).

including into the Methodical Guidelines on Establishing and Substantiating Safe Standards for Contents of Chemicals and Biological Agents in Food Products per Health Risk Indicators[8] approved by the Eurasian Economic Commission as well as into the Guide R 2.1.10.3968-23 Health Risk Assessment upon Exposure to Chemical Pollutants in the Environment[9].

Conclusion. Our findings revealed the significant dose-dependent relationship between the administered NDMA dose and changes in the activity of liver enzymes; this gives evidence of hepatotoxic effects produced by the analyzed chemical. Among all the analyzed biomarkers, GGT turned out to have the highest diagnostic sensitivity together with revealed pronounced dose dependence ($p \leq 0.05$).

We established RfD / ADI for NDMA equal to $5.73 \cdot 10^{-6}$ mg/kg of body weight based on BMDL (0.0055 mg/kg of body weight) using the relevant uncertainty factors (interspecies extrapolation, extrapolation of the experimental conditions, use of BMDL and repeated exposure taken into account).

We identified model parameters for the dose – response relationship, which can be used for quantifying non-carcinogenic health risks ($b_0 = -2.94$, $b_1 = 35.96$) caused by liver dysfunction.

Therefore, the conducted experimental study that involved using effect biomarkers made it possible to establish quantitative parameters for assessing non-carcinogenic health risks caused by repeated oral NDMA exposure.

The suggested parameters for risk assessment make a valuable contribution to the system of knowledge about formation of risks caused by chemical contaminants and can be recommended for inclusion into relevant regulatory and methodical documents.

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References

1. Nasr N.E.H., Metwaly M.G., Ahmed E.O., Fares A.R., ElMeshad A.N. Investigating the root cause of N-nitrosodimethylamine formation in metformin pharmaceutical products. *Expert Opin. Drug Saf.*, 2021, vol. 20, no. 7, pp. 855–862. DOI: 10.1080/14740338.2021.1917547

2. Gomm W., Röthlein C., Schüssel K., Brückner G., Schröder H., Heß S., Frötschl R., Broich K., Haenisch B. N-Nitrosodimethylamine-Contaminated Valsartan and the Risk of Cancer – A Longitudinal Cohort Study Based on German Health Insurance Data. *Dtsch Arztebl. Int.*, 2021, vol. 118, no. 21, pp. 357–362. DOI: 10.3238/arztebl.m2021.0129

3. Li Y., Hecht S.S. Metabolic Activation and DNA Interactions of Carcinogenic *N*-Nitrosamines to Which Humans Are Commonly Exposed. *Int. J. Mol. Sci.*, 2022, vol. 23, no. 9, pp. 4559. DOI: 10.3390/ijms23094559

4. Huang S., Fu W., Fang Q., Ni L., Zheng R., Yong L., Huang Z., Pang J. [et al.]. Occurrence and carcinogenic risk assessment of *N*-nitrosamines in some dried aquatic products in China. *Food Control*, 2023, vol. 152, no. 7, pp. 109845. DOI: 10.1016/j.foodcont.2023.109845

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[8] Metodicheskie ukazaniya po ustanovleniyu i obosnovaniyu gigienicheskikh normativov soderzhaniya khimicheskikh primesei, biologicheskikh agentov v pishchevoi produktsii po kriteriyam riska dlya zdorov'ya cheloveka [Methodical guidelines on establishing and substantiating safe standards for contents of chemicals and biological agents in food products per health risk indicators]. *EEC*. Available at: https://eec.eaeunion.org/upload/medialibrary/3ae/MU-po-ustanovleniyu-i-obosnovaniyu-gigienicheskikh-normativov.pdf (June 11, 2025) (in Russian).

[9] Guide R 2.1.10.3968-23. Rukovodstvo po otsenke riska zdorov'yu naseleniya pri vozdeistvii khimicheskikh veshchestv, zagryaznyayushchikh sredu obitaniya [Health Risk Assessment upon Exposure to Chemical Pollutants in the Environment]. Moscow, Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, 2023, 221 p. (in Russian).

5. Zhang H., Lu L., Zhao C., Liu Q., Zhou Q., Zhang Y., Pu Y., Wang S. [et al.]. Lipid metabolism disorders contribute to hepatotoxicity of ICR mice induced by nitrosamines exposure. *Environ. Int.*, 2022, vol. 167, pp. 107423. DOI: 10.1016/j.envint.2022.107423

6. Ugwueze C.V., Ezeude C.M., Abonyi C.M., Nwatu C.B., Ukwaja K.N., Young E.E. N-Nitrosodimethylamine (NDMA) and Some Prescribed Medicines: A Mini-review. *International Journal of Medicine and Health Development*, 2022, vol. 27, no. 2, pp. 109–113. DOI: 10.4103/ijmh.IJMH_15_21

7. Lathouri M., Korre A., Dusinska M., Durucan S. Human Health hazard assessment strategy for amine emissions around PCC facilities. Deliverable D3.3. Trondheim, Norway, Sustainable OPEration of post-combustion Capture plants (SCOPE), 2022, 67 p.

8. Rodríguez-Carrillo A., Mustieles V., Salamanca-Fernández E., Olivas-Martínez A., Suárez B., Bajard L., Baken K., Blaha L. [et al.]. Implementation of effect biomarkers in human biomonitoring studies: A systematic approach synergizing toxicological and epidemiological knowledge. *Int. J. Hyg. Environ. Health*, 2023, vol. 249, pp. 114140. DOI: 10.1016/j.ijheh.2023.114140

9. Usunomena U., Joshua A.A., Okugbo O.T., Evuen U.F., Osibemhe M., Okolie N.P. N-nitrosodimethylamine (NDMA), Liver Function Enzymes, Renal Function Parameters and Oxidative Stress Parameters: A Review. *British Journal of Pharmacology and Toxicology*, 2012, vol. 3, no. 4, pp. 165–176.

10. Thakur S., Kumar V., Das R., Sharma V., Kumar Mehta D. Biomarkers of Hepatic Toxicity: An Overview. *Curr. Ther. Res. Clin. Exp.*, 2024, vol. 100, pp. 100737. DOI: 10.1016/j.curtheres.2024.100737

11. Suvorov D.V., Shur P.Z., Zelenkin S.E. On the issue of determining tolerable daily intake of total N-nitrosamines for toddlers. *Health Risk Analysis*, 2024, no. 4, pp. 72–80. DOI: 10.21668/health.risk/2024.4.07.eng

12. Janmeda P., Jain D., Chaudhary P., Meena M., Singh D. A systematic review on multipotent carcinogenic agent, N-nitrosodiethylamine (NDEA), its major risk assessment, and precautions. *J. Appl. Toxicol.*, 2024, vol. 44, no. 8, pp. 1108–1128. DOI: 10.1002/jat.4574

13. Bai C., Zhang M., Zhang Y., He Y., Dou H., Wang Z., Wang Z., Li Z., Zhang L. Gamma-Glutamyltransferase Activity (GGT) is a Long-Sought Biomarker of Redox Status in Blood Circulation: A Retrospective Clinical Study of 44 Types of Human Diseases. *Oxid. Med. Cell. Longev.*, 2022, vol. 2022, pp. 8494076. DOI: 10.1155/2022/8494076

14. Priya S., Kma L. Modulation of EphA7 and pEphA7 Protein Expression: Potential Biomarkers for Early Detection of Hepatocellular Carcinoma. *Asian Pacific Journal of Cancer Prevention*, 2022, vol. 23, no. 8, pp. 2843–2850. DOI: 10.31557/APJCP.2022.23.8.2843

15. George J., Tsutsumi M., Tsuchishima M. Alteration of Trace Elements during Pathogenesis of N-Nitrosodimethylamine Induced Hepatic Fibrosis. *Sci. Rep.*, 2019, vol. 9, no. 1, pp. 708. DOI: 10.1038/s41598-018-37516-4

16. A review of the reference dose and reference concentration processes. Washington, DC, U.S. Environmental Protection Agency, 2002, 192 p.

17. European Food Safety Authority (EFSA), Hernández-Jerez A., Bennekou S.H., Hoogenboom L., Mcardle H., Pieper C., Schwerdtle T., Van Loveren H. [et al.]. Conceptual basis for the development of guidance for the use of biomarkers of effect in regulatory risk assessment of chemicals. *EFSA J.*, 2024, vol. 22, no. 12, pp. e9153. DOI: 10.2903/j.efsa.2024.9153

18. Bizjak T., Capodiferro M., Deepika D., Dinçkol Ö., Dzhedzheia V., Lopez-Suarez L., Petridis I., Runkel A.A. [et al.]. Human Biomonitoring Data in Health Risk Assessments Published in Peer-Reviewed Journals between 2016 and 2021: Confronting Reality after a Preliminary Review. *Int. J. Environ. Res. Public Health*, 2022, vol. 19, no. 6, pp. 3362. DOI: 10.3390/ ijerph19063362

19. Bonzini M., Leso V., Iavicoli I. Towards a toxic-free environment: perspectives for chemical risk assessment approaches. *Med. Lav.*, 2022, vol. 113, no. 1, pp. e2022004. DOI: 10.23749/mdl.v113i1.12748

20. Viegas S., Zare Jeddi M., Hopf N.B., Bessems J., Palmen N., Galea K.S., Jones K., Kujath P. [et al.]. Biomonitoring as an Underused Exposure Assessment Tool in Occupational Safety and Health Context-Challenges and Way Forward. *Int. J. Environ. Res. Public Health*, 2020, vol. 17, no. 16, pp. 5884. DOI: 10.3390/ijerph17165884

Suvorov D.V., Shur P.Z., Zelenkin S.E., Lir D.N., Nguyen Van Cuong, Nguyen Thi Hong Ngoc, Tran Cao Son. Substantiating indicators for assessing population health risks based on experimental investigation applying biomarkers of effect produced by N-nitrosamines. Health Risk Analysis, 2025, no. 3, pp. 95–103. DOI: 10.21668/health.risk/2025.3.10.eng

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