Biotechnic & Histochemistry

ISSN: 1052-0295 (Print) 1473-7760 (Online) Journal homepage: https://www.tandfonline.com/loi/ibih20

A stereological study of the effects of mercury inhalation on the cerebellum

Berrin Zuhal Altunkaynak, Nilgün Akgül, Ahmad Yahyazedeh, Emine Makaracı & Hayati Murat Akgül

To cite this article: Berrin Zuhal Altunkaynak, Nilgün Akgül, Ahmad Yahyazedeh, Emine Makaracı & Hayati Murat Akgül (2019) A stereological study of the effects of mercury inhalation on the cerebellum, Biotechnic & Histochemistry, 94:1, 42-47, DOI: 10.1080/10520295.2018.1493224

To link to this article: https://doi.org/10.1080/10520295.2018.1493224

Published online: 06 Aug 2018.

Submit your article to this journal

Article views: 111

View related articles

View Crossmark data

Citing articles: 1 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=ibih20

BIOTECHNIC & HISTOCHEMISTRY
2019, VOL. 94, NO. 1, 42–47
https://doi.org/10.1080/10520295.2018.1493224

Taylor & Francis
Taylor & Francis Group

A stereological study of the effects of mercury inhalation on the cerebellum

Berrin Zuhal Altunkaynak[a], Nilgün Akgül[b], Ahmad Yahyazedeh[c], Emine Makaracı[d], and Hayati Murat Akgül[e]

[a]Department of Histology and Embryology, Faculty of Medicine, Okan University, İstanbul; [b]Department of Restorative Dentistry, Faculty of Dentistry, Atatürk University, Erzurum; [c]Department of Histology and Embryology, Faculty of Medicine, Karabük University, Karabük; [d]Department of Neurosciences, Health Sciences, Ondokuz Mayis University, Samsun, Turkey; [e]Department of Oral Diagnose and Radiology, Faculty of Dentistry, Pamukkale University, Denizli, Turkey

ABSTRACT
Mercury in the environment that arises from organic and inorganic sources can cause irreversible damage to the nervous system. Toxicity may be direct or may arise from interactions with other metals in the environment. We evaluated the possible effects of mercury vapor on rat cerebellum. Twelve adult female rats were divided into control and experimental groups. The rats in the experimental group were exposed to mercury vapor for 9 h/day for 45 days. Cerebellar tissue samples were evaluated using stereology and for histopathology. The total number of Purkinje cells was estimated using a physical disector method. We found that in the experimental group, overall volume decreased and the number of Purkinje cells was reduced. We also found cellular damage including pycnotic nuclei, eosinophilic cytoplasm and vacuolization; these features were absent in the control group. We found that chronic exposure to inorganic mercury vapor is toxic to the cerebellum.

KEYWORDS
Cerebellum; mercury vapor; microscopy; rat; stereology; toxicity

Mercury is a heavy metal that can contaminate the environment; it is toxic to all living organisms. Mercury is found in various chemical forms; elemental mercury and methyl mercury compounds are of particular concern (Clarkson 1997). People are exposed to mercury by environmental poisoning, occupational practices and dental amalgam.

Mercury can damage both the immune and nervous systems (Goering et al. 2002; Geier et al. 2008). Neurological damage is particularly problematic and results in developmental defects, peripheral neuropathies and increased neurodegenerative changes (Monroe and Halvorsen 2009). Even low doses of mercury can be neurotoxic to fetuses (Marsh and Turner 1995), children (Grandjean and Weihe 1998) and adults (Yokoo et al. 2003).

The central nervous system is highly sensitive to reactive oxygen species (ROS) owing to its large concentration of unsaturated lipids and high oxidative metabolism. Relatively little is known, however, about the relation of inhaled mercury vapor to cell injury caused by oxidative stress. The central nervous system is considered particularly susceptible to mercury vapor toxicity (WHO (World Health Organization) 1991). Consequently, we investigated the effects of mercury inhalation on the volume and histological structure of the cerebellum, particularly the number of Purkinje cells, in the adult female rat.

Material and methods

Animals and procedures

Our study was approved by the Experimental Research and Application Center of Atatürk University. We used 12 adult 150–200 g 8–10-week-old female Wistar albino rats. Rats were obtained from the Experimental Animals Research and Application Center of Atatürk University (Erzurum, Turkey). Rats were randomly divided into experimental and control groups, each consisting of six animals.

During the experiment, the rats were kept under standardized conditions: a 12 h light:12 h dark cycle at 22 ± 2 °C at 50 ± 5% humidity. Animals were allowed access to food and water *ad libitum*. The experimental group was housed in a closed chamber and exposed to inorganic mercury by inhalation (Altunkaynak et al. 2016) at a dose of 1 mg/m^3 for 9 h/day; exposure was from 08:00 to 17:00 h each day for 45 days. The mercury concentration of the chamber was measured every hour using a Jerome Model 431-X mercury analyzer (Arizona Instruments, Phoenix AR). After 17:00 h, the mercury vapor unit attached to the chamber was turned off and the animals remained in the closed chamber without exposure to mercury vapor during the night (Altunkaynak et al. 2016, Yahyazedeh et al. 2017).

CONTACT BZ Altunkaynak berrinzuhal@gmail.com Department of Histology and Embryology, Faculty of Medicine, Okan University, İstanbul, Turkey
© 2018 The Biological Stain Commission

The control group rats were housed under identical conditions in a closed chamber, but without exposure to mercury vapor.

After six weeks, all rats were anesthetized by inhalation of 2–3% (v/v) sevoflurane (Sevorane® liquid 250 ml, Abbott, Istanbul, Turkey) in 100% oxygen. The rats were perfused intracardially with fixative as follows: 0.9% physiologic saline followed by 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS). Finally, the cerebella were removed.

Histology

The tissue samples were processed immediately after removal (Harorli et al. 2009). Cerebella were dehydrated through graded alcohols, cleared in xylene, then embedded in paraffin. Transverse serial sections were cut at 5 µm using a rotary microtome. The first section in the series to be analysed was selected at random from the first five sections and every successive fifth section was collected from the series to give a one fifth section sampling fraction. In this way, approximately 15–20 sections were obtained from each cerebellum. Slides were stained with hematoxylin and eosin (H & E) (Dursun et al. 2010) Stained sections were used for light microscopy and stereological analysis.

Stereology

Mean cerebellar volume

Estimation of cerebellar volume enables both assessment of numerical density of Purkinje cells and stereological computation of the total volume of the cerebellum. To do this, the unbiased Cavalieri method was applied to the light microscopic images (Akgül et al. 2016) and the point-counting method (Sengul et al. 2017). A point counting test grid was used to estimate the areas of the sections of cerebellum (Figure 1a, b). To ensure a significant coefficient of error (CE), an appropriate point density of the point counting grid was designed for interesting regions of sections (Zengin et al. 2013, Turkmen et al. 2016). Total volume of the cerebellum ($\sum V$) was estimated using the following formula (Güven et al. 2013, Çakır-Özkan et al. 2017):

$$\sum V(total) = tx\sum A$$

where t is the thickness sum of slices (including intervals) and $\sum A$ is the total sectional area obtained from the cerebellar samples. In other words, $\sum A$ is defined by:

$$\sum A = a(p) * \sum P$$

where $a(p)$ is the interval point area and $\sum P$ is the number of points hitting on areas of interest in the sections.

Mean numerical density and total number of purkinje cells

A physical disector method was used to compute mean numerical density and total number of Purkinje cells (Ulubay et al. 2015). Based on a pilot study, sections were obtained by systematic unbiased sampling. Section pairs 5 µm thick and at 10 µm intervals were selected randomly to provide 15–20 section pairs. This number is in the acceptable range for stereological analysis (Altunkaynak et al. 2011, Ayrancı et al. 2013). After taking photographs at x 400, an unbiased 20 × 20 cm counting frame (Alkan et al. 2017) was superimposed on the reference image and look-up sections. Purkinje cells were counted according to the principle of the physical dissector. Particle profiles observed in the reference section, but not in the look-up section, are considered dissector particles (Altunkaynak et al. 2012, Yilmaz et al. 2017) (Figure 1c, d). The mean numerical density of the Purkinje cells ($Nv_{(Purkinje\ cell)}/mm^3$) was calculated as:

$$N_V = \frac{\sum Q^-}{\sum V_{Disector}}$$

where $\sum Q$ is the total number of Purkinje cells counted and $\sum V = (t * A)$ is the total volume of the reference used for counting; t is the mean section thickness (5 µm), and A is the area of the unbiased counting frame.

Finally, the total number of Purkinje cells (TN) was estimated as follows:

$$TN(total\ Purkinje\ cells) = Nv * \sum V$$

where TN is total number of Purkinje cells, Nv is numerical density of Purkinje cells and $\sum V$ is total volume of cerebellum as described above.

Statistical analysis

Statistical evaluation was performed using IBM SPSS Statistics 15.0 software for Windows. The Mann Whitney-U test was used for comparisons of stereological data. Values for $p \leq 0.05$ were considered significant.

Results

Histology

Images of the cerebella of the control and mercury vapor exposed groups are shown in Figure 2. The morphology of the cerebellum and Purkinje cells was normal in the control

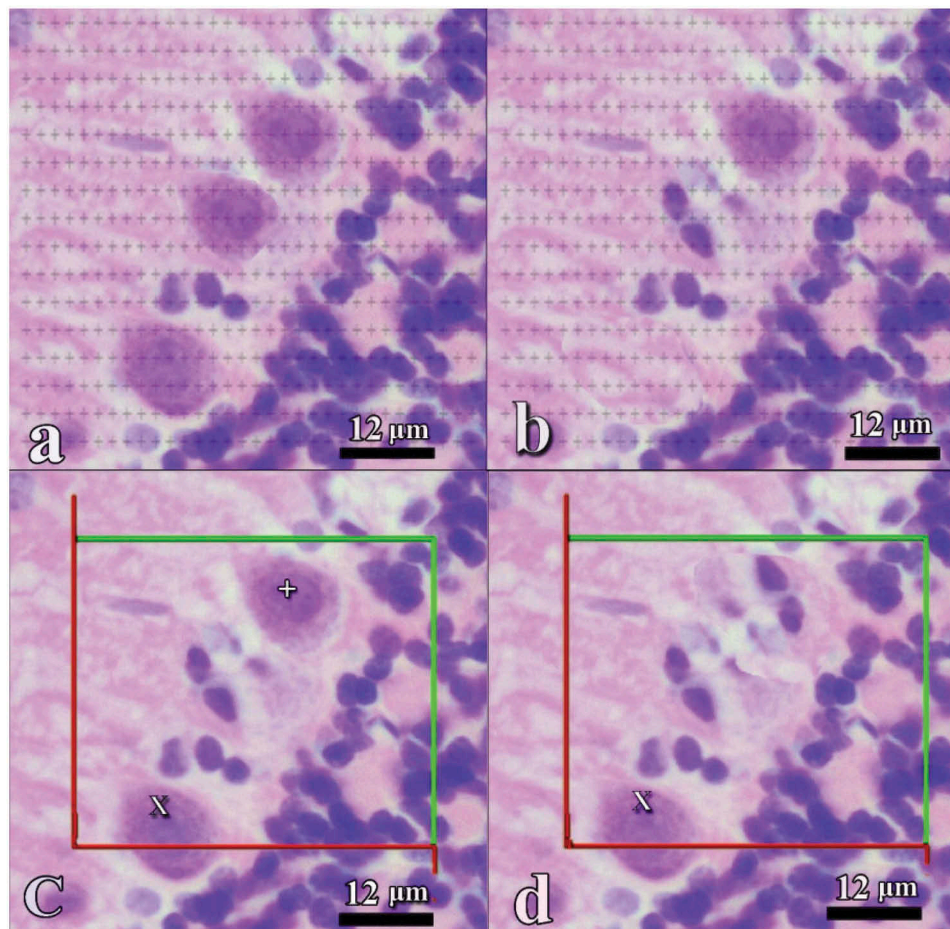

Figure 1. a, b) Estimation of cerebellar volume using the Cavalieri method. c, d) Procedures for applying the physical disector counting method. Note that (c) and (d) were the reference and look-up sections, respectively. (+) Counted as a dissector particle owing to lack of corresponding particle in look-up section while located within the frame of the reference section. (x) is a disector particle and is not counted, because it touched the exclusion line.

group (Figure 2a–c). Mercury vapor exposure caused damage to the cerebellum including gliosis, and peri-neuronal and perivascular vacuolization in the experimental group. The Purkinje cells in the experimental group also exhibited irregular cellular boundaries, eosinophilic cytoplasm and heterochromatic nuclei. Purkinje cells were seen rarely in the sections of the experimental group exposed to mercury vapor (Figure 2d–f).

Stereology

Mean cerebellar volume

A statistically significant reduction in volume was found in the mean volumes of the cerebella of the experimental group exposed to mercury compared to controls.

Mean numerical density and total number of purkinje cells

The physical dissector method demonstrated that cerebella of the experimental group contained significantly fewer Purkinje cells than the control group. Stereological data are summarized in Table 1.

Discussion

Several neurotoxic and nephrotoxic metals, including mercury, cause ROS and oxidative stress, which in turn cause in cell injury (Stohs and Bagchi 1995). Chen et al. (2005) reported that mercury can cause an increase in the concentration of free radicals with resulting DNA damage. The concentration of mercury was reported to be elevated in kidney and brain following long term mercury vapor exposure (Goering et al. 2002). Exposure to Hg has been shown

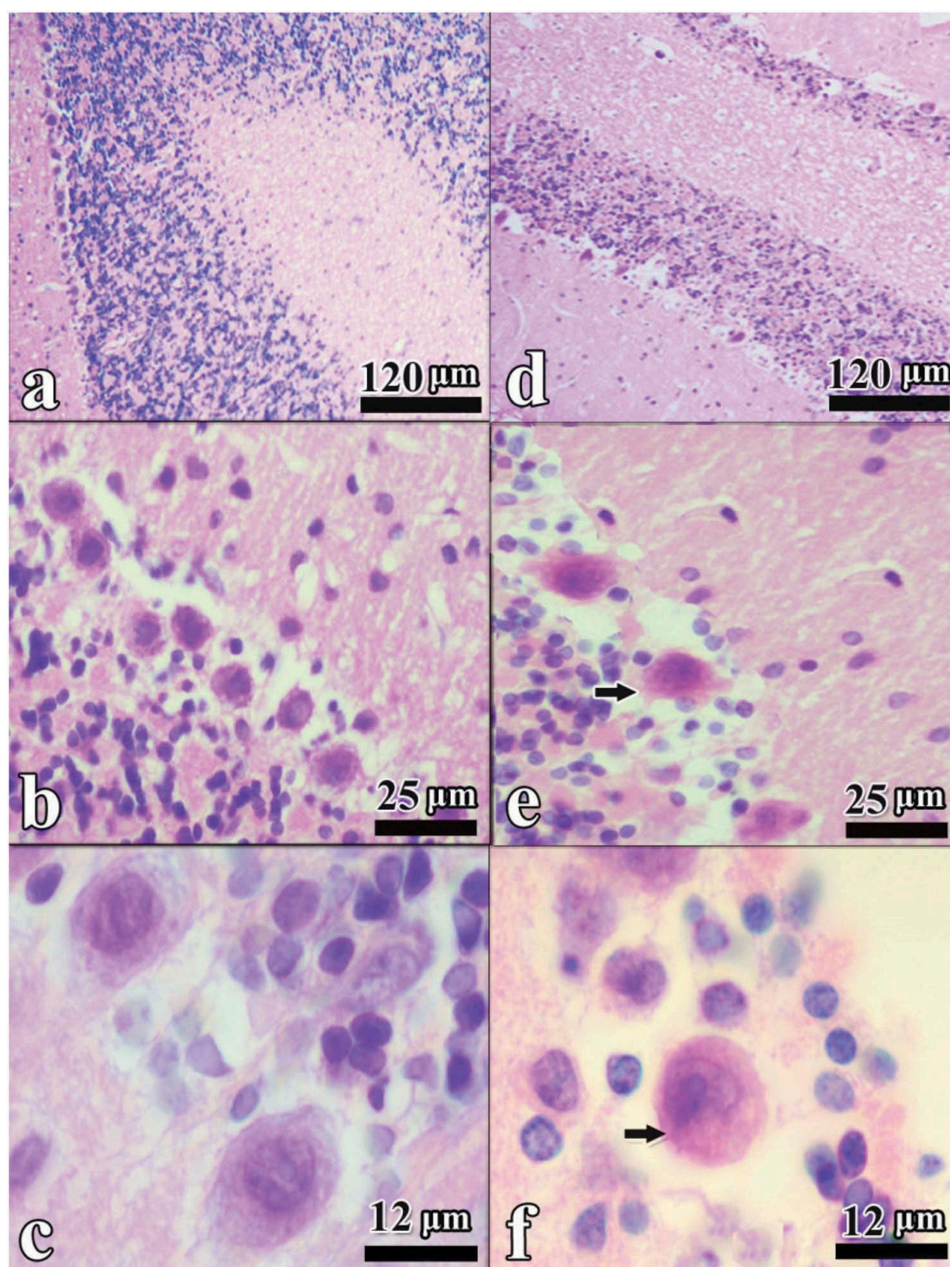

Figure 2. Example light microscopic images of cerebellum sections of control and mercury exposed experimental groups stained with H & E. a–c) Control group at 10, 40 and 100, respectively. d–f) Experimental group at 10, 40 and 100 x, respectively. Arrows: degenerated Purkinje cells with eosinophilic cytoplasm, dark stained nuclei and irregular cell boundaries.

Table 1. Mean volume of cerebellum and mean numerical density, total number and mean nuclear height of Purkinje cells in control and mercury exposed groups.

Estimations	Control	Exposed to mercury	CE	CV
Mean volume of cerebellum (cm^3)	2.3	2.1	0.035	0.054
Mean numerical density of Purkinje cells (cell/mm^3)	153,000	147,600	0.006	0.08
Total number of Purkinje cells	352,000	310,000	0.003	0.04

to produce metallothionein in brains of experimental animals (Yasutake et al. 1998).

We report here the effects of mercury vapor on female rat cerebellum. We detected loss of Purkinje cells and an increased number of Purkinje cells with shrunken cell bodies and pycnotic nuclei in rats exposed to mercury vapor for 6 weeks compared to control animals. Although the report by Fukuda (1971) was comparable to our investigation, these investigators did not demonstrate morphological

alteration in rabbit brain. Furthermore, no perceptible pathological changes were observed in squirrel monkey brain exposed to a single dose of 8 mg mercury (Berlin et al. 1975).

Although the literature contains many reports concerning the effects of organic mercury, there are few reports concerning the effects of inhaled Hg vapor. We found no reports of stereological and histological examination of the cerebellum following mercury inhalation (Fukuda 1971, Berlin et al. 1975). We conclude that mercury vapor causes loss of the Purkinje cells and that the vapor has deleterious effects on the cerebellum structure.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Akgül N, Altunkaynak BZ, Altunkaynak ME, Deniz ÖG, Ünal D, Akgül HM. 2016. Inhalation of mercury vapour can cause the toxic effects on rat kidney. Ren Fail. 38:465–473.

Alkan I, Altunkaynak BZ, Altun G, Erener E. 2017. The investigation of the effects of topiramate on the hypothalamic levels of fat mass/obesity-associated protein and neuropeptide Y in obese female rats. Nutr Neurosci. 15:1–10.

Altunkaynak BZ, Ozbek E, Aydin N, Altunkaynak ME, Vuraler O, Unal B. 2011. Effects of haloperidol on striatal neurons: relation to neuronal loss (a stereological study). Fol Neuropathol. 49:21–27.

Altunkaynak BZ, Unal D, Altunkaynak ME, Halici Z, Kalkan Y, Keles ON, Aksak S, Selli J, Unal B. 2012. Effects of diabetes and ovariectomy on rat hippocampus (a biochemical and stereological study). Gynecol Endocrinol. 28:228–233.

Altunkaynak BZ, Akgül N, Yahyazadeh A, Altunkaynak ME, Turkmen AP, Akgül HM, Ünal B. 2016. Effect of mercury vapor inhalation on rat ovary: stereology and histopathology. J Obstet Gynaecol Res. 42:410–416.

Ayrancı E, Altunkaynak BZ, Aktaş A, Rağbetli MÇ, Kaplan S. 2013. Prenatal exposure of diclofenac sodium affects morphology but not axon number of the median nerve of rats. Fol Neuropathol. 51:76–86.

Berlin M, Blomstrand C, Grant CA, Hamberger A, Trofast J. 1975. Tritiated methylmercury in the brain of squirrel monkeys. Arch Env Health. 30:591–597.

Çakır-Özkan N, Eğri S, Bekar E, Altunkaynak BZ, Kabak YB, Kıvrak EG. 2017. The use of sequential VEGF- and BMP2-releasing biodegradable scaffolds in rabbit mandibular defects. J Oral Maxillofac Surg. 75:221.e1–221.e14.

Chen C, Qu L, Li B. 2005. Increased oxidative DNA damage, as assessed by urinary 8-hydroxy-2′-deoxyguanosine concentrations, and serum redox status in persons exposed to mercury. Clin Chem. 51:759–767.

Clarkson TW. 1997. The toxicology of mercury. Crit Rev Clin Lab Sci. 34:369–403.

Dursun H, Albayrak F, Uyanik A, Keleş NO, Beyzagül P, Bayram E, Halici Z, Altunkaynak ZB, Süleyman H, Okçu N, et al. 2010. Effects of hypertension and ovariectomy on rat hepatocytes. Are amlodipine and lacidipine protective? (A stereological and histological study.). Turk J Gastroenterol. Dec;21(4):387–395.

Fukuda K. 1971. Metallic mercury induced tremor in rabbits and mercury content of the central nervous system. Br J Ind Med. 28:308–311.

Geier DA, King PG, Sykes LK, Geier MR. 2008. A comprehensive review of mercury provoked autism. Ind J Med Res. 128:383–411.

Goering PL, Morgan DL, Ali SF. 2002. Effects of mercury vapor inhalation on reactive oxygen species and antioxidant enzymes in rat brain and kidney are minimal. J Appl Toxicol. 22:167–172.

Grandjean P, Weihe P. 1998. Cognitive performance of children prenatally exposed to "safe" levels of methyl mercury. Env Res. 77:165–172.

Güven D, Altunkaynak BZ, Ayrancı E, Kaplan S, Bildircin FD, Kesim Y, Rağbetli MÇ. 2013. Stereological and histopathological evaluation of ovary and uterine horns of female rats prenatally exposed to diclofenac sodium. J Obstet Gynaecol. 33:258–263.

Harorli OT, Bayindir YZ, Altunkaynak Z, Tatar A, 2009. Cytotoxic effects of TEGDMA on THP-1 cells in vitro. Med Oral Pathol Oral Cir Bucal.

Marsh DO, Turner MD. 1995. Fetal methylmercury study in Peruvian fish-eating population. Neurotoxicology. 16:717–726.

Monroe RK, Halvorsen SW. 2009. Environmental toxicants inhibit neuronal Jak tyrosine kinase by mitochondrial disruption. Neur Toxicol. 30:589–598.

Sengul AT, Buyukkkarabacak YB, Altunkaynak BZ, Yetim TD, Altun GY, Sengul B, Basoglu A. 2017. Effects of platelet-rich plasma on cartilage regeneration after costal cartilage resection: a stereological and histopathological study. Acta Chir Belg. 117:21–28.

Stohs SJ, Bagchi D. 1995. Oxidative mechanisms in the toxicity of metal ions. Free Rad Biol Med. 18:321–336.

Turkmen AP, Kaplan S, Aksoy A, Altunkaynak B, Yurt K, Elibol E, Çokluk C, Onger M. 2016. Effects of diclofenac sodium on the hippocampus of rats with acute subdural hematoma: histological, stereological, and molecular approach. Fol Neuropathol. 54:167–179.

Ulubay M, Yahyazadeh A, Ög D, Kıvrak EG, Altunkaynak BZ, Erdem G, Kaplan S. 2015. Effects of prenatal 900 MHz electromagnetic field exposures on the histology of rat kidney. Int J Radia Biol. 91:35–41.

WHO (World Health Organization). 1991. Environmental health criteria. 118. Inorganic mercury. Geneva: World Health Organization.

Yahyazedeh A, Altunkaynak BZ, Akgül N, Akgül HM. 2017. A histopathological and stereological study of liver damage in female rats caused by mercury vapor. Biotech & Histochem. 92:338–346.

Yasutake A, Nakano A, Hirayama K. 1998. Induction by mercury compounds of brain metallothionein in rats: hgO exposure induces long-lived brain metallothionein. Arch Toxicol. 72:187–191.

Yilmaz S, Ozden B, Bas B, Altun G, Altunkaynak BZ. 2017. Could calcified triglyceride bone cement be an alternative

graft material in maxillary sinus augmentation? J Craniofac Surg. 28:97–103.

Yokoo EM, Valente JG, Grattan L, Schmidt SL, Platt I, Silbergeld EK. 2003. Low level methyl mercury exposure effects neuropsychological function in adults. Env Health. 2:8.

Zengin H, Kaplan S, Tümkaya L, Altunkaynak BZ, Rağbetli MÇ, Altunkaynak ME, Yilmaz O. 2013. Effect of prenatal exposure to diclofenac sodium on the male rat arteries: a stereological and histopathological study. Drug Chem Toxicol. 36:67–78.