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Toxicopathological changes induced by combined exposure to noise and toluene in New Zealand White rabbits

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Noise and toluene can have significant adverse effects on different systems in the human body, but little is known about their combination. The aim of this study was to see how their combined action reflects on serum levels of inflammatory cytokines tumour necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β), body weight, and pathological changes in the heart, lung, stomach, and spleen tissues. To do that we exposed New Zealand rabbits to 1000 mg/L toluene and 100 dB of white noise in a chamber specifically designed for the purpose over two consecutive weeks. Serum levels of TNF-α and IL-1β were measured with the enzyme-linked immunosorbent assay (ELISA), whereas Bax and Bcl-2 expressions in tissues were determined with real-time polymerase chain reaction (PCR). Noise and toluene changed TNF-α and IL-1β serum levels on different days following the end of exposure and significantly increased the Bax/Bcl-2 ratio in the lung and spleen. In addition, they induced different pathological changes in the heart, lung, spleen, and stomach tissues. This study has confirmed that exposure to noise and toluene can induce a range of toxicopathological changes, probably by inducing inflammatory pathways and apoptosis, but their combined effects look weaker than those of its components, although histopathological findings suggest the opposite.

KEY WORDS: body weight; environmental exposure; heart; IL-1β; industrial toxicology, apoptosis; lung; spleen; stomach; TNF-α

We know today that noise can affect health beyond loss of hearing (1). Exposure to noise can disturb physiological processes and affect systems such as cardiovascular (2), digestive (3, 4), immune (5–7), endocrine (8), and nervous (9). We also know that toluene – used as solvent in many products and industries (10–14), including vehicle fuel (15) – can at high concentrations cause nervous system disorders (16, 17), cardiovascular changes (18), and respiratory problems (19).

However, there are a number of industries in which exposure to both is quite common, such as chemical and petrochemical industry (17). The issue of combined effects of various physical and chemical factors on health has been a controversial and much-disputed subject in the field of environmental health and toxicology in recent years (20, 21). Considering the prevalence of toluene and noise in different industries and the lack of scientific studies of their combined effects, our aim was to fill that gap and get new insights into some toxicological effects related to inflammatory and apoptotic processes and resulting pathological changes in various organs usually affected by the two. Our research hypothesis was that combined exposure to noise and toluene should induce more severe effects and pathological changes in the lung, heart, spleen, and stomach tissues than its components alone.

MATERIALS AND METHODS

Experimental design and animals

For this study we used 24 four months old New Zealand white rabbits weighing 2.83±0.5 kg. The rabbits were housed in wire mesh cages at 21±5 °C (55–70 % humidity) and had free access to standard pellet and water. Animals were randomised into four groups of six: control, noise group exposed to 100 dB of noise, toluene group exposed to 1000 mg/L of toluene, and combined group exposed to noise and toluene. Exposure to toluene and/or noise was set up in an exposure chamber specifically designed for this purpose and lasted eight hours a day for 14 days. The control group was also placed in the exposure chamber for eight hours but was not exposed to either toluene or noise. All environmental variables (such as humidity, temperature, lightness, exposure and mixer chambers, and airflow rate) were identical for all groups.

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The study was approved by the National Committee on Ethics in Iran's Biomedical Research (approval code: IR.TBZMED.REC.1396.953).

Experimental exposure chamber

The chamber was made of polycarbonate 50×60×90 cm panes as described in earlier studies (22, 23) in order to have noise distributed equally across the chamber. The chamber was attached to another mixer chamber as shown in Figure 1. Exposure conditions, including temperature (23±5 °C) and humidity (65–80 %) were identical for all groups.

Noise generation system

White noise (100±5 dB at 50–20,000 Hz) was generated by the Audacity® 1.3.12 Beta software (Carnegie Mellon University, Pittsburgh, PA, USA), amplified, and delivered to noise-exposed groups continuously over eight hours through a speaker (Figure 1). The level of noise was constantly monitored with the Cool Edit Pro 2.1© software (Adobe, San Jose, CA, USA) every 30 min using a real-time sound analyser (TES 1358; TES Electrical Electronic Corp, Taipei, Taiwan) both in pilot tests and in experiments. The clean air flow rate was 33 L/min for the control and noise-exposed groups.

Toluene vapour exposure

Toluene vapour was obtained by impinging extra pure liquid toluene (Merck, Darmstadt, Germany) in a 250 mL impinger and diluting it with fresh air to reach the concentration of 1000±50 mg/L. Clean air flow rate was 30 L/min and toluene vapour flow rate was 3 L/min to match the flow for control and noise-exposed animals. The starting liquid toluene volume in the impinger was 100 mL, and 20 mL were further injected every 90 min. These flow rates and toluene liquid volume in the impinger were obtained in pilot tests to produce a relatively constant and uniform concentration of 1000 mg/L as determined with a PhoCheck+ 5000Ex volatile organic compound (VOC) detector (Ion Science, Cambs, UK). Toluene concentration of 1000 mg/L and its purity in the exposure chamber were verified by gas chromatography-mass spectrometry (Agilent 6890/5973, Santa Clara, CA, USA) according to the method no. 1021 recommended by the Occupational Safety and Health Administration (OSHA). Toluene concentration was continuously checked at checking outlets using the same VOC detector used in pilot experiments.

Blood collection

Blood was collected from the marginal vein on rabbit ear into serum gel and clot activator tubes at five time points before centrifuge: immediately before the beginning of 14-day exposure and on days 0, 3, 7, and 14 after the end of 14-day exposure. One hour after collection, the samples were centrifuged at 706 *g* for 15 min, and the obtained serum transferred to micro tubes with a sampler and stored in a freezer at -80 °C until blood from all groups had been sampled and biochemistry tests initiated.

Determination of animal body weight

All animals were weighed with a digital scale immediately before each blood sampling.

Assessment of serum cytokines

Inflammatory cytokines, tumour necrosis factor alpha (TNF-α; Cat. No. CK-E91014) and interleukin 1 beta (IL-1β; Cat. No. CK-E80175), were measured with the rabbit enzyme-linked immunosorbent assay (ELISA) kit (Hangzhou Eastbiopharm, Hangzhou, China) according to manufacturer's instructions. The ELISA plate reader and washer used in this study were State Fax 2100 and 2600, respectively (Awareness Technology., Palm City, FL, USA).

Tissue sampling

After 14 days of exposure, the rabbits were anaesthetised with a mixture of 35 mg/kg ketamine and 5 mg/kg xylazine (Rotexmedica, Trittau, Germany) injected intramuscularly. When the animals were completely senseless, their heart, lung, spleen, and stomach tissues were dissected and samples (1×1×1 cm) fixed in 10 % pH 7.2 formaldehyde solution (Merck).

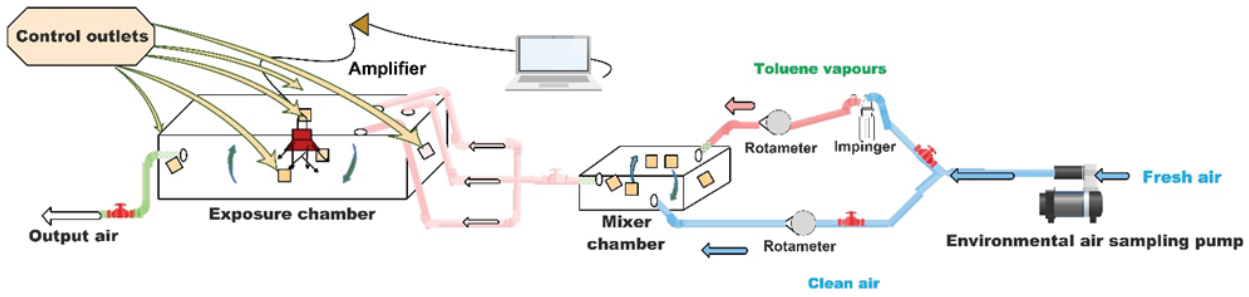

Figure 1 Exposure setup

Histological analysis of tissue samples

The obtained samples were dried, cleared, impregnated with paraffin, and moulded using an MSLTS01 automated tissue processor (MedSingLong Global Group, Guangdong, China). Paraffin blocks were then cut into 5-µm thick sections with a Leitz microtome 1512 (Leica, Wetzlar, Germany). Finally, the slides were stained with haematoxylin-eosin (Thermo Fisher Scientific. Geel, Belgium) and assessed using a Nikon Eclipse E100 light microscope (100× magnification) (Nikon, Tokyo Japan).

Real-time PCR analysis

Total RNA was isolated from tissue samples with Trizol (Yekta Tajhiz Azma, Tehran, Iran) and RNAiso Plus (TaKaRa, Dalian, China) reagents and its high purity determined with a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at the absorbance ratio of 260 nm/280 nm, which ranged from 1.8 to 2.0. The quality and quantity of extracted RNA extracted were also proven with agarose gel. Afterwards, 1 µg of RNA was used to synthesise first-strand complementary DNA (cDNA) using a Primescript RT reagent kit with a gDNA Eraser (Takara Bio, Dalian, China) according to the manufacturer's instructions. Real-time quantitative reverse transcription (qRT) PCR was carried out with a conventional thermal cycler (LightCycler® 96, F. Hoffmann-La Roche, Basel, Switzerland) and the cyanine SYBR® Green I dye (Yekta Tajhiz Azma) and analysed using the OLIGO 7 primer analysis software (Molecular Biology Insights, Colorado Springs, CO, USA). Complementary DNA (cDNA) of each sample was distilled to a working concentration of 4 pmol/L. To determine the expression ratio of the *Bax* and *Bcl-2* genes as markers of apoptosis, we used a total volume of 10 µL reaction solution containing 5 µL SYBR PremixEX Taq polymerase, 0.3 µL of primer mix (forward and reverse), 3.7 µL of ultrapure water treated with diethyl pyrocarbonate (DEPC, PT-P560, Protech Technology Enterprise, Taipei, Taiwan), and 1 µL of sanitised cDNA. Primer sequences are presented in Table 1. The PCR cycling protocol started with denaturation at 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s, annealing at 59 °C for 30 s, and extension at 72 °C for 30 s. The standard curves were made using a 10-fold dilution series of cDNA for each treatment and linear regression model.

Statistical analysis

Cytokine data were estimated using generalised estimated equations (GEE) run on the IBM SPSS Statistics software (IBM SPSS Statistics for Windows, version 25.0.0.0, Armonk, NY, USA). Bax, Bcl-2, and tissue weights were compared using the analysis of variance (ANOVA) and Tukey's method run on the Minitab statistics package version 18 (Minitab, State College, PA, USA).

RESULTS AND DISCUSSION

Effects on body and organ weight and inflammation markers

Figure 2 shows that body weight in the toluene and combined group dropped initially and recovered by day 14 after exposure

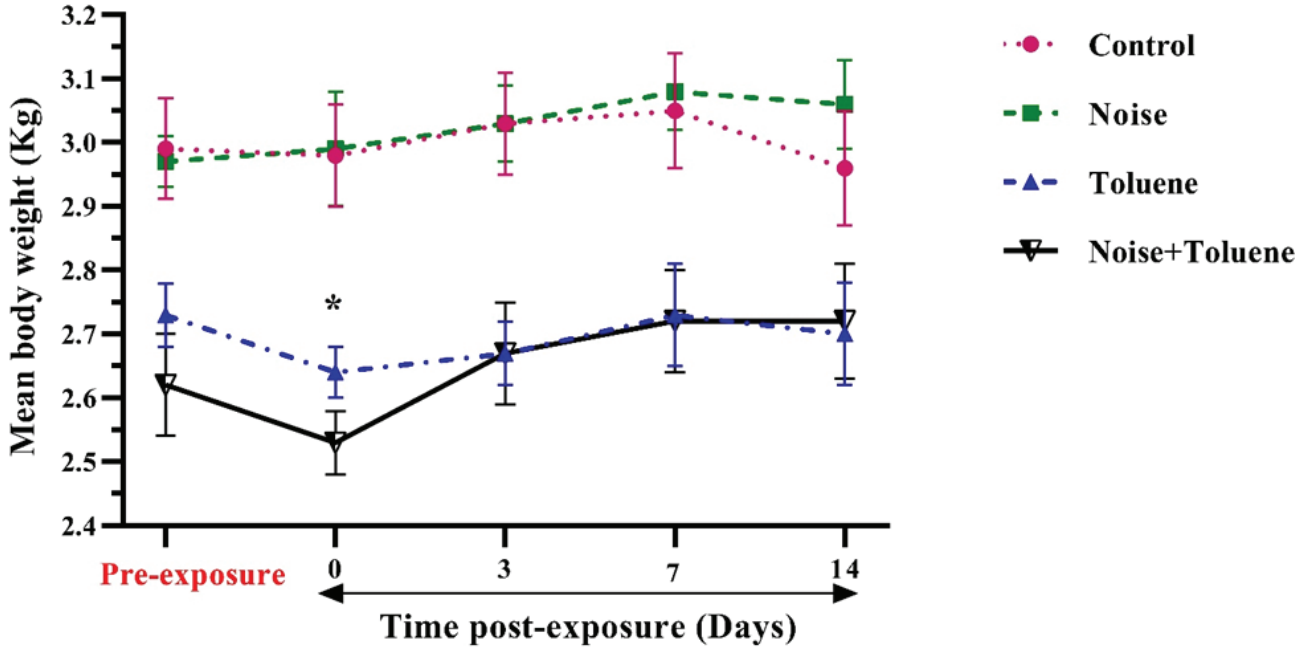

Figure 2 Changes in body weight by study groups; each point represents mean ± SE (n=6). The asterisk (*) marks significant difference in body weight between day 0 and pre-exposure measurement in the toluene and combined groups (P<0.05)

ended, whereas noise alone did not significantly affect body weight, which, in fact increased steadily by post-exposure day 14. Relative organ tissue weights, in turn, did not significantly differ between the groups (Figure 3).

Figure 4 shows changes in serum TNF-α and IL-1β across post-exposure days. On day 7 post exposure, IL-β in the combined group was significantly higher than in the noise group and significantly lower than in control. TNF-α level in the combined group was significantly higher than in the noise group immediately after the end of 14-day exposure (day 0).

Previous animal studies have generally shown lowering of interleukin and TNF-α in response to toluene exposure (24, 25). Reports on noise, in turn, are inconclusive, as some studies reported TNF-α increase at 100 dB over 15 (26) and 30 exposure days in rats (5), whereas others reported a drop at 45–75 dB over a 2-week exposure in mice (27) and mixed findings in in rats exposed to 100 dB for 15 days (28). Similar can be said for interleukin response to noise exposure (5, 28, 29), and more experiments are needed to establish specific effects of noise on cytokine behaviour.

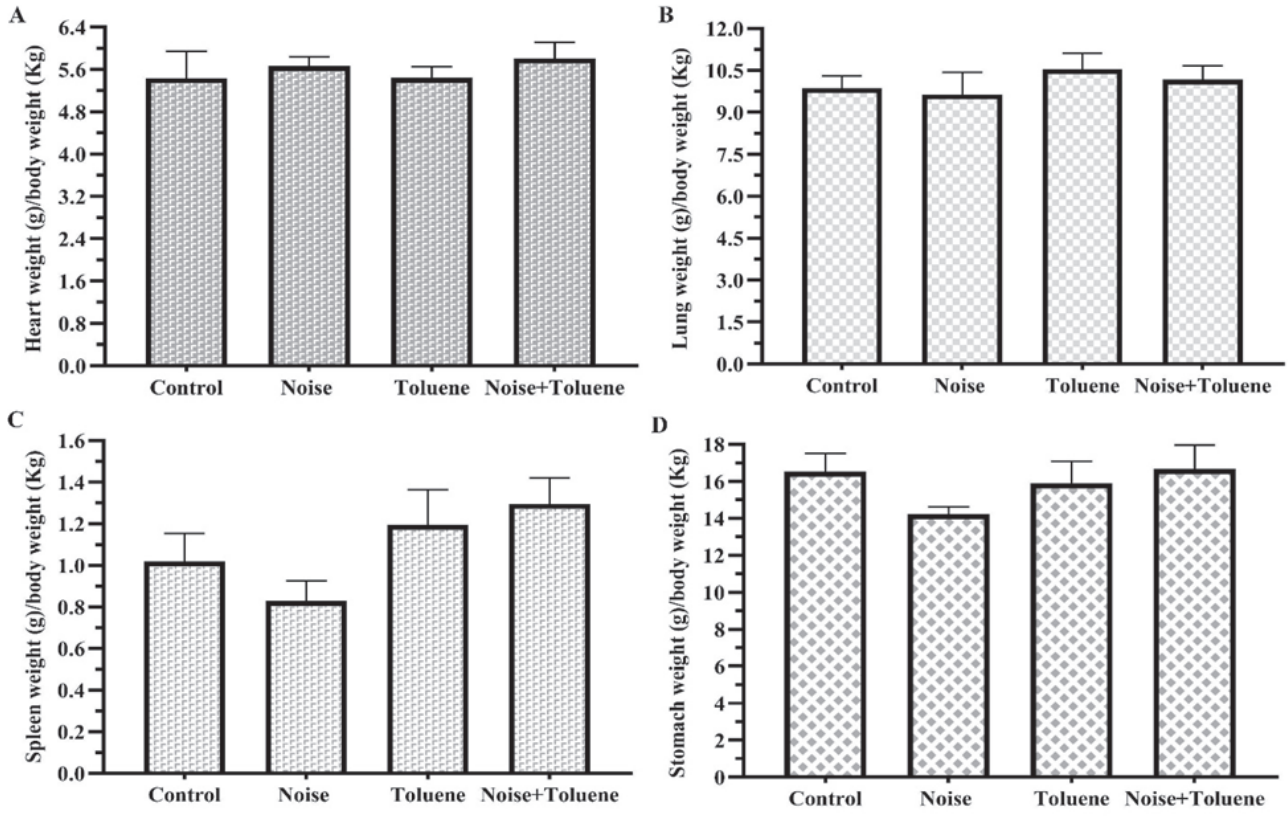

Figure 3 Mean (±SD) relative tissue weights (tissue/body weight) by study groups (n=6) and organs: heart (A), lung (B), spleen (C), and stomach (D)

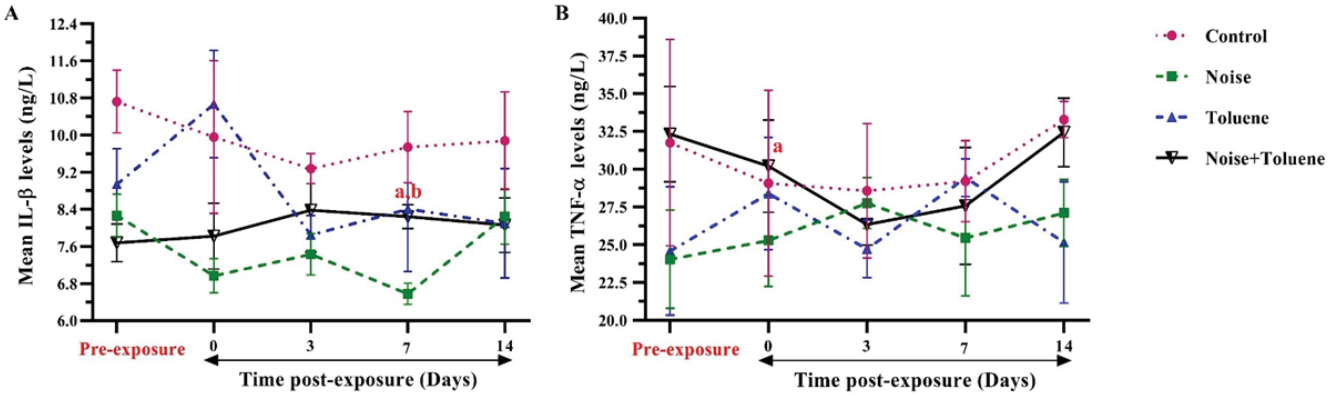

Figure 4 Changes in IL-β (A) and TNF-α (B) levels by study groups; each point represents mean ± SE (n=6). [a] – significantly higher in the combined than the noise group (P<0.05); [b] – significantly lower in the combined than control group (P<0.05)

On the other hand, the effects of combined exposure to noise and toluene on IL-β and TNF-α have not yet been studied, and this study points to their decrease. Tables 2–4 show generalised estimating equations for IL-β, TNF-α, and body weight with respect to control over the 14 post-exposure days. The values presented there have been adjusted to pre-exposure and control values to remove the effect of confounding variables. Combined effects (synergism, antagonism) were calculated based on a study by Piggott et al. (30). Noise and toluene had both antagonistic and synergistic effects on IL-β, TNF-α, and body weight (Table 2) and interacted differently (synergism and antagonism) on different post-exposure days, which may point to the activation of mechanisms of homeostasis in the body to balance and neutralise their combined effects.

Gene expression changes

Figure 5 shows that combined exposure to noise and toluene increased *Bax* expression significantly in all tissues compared to control. *Bcl-2* expression significantly increased in the heart tissue but decreased in the lung and spleen tissues. As for the Bax/Bcl-2 ratio, combined exposure increased it in lung and spleen but not heart and stomach tissues. As for toluene and noise alone findings,

Table 1 Primers used for quantitative RT-PCR analyses

Gene	Primer sequence (5'-3')	length (bp)
Bax	Forward: AGGTCTTTTTCCGAGTGGCAGC Revers: GCGTCCCAAAGTAGGAGAGGAG	234
Bcl-2	Forward: GACGACTTCTCCCGCCGCTAC Revers: CGGTTCAGGTACTCAGTCATCCAC	245
GAPDH	Forward: GCCAAAAGGGTCATCATCTCTGC Revers: GGTCACGAGTCCTTCCACGATAC	183

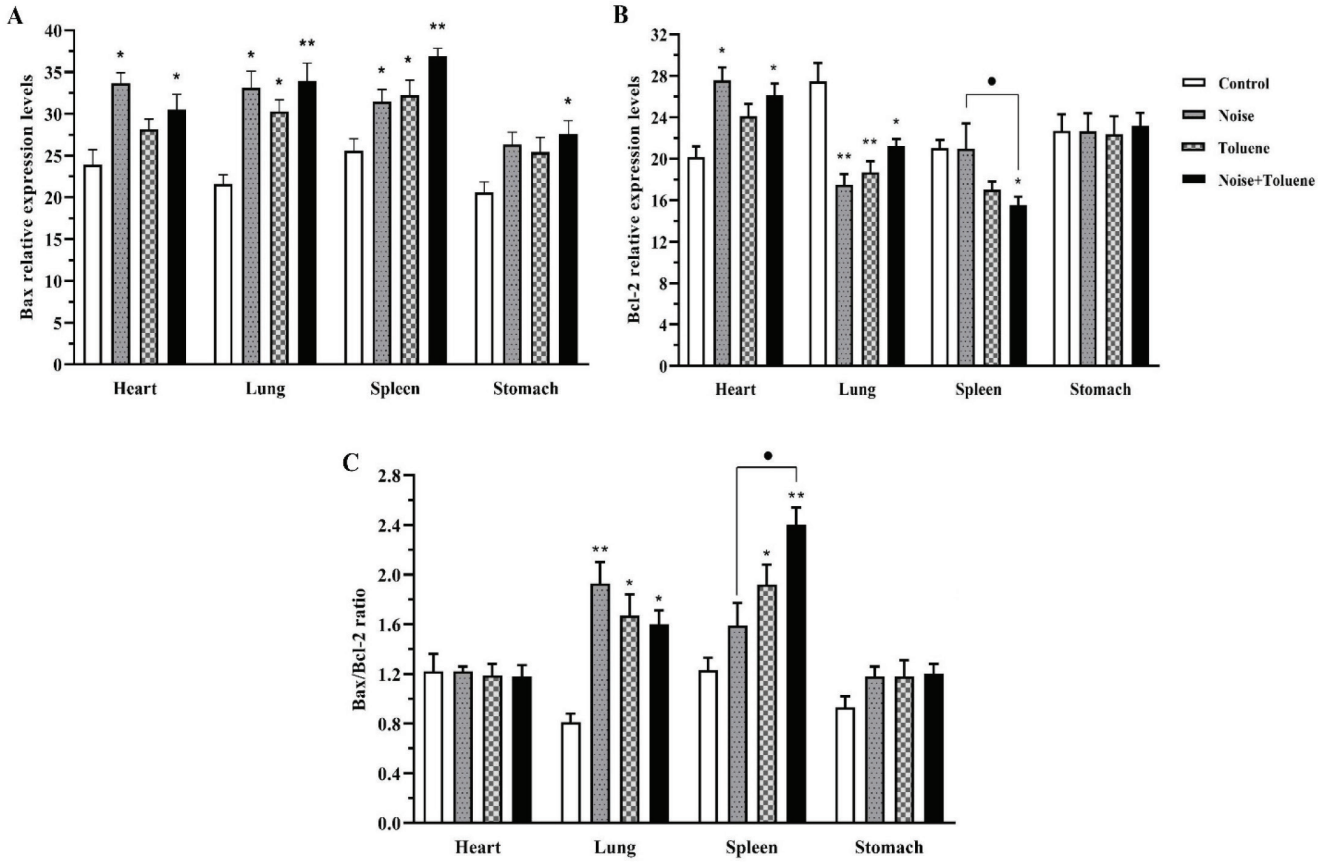

Figure 5 Changes in the expression of Bax (A), *Bcl-2* (B), and the *Bax*/Bcl-2 ratio (C) in heart, lung, spleen, and stomach tissues by study groups. Each bar represents maen±SE values (n=6). Asterisks represent significant differences compared to control (* P<0.05, ** P<0.001). The circle symbol (●) represents a significant difference between the noise and combined groups (P<0.05)

our results corroborate increases in *Bax* expression reported earlier (31–34), albeit in different organs.

In addition, noise and toluene did not have a consistent pattern of either synergistic or antagonistic interaction, judging by *Bax* and *B-Cl2* expression in these organs (Table 3) and relative organ tissue weights (Table 4).

Histopathological findings

Figure 6 compares heart tissues between the groups. In the control group it was normal. In the noise group it showed congestion and dilatation in the heart veins, slight and diffused fibrotic necrosis, but not inflammatory infiltrates. In the toluene group we found a slight fibrotic necrosis (milder than the one in the noise group), lymphocyte infiltrates, and congestion with local degenerative changes (also milder than in the noise group). In the combined group we observed congestion, tissue inflammation, and fibrotic necrosis with dilatation of the veins similar to the one seen in the noise and toluene groups. Altogether, our findings indicate that toluene and noise induce lymphocyte infiltration, dilation, and congestion in the heart tissue. Similar findings were reported by others. In animal studies, noise was reported to induce significant narrowing of myocardial muscle fibres (35), dilated veins in the pericardium and endocardium, deposit in arteries in the myocardium, damaged endothelium of the veins, inflammatory cells (36), myocardial vascular congestion and dilation, mild hyperaemia, and degeneration of myocardial cells (37). Toluene was reported to cause congestion and oedema in the heart tissue in male Wistar rats (18).

In humans, Assunta et al. (38) reported that workers exposed to industrial noise at their workplace had higher incidence of hypertension than other workers.

Figure 7 gives an insight into lung tissue changes. Control, as expected, showed normal structure. The noise group showed alveolar obliteration, also visible in other exposed groups, and lymphocyte infiltrates in areas near the *hilum pulmonis*, which were also noted in the toluene group. The toluene group also showed indiscernible emphysema in the alveoli. The most prominent lymphocyte infiltration, however, was found in the combined group along with diffuse alveolar haemorrhage and intra-alveolar septal thickening, which was not observed in the other groups.

In animal studies, noise has already been associated with interalveolar septal thickening and alveolar obliteration, fibrosis, peribronchiolar infiltration, interstitial infiltration and deposition of collagen fibres, thickened walls of alveolar blood vessels, and apoptotic changes (35), emphysema, pneumonia, oedema, congestion, cholesterol crystals, and granular tissue formation (39). Toluene, in turn, has been associated with inflammatory cell infiltration around small airways and mucosal epithelium, oedema, and alveolar haemorrhage, wall thickening of bronchial cells and terminal bronchioles, and distension of alveolar septa (19).

Figure 8 looks at histopathological changes in the stomach tissue across the groups. Compared to normal stomach wall thickness in

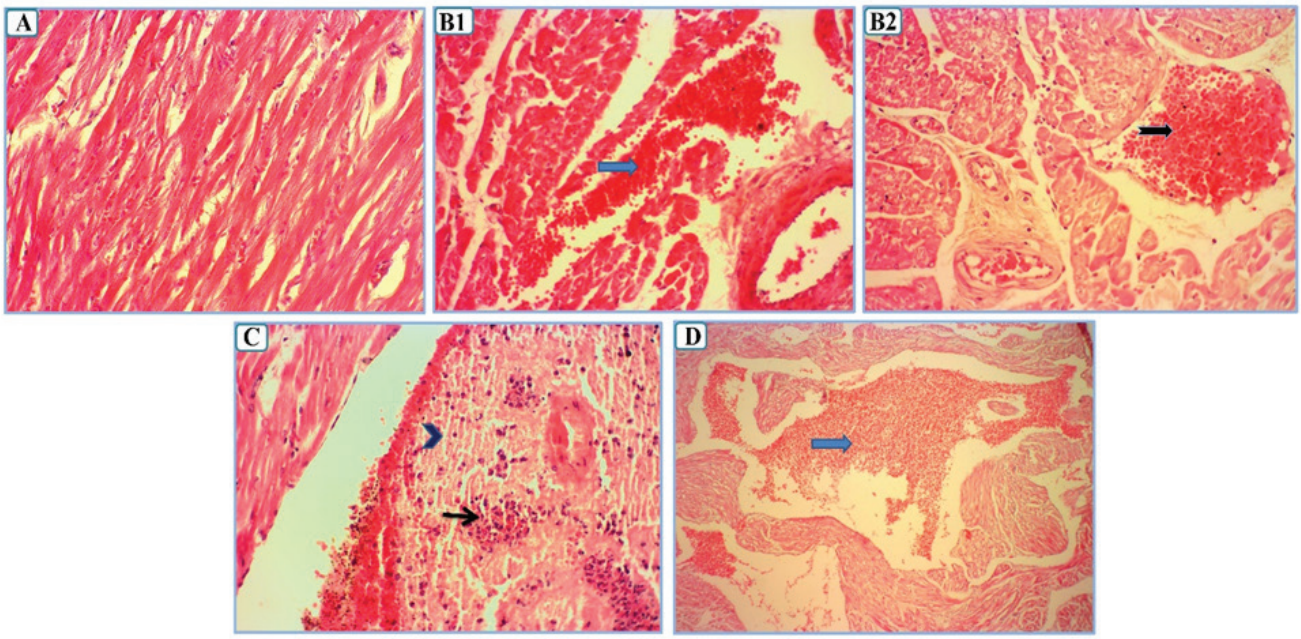

Figure 6 Representative appearances of the heart tissue stained with H&E. The photographs were taken at 10× (B2 and D) and 40× (A, B1, and C) magnification. The control group (A) had a normal structure, however, congestion (thick blue arrow) and vessel dilation (thick black arrow with blue outline) appeared in the noise exposure group (B1 and B2). Additionally, myocardial local degenerative changes (thick black arrow head with blue outline) and lymphocyte infiltration (narrow black arrow) observed in the toluene exposure group (C). Furthermore, congestion (thick blue arrow) was visible in the simultaneous exposure group (D)

Table 2 GEE analysis for body weights and the immunological parameters in respect to control over the 14 days after the end of exposure to noise and/or toluene in New Zealand rabbits [(values are modified based on pre-exposure and control values to remove the effects of confounding variables. Combined effects (synergism, antagonism) were calculated as described in a study by Piggott et al. (30)]

Time point post exposure	Parameter	Noise group	Toluene group	Combined group	Interaction type
Day 0	TNF-α	3.927±2.636	6.477±3.862	0.560±2.439	+Antagonism
	IL-β	-0.535±1.412	**2.490[b]±0.689**	0.900±1.462	+Antagonism
	Body weight	0.028±0.091	-0.075±0.090	**-0.081[a]±0.036**	-Synergism
Day 3	TNF-α	6.895±9.150	3.320±4.591	-2.800±6.901	-Synergism
	IL-β	0.600±1.215	0.350±0.930	2.140±1.242	+Synergism
	Body weight	0.018±0.076	**-0.097[a]±0.038**	0.011±0.020	-Antagonism
Day 7	TNF-α	3.981±10.624	7.406±5.010	-2.180±5.128	-Synergism
	IL-β	-0.703±0.687	0.447±2.221	1.540±0.917	+Synergism
	Body weight	0.038±0.059	-0.06±0.036	0.031±0.020	-Antagonism
Day 14	TNF-α	1.525±7.446	-0.975±7.747	-1.420±2.416	-Synergism
	IL-β	0.821±0.809	-0.004±0.811	1.220±0.973	+Synergism
	Body weight	0.116±0.096	-0.003±0.043	**0.121[b]±0.027**	+Synergism

[a] P<0.05; [b] P<0.001. All values are the β value±SE (standard error)

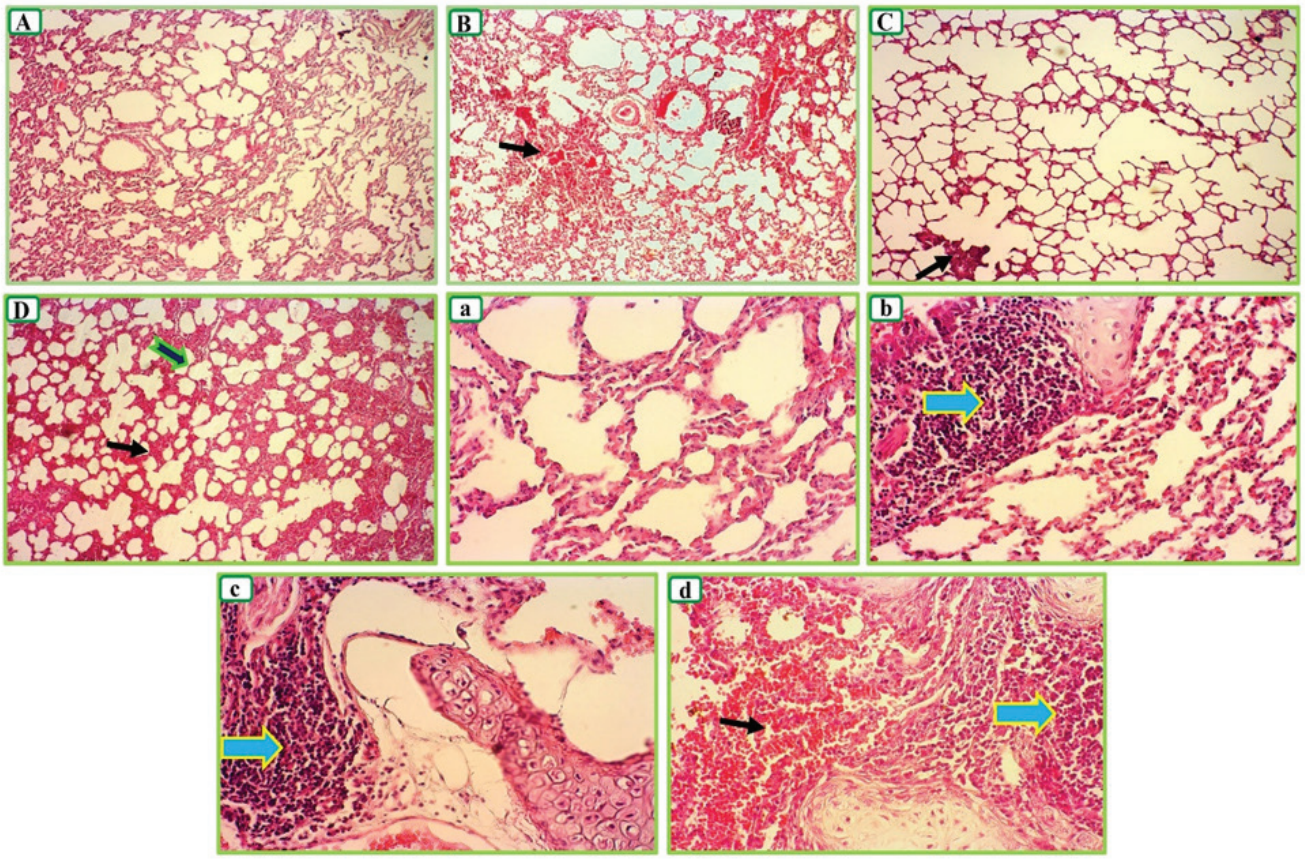

Figure 7 Representative appearances of the lung tissue stained with H&E. The photographs were taken at 10× (A, B, C, and D) and 40× (a, b, c, and d) magnification. The control group (A and a) had a normal structure. Nevertheless, mild alveolar dilation appeared in noise, toluene, and simultaneous exposure groups. Moreover, imperceptible emphysema appeared in the toluene exposure group. Symbols denote parenchymal hemorrhage (narrow black arrow), inter-alveolar septal thickening (thick black arrow with green outline), and lymphocyte infiltration (thick blue arrow with yellow outline)

Table 3 Coefficients of ANOVA analysis for different apoptosis indices in the heart, lung, spleen, and stomach on day 14 post exposure (values are modified based on pre-exposure and control values to remove the effects of confounding variables)

Tissue	Parameter	Noise group	Toluene group	Combined group
Heart	Bax	4.58±1.34	-0.88±1.34	1.44±1.34
	Bcl-2	3.08±1.01	-0.39±1.01	1.62±1.01
	Bax/Bcl-2	0.0217±0.0882	-0.0138±0.0882	-0.0233±0.0882
Lung	Bax	3.39±1.46	0.54±1.46	4.19±1.46
	Bcl-2	-3.71±1.06	-2.55±1.06	-0.00±1.06
	Bax/Bcl-2	0.430±0.125	0.165±0.125	0.102±0.125
Spleen	Bax	-0.08±1.24	0.70±1.24	5.35±1.24
	Bcl-2	2.33±1.21	-1.61±1.21	-3.10±1.21
	Bax/Bcl-2	-0.192±0.131	0.131±0.131	0.617±0.131
Stomach	Bax	1.34±1.34	0.45±1.34	2.60±1.34
	Bcl-2	-0.08±1.39	-0.35±1.39	0.46±1.39
	Bax/Bcl-2	0.0598±0.0884	0.0578±0.0884	0.0738±0.0884

All values are mean ± SE (standard error)

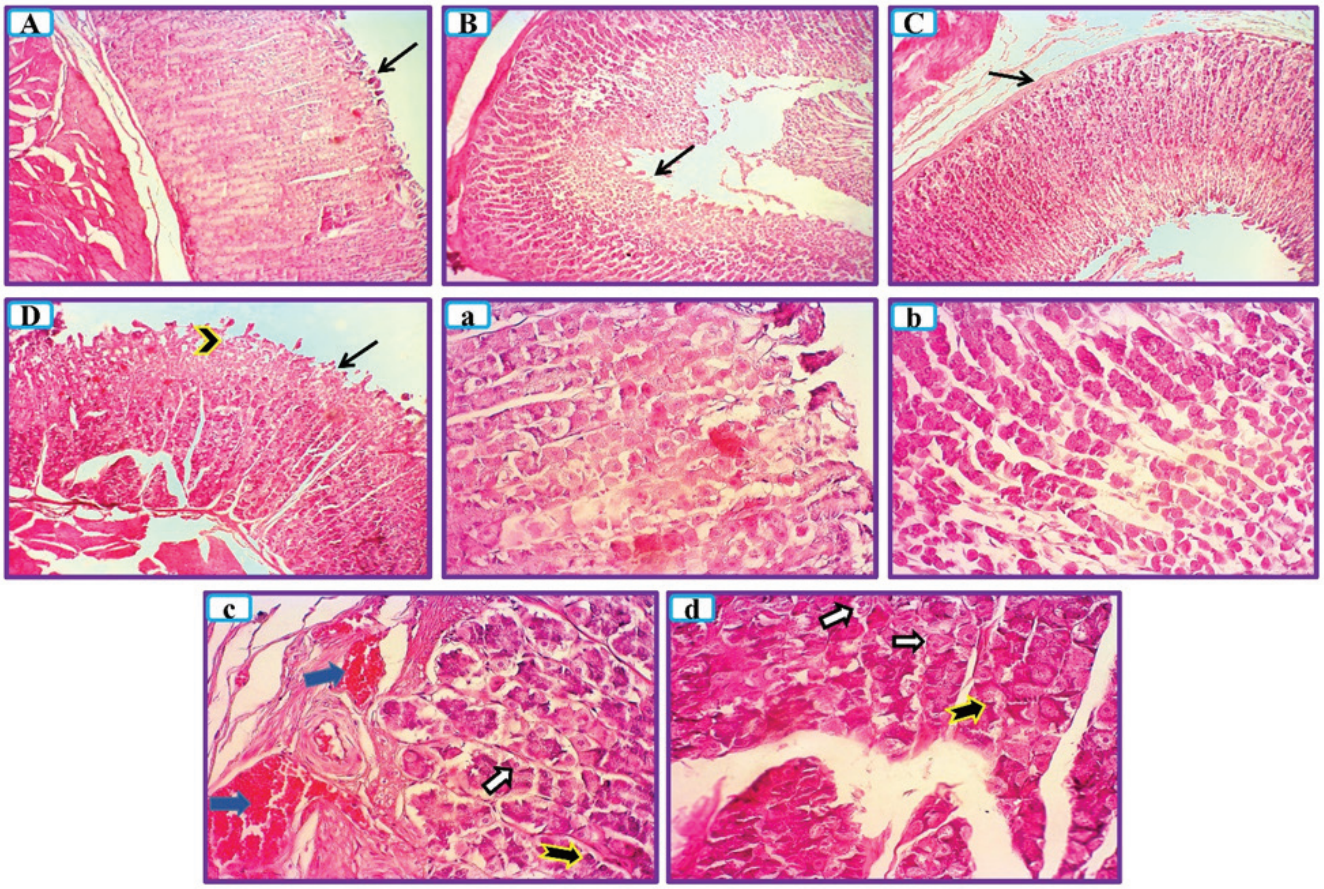

Figure 8 Representative appearances of the stomach tissue stained with H&E. The photographs were taken at 10× (A, B, C, and D) and 40× (a, b, c, and d) magnification. The control group appeared in normal condition (A and a). The noise exposure group (B and b) observed with cellular swelling. The toluene exposure group (C and c) and the simultaneous exposure group (D and d) appeared more destructed than the noise exposure group. Symbols denote glycocalix layer (narrow black arrow), epithelium exfoliation (thick black arrow head with yellow outline), congestion (thick blue arrow), pyknotic cells (thick black arrow with yellow outline), and glandular cell disorganisation (thick white arrow with black outline)

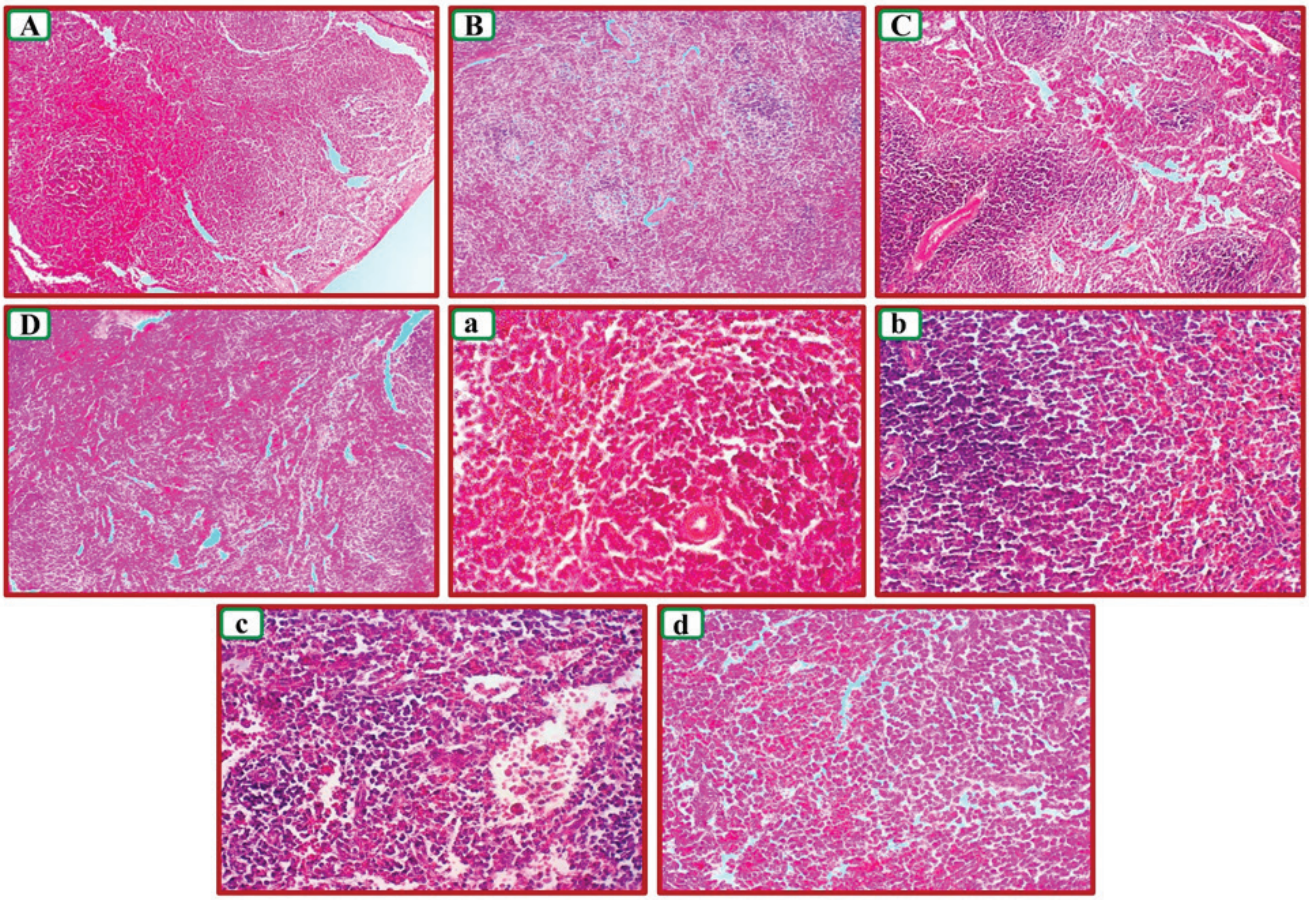

Figure 9 Representative appearances of the spleen tissue stained with H&E. The photographs were taken at 10× (A, B, C, and D) and 40× (a, b, c, and d) magnification. The control group (A and a) presented with normal tissue. The noise group (B and b), the toluene group (C and c), and the combined group (D and d) showed some pathological changes in lymphoid tissue. The noise group showed larger lymphoid tissue, yet smaller than the toluene group. It had more dilated and bloodier sinusoids than control and the toluene group. The combined group showed the same as its components but the changes were more prominent

the control group, the noise group exhibited a thinner glycocalyx and glycoprotein layer (mucosal layer) at the apex of gastric glands, cellular swelling and disorder. The toluene group showed pyknotic cells in gastric pits, swelled gastric glands, and congestion between mucosa and submucosa. Changes in the combined group were more severe, which might indicate synergistic effects of noise and toluene on the stomach tissue. The mucosal layer was thinned, and the appearance of pyknotic cells increased. We also noticed scattered cellular swelling and disorder in the epithelial parenchyma and epithelial exfoliation.

Earlier noise animal studies reported fibrous thickening of gastric lesions and media, massive collagen deposition, rupture of the inner elastic lamina (4), massive death of epithelial cells and cell degeneration without inflammation (40), cell shedding, pyknotic nuclei and separated basal lamina in mucosal cells, thickened mucosa, mononuclear cell infiltrates, vacuolated cytoplasm in parietal cells, and increased percentage of collagen fibres in fundic mucosa (3).

Figure 9 shows histopathological changes in the spleen across groups. Compared to normal spleen tissue in the control group, the noise group showed larger lymphoid tissue, yet smaller than the toluene group. However, it had more dilated and bloodier sinusoids than control and the toluene group. The latter showed disrupted areas around the white and red pulp resulting from lymphocyte proliferation and extensive lymphoid tissue. The combined group showed the same as its components but the changes were more noticeable, which may also suggest some kind of additive or synergistic effect.

Similar to our findings, earlier animal studies associate noise with large cells with pale vesicular irregular nuclei and acidophilic cytoplasm scattered in the white pulp and hyperaemia (37). Toluene studies report fibrosis, capsule wall thinning, increased internal diameter of the central arteriole in the white pulp, central arteriole wall thickening, spleen weight loss, extramedullary haematopoiesis in the red pulp, and increased white pulp area (41, 42).

Table 4 Coefficients of ANOVA analysis for relative tissue weights (tissue/body weight) in different groups taken on day 14 post exposure [(values are modified based on pre-exposure and control values to remove the effects of confounding variables. Combined effects (synergism, antagonism) were calculated as described in a study by Piggott et al. (30).]

Tissue	Noise group	Toluene group	Combined group	Interaction type
Heart	0.075±0.279	-0.146±0.279	0.222±0.279	+Synergism
Lung	-0.416±0.506	0.488±0.506	0.120±0.506	-Antagonism
Spleen	-0.264±0.116	0.121±0.116	0.203±0.116	+Synergism
Stomach	-1.611±0.891	0.056±0.891	0.850±0.891	+Synergism

All values are the coefficient value ± SE (standard error of coefficient)

Possible mechanisms of action

According to previous studies, noise and toluene can induce secretion of stress hormones (43–45) which produce reactive oxygen species (ROS) (46, 47). ROS, in turn, can induce apoptotic mechanisms through the expression of Bax and inhibition of Bcl-2 (48) and immune response through increased white blood cell, macrophage, immunoglobulin, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-ϰB) production, which secrete inflammatory cytokines (49). As a result, the immune system stops producing inflammatory cytokines after the early days of exposure by secreting some anti-inflammatory cytokines such as IL-10 (50, 51) to counteract inflammatory response (52, 53). In addition, an earlier study (7) suggests that acute exposure to noise enhances and chronic exposure suppress immune response.

Limitations

Our findings are somewhat limited for interpretation by small sample size and design that did not allow for several toluene concentrations and noise intensities, nor did it include other immunological parameters that could shed more light on mechanisms of action. It is also unfortunate that the study did not include histochemistry or more specific tissue staining.

CONCLUSION

Our study of combined exposure to noise and toluene is inconclusive, as it exhibited both synergistic and antagonistic effects in studied organs. Overall, however, the effects seem weaker than those of its components, yet the histopathological findings suggest the opposite.

Future studies should overcome its limitations with a larger sample size, different toluene concentrations, different noise intensities, longer exposure, and with different animals to reach more reliable insights into the histopathological changes in different organs.

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None to declare. The authors alone are responsible for the content of this study.

REFERENCES

1. Stansfeld SA, Matheson MP. Noise pollution: non-auditory effects on health. Br Med Bull 2003;68:243–57. doi: 10.1093/bmb/ldg033
2. Babisch W. Stress hormones in the research on cardiovascular effects of noise. Noise Health 2003;5:1–11. PMID: 12631430
3. Ahmed SM, Abdelrahman SA, Hassan EZ. Effect of low frequency noise on fundic mucosa of adult male albino rats and the role of vitamin E supplementation (histological and immunohistochemical study). J Clin Exp Pathol 2015;5:1000256. doi: 10.4172/2161-0681.1000256
4. Fonseca J, Martins-dos-Santos J, Oliveira P, Laranjeira N, Aguas A, Castelo-Branco N. Noise-induced gastric lesions: a light and electron microscopy study of the rat gastric wall exposed to low frequency noise. Arq Gastroenterol 2012;49:82–8. doi: 10.1590/s0004-28032012000100014
5. Cui B, Gai Z, She X, Wang R, Xi Z. Effects of chronic noise on glucose metabolism and gut microbiota–host inflammatory homeostasis in rats. Sci Rep 2016;6:36693. doi: 10.1038/srep36693
6. Pascuan CG, Uran SL, Gonzalez-Murano MR, Wald MR, Guelman LR, Genaro AM. Immune alterations induced by chronic noise exposure: comparison with restraint stress in BALB/c and C57Bl/6 mice. J Immunotoxicol 2014;11:78–83. doi: 10.3109/1547691X.2013.800171
7. Zheng KC, Ariizumi M. Modulations of immune functions and oxidative status induced by noise stress. J Occup Health 2007;49:32–8. doi: 10.1539/joh.49.32
8. Barbaresco GQ, Reis AV, Lopes GD, Boaventura LP, Castro AF, Vilanova TC, Da Cunha Junior EC, Pires KC, Pôrto Filho R, Pereira BB. Effects of environmental noise pollution on perceived stress and cortisol levels in street vendors. J Toxicol Environ Health A 2019;82:331–7. doi: 10.1080/15287394.2019.1595239
9. Rabat A, Bouyer JJ, George O, Le Moal M, Mayo W. Chronic exposure of rats to noise: relationship between long-term memory deficits and slow wave sleep disturbances. Behav Brain Res 2006;171:303–12. doi: 10.1016/j.bbr.2006.04.007
10. Koh SM, Dixon JB. Preparation and application of organo-minerals as sorbents of phenol, benzene and toluene. Appl Clay Sci 2001;18:111–22. doi: 10.1016/S0169-1317(00)00040-5

11. Auvinen J, Wirtanen L. The influence of photocatalytic interior paints on indoor air quality. Atmos Environ 2008;42:4101–12. doi: 10.1016/j.atmosenv.2008.01.031
12. Barzegar A, Mortazavi B, Asilian H, Kazemian H. Catalytic degradation of toluene by manganese oxide catalyst loaded on a natural zeolite support. Sci J Rev 2014;3:345–52.
13. Shangol AB, Mortazavi SB, Asilian H, Kazemian H. Elimination of toluene vapours using natural zeolite treated by copper oxide. J Kermanshah Univ Med Sci 2013;17(7):e74461.
14. Joshi DR, Adhikari N. An overview on common organic solvents and their toxicity. J Pharm Res Int 2019;28:1–18. doi: 10.9734/JPRI/2019/v28i330203
15. Kodavanti PR, Royland JE, Moore-Smith DA, Besas J, Richards JE, Beasley TE, Evansky P, Bushnell PJ. Acute and subchronic toxicity of inhaled toluene in male Long–Evans rats: oxidative stress markers in brain. Neurotoxicology 2015;51:10–19. doi: 10.1016/j.neuro.2015.09.001
16. Win-Shwe TT, Fujimaki H. Neurotoxicity of toluene. Toxicol Lett 2010;198:93–9. doi: 10.1016/j.toxlet.2010.06.022
17. Bowen SE, Hannigan JH. Developmental toxicity of prenatal exposure to toluene. AAPS J 2006;8:E419–24. doi: 10.1007/BF02854915
18. Taş U, Ekici F, Koç F, Söğüt E, Ayan M, Kuloglu T, Arici A, Özyurt B. Acute cardiotoxic effects of high dose toluene: an experimental study. Anadolu Kardiyol Derg 2013;13:3–8. doi: 10.5152/akd.2013.001
19. Kanter M. Thymoquinone attenuates lung injury induced by chronic toluene exposure in rats. Toxicol Ind Health 2011;27:387–95. doi: 10.1177/0748233710387630
20. Abouee-Mehrizi A, Rasoulzadeh Y, Mehdipour A, Alihemmati A, Rahimi E. Hepatotoxic effects caused by simultaneous exposure to noise and toluene in New Zealand white rabbits: a biochemical and histopathological study. Ecotoxicology 2021;30:154–63. doi: 10.1007/s10646-020-02288-z
21. Gupta RC, editor. Veterinary Toxicology: Basic and Clinical Principles. 2nd ed. Cambridge (MA): Academic Press, 2012.
22. Cobo P, Murillo-Cuesta S, Cediel R, Moreno A, Lorenzo-García P, Varela-Nieto I. Design of a reverberant chamber for noise exposure experiments with small animals. Appl Acoust 2009;70:1034–40. doi: 10.1016/j.apacoust.2009.03.005
23. Moreno A, Ruiz J, de la Colina C. Re-visiting Bolt's criterion for homogeneous distribution of normal frequencies in rectangular enclosures. In: Comunicacion presentada en los siguientes congresos: II Congreso Iberoamericano de Acustica. XXXI Congreso Nacional de Acustica – TecniAcustica 2000. II Jornadas Iberoamericanas de Ultrasonidos. II Congreso Iberico de Acustica. EAA Symposium on Architectural Acoustics. Madrid, 2000. p. 1–6.
24. Win-Shwe TT, Kunugita N, Nakajima D, Yoshida Y, Fujimaki H. Developmental stage-specific changes in immunological biomarkers in male C3H/HeN mice after early life toluene exposure. Toxicol Lett 2012;208:133–41. doi: 10.1016/j.toxlet.2011.10.015
25. Fujimaki H, Yamamoto S, Hojo R, Sato F, Kunugita N, Arashidani K. Effect of long-term exposure to low-level toluene on airway inflammatory response in mice. Toxicol Lett 2007;168:132–9. doi: 10.1016/j.toxlet.2006.11.008
26. Loganathan S, Srinivasan S, Wankhar W, Chodhary AK, Rathinasamy S. Noise exposure effect on selective inflammatory markers of Wistar albino rats immune organs and role *of Scoparia dulcis*. J Biol Active Products Nat 2017;7:294–310. doi: 10.1080/22311866.2017.1334587
27. Hou N, Zhang X, Zhao L, Zhao X, Li Z, Song T, Huang C. A novel chronic stress-induced shift in the Th1 to Th2 response promotes colon cancer growth. Biochem Biophys Res Commun 2013;439:471–6. doi: 10.1016/j.bbrc.2013.08.101
28. Sundareswaran L, Srinivasan S, Wankhar W, Sheeladevi R. Effect of *Scoparia dulcis* on noise stress induced adaptive immunity and cytokine response in immunized Wistar rats. J Ayurveda Integr Med 2017;8:13–9. doi: 10.1016/j.jaim.2016.10.004
29. Kim A, Sung JH, Bang JH, Cho SW, Lee J, Sim CS. Effects of self-reported sensitivity and road-traffic noise levels on the immune system. PLoS One 2017;12:e0187084. doi: 10.1371/journal.pone.0187084
30. Piggott JJ, Townsend CR, Matthaei CD. Reconceptualizing synergism and antagonism among multiple stressors. Ecol Evol 2015;5:1538–47. doi: 10.1002/ece3.1465
31. Demır M, Cicek M, Eser N, Yoldaş A, Sısman T. Effects of acute toluene toxicity on different regions of rabbit brain. Anal Cell Pathol 2017;2805370. doi: 10.1155/2017/2805370
32. Tas U, Ogeturk M, Meydan S, Kus I, Kuloglu T, Ilhan N, Kose E, Sarsilmaz M. Hepatotoxic activity of toluene inhalation and protective role of melatonin. Toxicol Ind Health 2011;27:465–73. doi: 10.1177/0748233710389853
33. Frenzilli G, Ryskalin L, Ferrucci M, Cantafora E, Chelazzi S, Giorgi FS, Lenzi P, Scarcelli V, Frati A, Biagioni F, Gambardella S, Fallen A, Fornai F. Loud noise exposure produces DNA, neurotransmitter and morphological damage within specific brain areas. Front Neurosci 2017;11:49. doi: 10.3389/fnana.2017.00049
34. Gröschel M, Basta D, Ernst A, Mazurek B, Szczepek AJ. Acute noise exposure is associated with intrinsic apoptosis in murine central auditory pathway. Front Neurosci 2018;12:312. doi: 10.3389/fnins.2018.00312
35. Abu-Amara TM, Abdelhay WM, Elsharawy AF, Mohamed LS, Mourad SE, Abdelghany AH, Taha NM. Effect of crowding stress on lung and heart of the adult albino rats and the possible protective role of sulpiride. Egypt J Hosp Med 2014;57:580–97. doi: 10.12816/008490
36. Gannouni N, Mhamdi A, El May M, Tebourbi O, Rhouma KB. Morphological changes of adrenal gland and heart tissue after varying duration of noise exposure in adult rat. Noise Health 2014;16:416–21. doi: 10.4103/1463-1741.144424
37. Xue L, Zhang D, Wang T, Shou X. Effects of high frequency noise on female rat's multi-organ histology. Noise Health 2014;16:213–7. doi: 10.4103/1463-1741.137048
38. Assunta C, Ilaria S, Gianfranco T, Teodorico C, Carmina S, Anastasia S, Roberto G, Francesco T, Valeria RM. Noise and cardiovascular effects in workers of the sanitary fixtures industry. Int J Hyg Environ Health 2015;218:163–8. doi: 10.1016/j.ijheh.2014.09.007
39. Zymantiene J, Zelvyte R, Pampariene I, Aniuliene A, Juodziukyniene N, Kantautaite J, Oberauskas V. Effects of long-term construction noise on health of adult female Wistar rats. Pol J Vet Sci 2017;20:155–65. doi: 10.1515/pjvs-2017-0020
40. Da Fonseca J, Dos Santos JM, Branco NC, Alves-Pereira M, Grande N, Oliveira P, Martins AP. Noise-induced gastric lesions: a light and scanning electron microscopy study of the alterations of the rat gastric mucosa induced by low frequency noise. Cent Eur J Public Health 2006;14:35–8. doi: 10.21101/cejph.a3362
41. Naeimi N, Adeli HR, Zare K. Study of toluene effect on blood parameters and spleen tissue in NMRI albino male mice. JNKUMS 2017;8:481–95. doi: 10.18869/acadpub.jnkums.8.3.481

42. Voloshin VN, Koveshnikov VG, Voloshina IS. Morphology of the spleen in adult albino rats after whole-body exposure to low-level of toluene. Int J Anat Res 2014;2:421–30.
43. Farzadinia P, Bigdeli M, Akbarzadeh S, Mohammadi M, Daneshi A, Bargahi A. Effect of noise pollution on testicular tissue and hormonal assessment in rat. Andrologia 2016;48:957–61. doi: 10.1111/and.12524
44. Kaiser K, Devito J, Jones CG, Marentes A, Perez R, Umeh L, Weickum RM, McGovern KE, Wilson EH, Saltzman W. Effects of anthropogenic noise on endocrine and reproductive function in White's treefrog, *Litoria caerulea*. Conserv Physiol 2015;3(1):cou061. doi: 10.1093/conphys/cou061
45. Münzel T, Daiber A, Steven S, Tran LP, Ullmann E, Kossmann S, Schmidt FP, Oelze M, Xia N, Li H, Pinto A, Wild P, Pies K, Schmidt ER, Rapp S, Kröller-Schön S. Effects of noise on vascular function, oxidative stress, and inflammation: mechanistic insight from studies in mice. Eur Heart J 2017;38:2838–49. doi: 10.1093/eurheartj/ehx081
46. Espinoza MB, Aedo JE, Zuloaga R, Valenzuela C, Molina A, Valdés JA. Cortisol induces reactive oxygen species through a membrane glucocorticoid receptor in rainbow trout myotubes. J Cell Biochem 2017;118:718–25. doi: 10.1002/jcb.25676
47. Flaherty RL, Owen M, Fagan-Murphy A, Intabli H, Healy D, Patel A, Allen MC, Patel BA, Flint MS. Glucocorticoids induce production of reactive oxygen species/reactive nitrogen species and DNA damage through an iNOS mediated pathway in breast cancer. Breast Cancer Res 2017;19:35. doi: 10.1186/s13058-017-0823-8
48. Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta 2016;1863:2977–92. doi: 10.1016/j.bbamcr.2016.09.012
49. Ko EY, Cho SH, Kwon SH, Eom CY, Jeong MS, Lee W, Kim SY, Heo SJ, Ahn G, Lee KP, Jeon YJ, Kim KN. The roles of NF-κB and ROS in regulation of pro-inflammatory mediators of inflammation induction in LPS-stimulated zebrafish embryos. Fish Shellfish Immunol 2017;68:525–9. doi: 10.1016/j.fsi.2017.07.041
50. Curtin NM, Mills KH, Connor TJ. Psychological stress increases expression of IL-10 and its homolog IL-19 via β-adrenoceptor activation: Reversal by the anxiolytic chlordiazepoxide. Brain Behav Immun 2009;23:371–9. doi: 10.1016/j.bbi.2008.12.010
51. Téllez-Martínez D, Batista-Duharte A, Silva VP, Fuentes DP, Ferreira LS, Polesi MC, Costa CB, Carlos IZ. Adaptive stress response induced by toluene increases *Sporothrix schenckii* virulence and host immune response. bioRxiv 2019:539775. doi: 10.1101/539775
52. Kasten KR, Muenzer JT, Caldwell CC. Neutrophils are significant producers of IL-10 during sepsis. Biochem Biophys Res Commun 2010;393:28–31. doi: 10.1016/j.bbrc.2010.01.066
53. Said EA, Dupuy FP, Trautmann L, Zhang Y, Shi Y, El-Far M, Hill BJ, Noto A, Ancuta P, Peretz Y, Fonseca SG, Van Grevenynghe J, Boulassel MR, Bruneau J, Shoukry NH, Routy J-P, Douek DC, Haddad EK, Sekaly R-P. Programmed death-1–induced interleukin-10 production by monocytes impairs $CD4^+$ T cell activation during HIV infection. Nat Med 2010;16:452–9. doi: 10.1038/nm.2106

Toksikopatološke promjene izazvane kombiniranom izloženosti buci i toluenu u novozelandskih bijelih kunića

Buka i toluen mogu prouzročiti značajne štetne posljedice u različitim organskim sustavima u ljudi, ali je malo poznato kako djeluju zajedno. Stoga je cilj ovog istraživanja bio utvrditi kako izloženost njihovoj kombinaciji utječe na razine upalnih citokina u serumu [tumorskoga nekrotskog čimbenika alfa (TNF-α) i interleukina 1 beta (IL-1β)], na tjelesnu masu te na apoptotske i histopatološke promjene na srčanom, plućnom, želudčanom i slezenskom tkivu. U tu smo svrhu novozelandske kuniće izložili buci (100 dB) i/ili toluenu (1000 mg/L) u posebnoj komori u neprekidnom trajanju od dva tjedna. Razine TNF-α i IL-1β izmjerene su u 14 dana nakon svršetka izloženosti pomoću imunoenzimske metode ELISA, a ekspresije proteina Bax i Bcl-2 povezanih s apoptozom pomoću kvantitativne polimerazne lančane reakcije (engl. *real-time polymerase chain reaction*, PCR). Buka i toluen, zasebno i u kombinaciji, doveli su do različitih promjena razina TNF-α i IL-1β u serumu te značajno povećali omjer proteina Bax/Bcl-2 u plućima i slezeni. Usto su prouzročili različite patološke promjene u srčanom, plućnom, slezenskom i želučanom tkivu. Istraživanje je potvrdilo da izloženost buci i toluenu dovodi do cijelog niza toksikopatoloških promjena, vjerojatno tako što pokreće upalne putove i mehanizme apoptoze. Također se doima da kombinirana izloženost ima slabije djelovanje od djelovanja svake sastavnice zasebno, premda histopatološki nalazi upućuju na suprotno.

KLJUČNE RIJEČI: apoptoza; IL-1β; industrijska toksikologija, okolišna izloženost; pluća; slezena; srce; tjelesna masa; TNF-α; želudac