2008-12-15

Ototoxic effects of industrial chemicals**

Ethyl benzene

A. Vyskocil[1]*, G. Truchon[2], T. Leroux[3], F. Lemay[2], M. Gendron[3], F. Gagnon[1], S. Botez[2], N. El Majidi[1], S.Lim[1], C. Émond[1], C. Viau[1]

Introduction

There is accumulating epidemiological evidence that exposure to some solvents, metals, asphyxiants and other substances in humans is associated with an increased risk of hearing loss. This project was undertaken to develop a toxicological database allowing the identification of possible ototoxic substances present in the work environment. Critical toxicological data were compiled for chemical substances included in the Quebec Occupational Health Regulation.

Methods

The data were evaluated only for realistic exposure concentrations up to the short-term exposure limit or ceiling value or five times the 8-h time weighted average exposure limit value(TWAEV) for human data and up to 100 times the 8-h TWAEV or ceiling value for animal studies.

Using a systematic weight of evidence approach, the information from both human and animal studies was examined.

At first, information from each source was given a weight of evidence qualifier for ototoxicity: strong, medium, weak, absent or "no study found". We took into consideration the following parameters: studied specie, number of subjects, exposure way, characteristics of control groups, exposure levels, audiometric and statistical tests, dose/effect relation. Table 1 shows how this information was combined to yield an overall assessment of the ototoxic potential of a given substance. Human data were generally given more weight in the overall assessment. When no human studies were available, which is different from the absence of evidence from the available human studies, the overall assessment was deemed the same as that from animal studies.

We built a weight of evidence table that allowed us to combine the information from both human and animal studies on ototoxicity of chemicals. Table 1 shows how the information from both types of studies were combined to yield an overall assessment and corollary conclusion about the ototoxicity of the investigated chemicals.

Human data were generally given more weight in the overall assessment. When no human studies were available, or when good quality human studies showed absence of evidence of an ototoxic effect, the overall assessment was one degree lower than that resulting from the animal studies. For example, a "strong" evidence from animal studies combined with an "absence" of evidence from the available human studies yielded a "medium" evidence overall.

Regarding the final conclusion about the ototoxic potential of chemical substances, all substances bearing a "strong evidence" of ototoxicity overall are considered "ototoxic". Those with "medium evidence" overall are rated "possibly ototoxic". We consider the ototoxic potential of those with only "weak evidence" as "non conclusive". Finally, those for which there is absence of evidence overall bear the mention "no evidence".

* Corresponding author : adolf.vyskocil@UMontreal.CA
** Production of this document was supported by the IRSST (Grant 099-542)

[1] Groupe de recherche interdisciplinaire en santé- Département de santé environnementale et santé au travail, Université de Montréal
[2] Institut de recherche Robert-Sauvé en santé et en sécurité du travail (IRSST)
[3] École d'orthophonie et d'audiologie, Université de Montréal

Table 1. Weight of evidence approach for the assessment of ototoxicity of various industrial chemicals

Strength of evidence about ototoxicity in assessed studies			Conclusion about ototoxicity
Human	**Animal**	**Overall**	
S	S	S	O
S	M	S	O
S	W	S	O
S	A	S	O
S	X	S	O
M	S	S	O
M	M	M	PO
M	W	M	PO
M	A	M	PO
M	X	M	PO
W	S	M	PO
W	M	W	NC
W	W	W	NC
W	A	W	NC
W	X	W	NC
A	S	M	PO
A	M	W	NC
A	W	W	NC
A	A	A	NE
A	X	A	NE
X	S	M	PO
X	M	W	NC
X	W	W	NC
X	A	A	NE

Indication of ototoxicity:

S = strong; M = medium; W = weak; A = absent; X = no study found

General conclusion about ototoxicity:

O = ototoxic substance; PO = possibly ototoxic substance; NC = non conclusive; NE = no evidence

---

Abbreviations

TWAEV : 8 h time weighed average exposure [limit] value in Quebec

D-TWAEV : Calculated inhaled dose for pulmonary ventilation of 10 m^3/d and body weight of 70 kg

Ceiling : Ceiling exposure [limit] value in Quebec

D-Ceiling : Calculated inhaled dose for pulmonary ventilation of 10 m^3/d and body weight of 70 kg

STEV : Short term exposure [limit] value in Quebec

C/D reported : Reported concentration or reported dose

CSU/DSU : Reported concentration expressed in standard units of mg/m^3 or reported dose expressed in standard units of mg/kg/d

Ratio : For concentrations CSU/TWAEV or CSU/Ceiling and for doses DSU/ D-TWAEV or DSU/D-Ceiling

ASM : Air sampling method

BM : Biological monitoring results

Ethyl benzene

Occupational exposure limits: TWAEV: 434 mg/m³ (100 ppm). STEV: 543 mg/m³ (125 ppm)

Conclusion about ototoxicity	Strength of evidence
Possibly ototoxic substance	From animal studies: **Strong** From human studies: **No study found** Overall: **Medium**

ANALYSIS OF ANIMAL STUDIES

Six studies on rats of two different strains and one study on guinea pig were identified. Five studies were performed in the same laboratory. An ototoxic effect was observed in 5 inhalation and 1 oral studies. Susceptibility to ethyl benzene is species dependent. Ethyl benzene causes a permanent damage to auditory system of the rat. The auditory system of the guinea-pig is not injured by ethyl benzene (Cappaert 2002). Ethyl benzene damages hair cells in the cochleae of rats. The important characteristic of ethyl benzene is higher susceptibility of outer hair cells (OHC) compared to inner hair cells. The effect is dose-related. Higher ethyl benzene concentrations lead to greater hair cell mortality. The mid-frequency hearing loss is most often reported. Morphologic examination determined a corresponding loss of OHC in the middle frequency region of the rat cochlea. Hair cell losses are not closely related to hearing threshold shifts in the rat (Cappaert 2001).

No chronic studies were identified. There is no ethyl benzene induced hearing loss for subacute exposure of rats up to about 300 ppm (Cappaert 2000) or for subchronic exposure of rats to 200 ppm (Gagnaire 2007). Concentrations greater than 300 ppm show threshold shifts directly related to ethyl benzene concentration (Cappaert 2000, Gagnaire 2007). Hair cell loss is a more sensitive endpoint than auditory threshold. The OHC losses were observed at 200 ppm (Gagnaire 2007).

ANALYSIS OF HUMAN STUDIES

No study was identified.

CONCLUSION

No human study was identified. In rats ethyl benzene affects the auditory function mainly in the cochlear mid-frequency range. Given the current evidence from animal studies, we recommend considering the ethyl benzene as a possibly ototoxic agent. Further studies with sufficient data on the exposure of workers to ethyl benzene are necessary to make a definitive conclusion.

Ethyl benzene [100-41-4]

Ethyl benzene
• TWAEV : 100 ppm | 434 mg/m³ D-TWAEV : 62 mg/kg/d

Population

Species : Rat Wistar # : 16 Sex : Males

Age :

Exposure

Route : Inhalation

Duration : 8 h/d; 5 d

C/D reported : 800 ppm

CSU/DSU :

Ratio : 8

ASM :

BM :

Remarks :

Tests

Test type • Effects reported	Precisions on test • Remarks
Reflex modification audiometry	Tone bursts at 4, 8, 12, 16, 20 and 24 kHz
• Threshold increased by about 25 dB, 1 and 4 weeks after the exposure irrespective of the stimulus frequency tested	• Test performed before and 1 and 4 weeks after the end of exposure
Electrocochleography	Tone bursts at 1, 2, 4, 8, 12, 16 and 24 kHz
• Threshold increased by 10 - 30 dB at all frequencies tested 8 and 11 weeks after the enf of exposure	• Test performed 8 - 11 weeks after the end of exposure
Light microscopy	
• Outer hair cells loss, especially in the upper basal and lower middle turns (corresponding to the mid-frequency region) to an extent of 65 %	• Histology performed immediately after electrocochleography

Action mechanism

Authors' conclusion

Hearing loss at 800 ppm in rats due to outer hair cells loss

Our conclusion

Ototoxic effect at 800 ppm in rats

Ethyl benzene [100-41-4]

Ethyl benzene
• TWAEV : 100 ppm | 434 mg/m³ D-TWAEV : 62 mg/kg/d

Population

Species : Rat Wag/Rij # : 8 Sex : Males

Age : 9 weeks

Exposure

Route : Inhalation

Duration : 8 h/d; 5 d

C/D reported : 300, 400 and 550 ppm

CSU/DSU :

Ratio : 3 - 5.5

ASM :

BM :

Remarks :

Tests

Test type • Effects reported	Precisions on test • Remarks
Distortion product otoacoustic emissions (DPOAE)	at 4, 5.6, 8, 11.3, 16 and 22.6 kHz L2 = L1-10 Ratio f2/f1 = 1.25
• 300 ppm : no effect 400 ppm : no effect 550 ppm : amplitude growth at 5, 6, 8 and 11.3 kHz	• Test performed 3 - 6 weeks after the end of exposure
Electrocochleography	at 1, 2, 4, 8, 12, 16 and 24 kHz
• 300 ppm : no effect 400 ppm : auditory threshold increased by 15 and 16 dB at 12 and 16 kHz, respectively 550 ppm : auditory threshold increased by 24, 31 and 22 dB at 8, 12 and 16 kHz, respectively	• Test performed 3 - 6 weeks after the end of exposure
Light microscopy	
• 300 ppm : no effect 400 ppm : 25 % outer hair cell (OHC) loss at 11- and 21-kHz region 550 ppm : 40 % and 75 % OHC loss at 11- and 21-kHz region, respectively	• Histology performed immediately after electrocochleography

Action mechanism

Authors' conclusion

Middle-frequency region of rats is affected after exposure to 400-550 ppm

Our conclusion

Ototoxic effect at 400 ppm in rats

Ethyl benzene [100-41-4]

Ethyl benzene
• TWAEV : 100 ppm | 434 mg/m³ D-TWAEV : 62 mg/kg/d

Population

Species : Rat Wag/Rij # : 8 Sex : Males

Age :

Exposure

Route : Inhalation

Duration : 8 h/d; 5 d

C/D reported : 300 and 400 ppm

CSU/DSU :

Ratio : 3 - 4

ASM :

BM :

Remarks :

Tests

Test type • Effects reported	Precisions on test • Remarks
Distortion product otoacoustic emissions (DPOAE)	at 4, 5.6, 8, 11.3, 16 and 22.6 kHz L2 = L1-10 Ratio f2/f1 = 1.25
• No effect	• Test performed 3 - 7 weeks after the end of exposure
Electrocochleography	at 1, 2, 4, 8, 12, 16 and 24 kHz
• No effect	• Test performed 3 - 7 weeks after the end of exposure
Light microscopy	
• Outer hair cells loss in the third row after 300 ppm and in the first, second and third row after 400 ppm. Outer hair cells loss located in the mid-frequency region of the cochlea	• Histology performed immediately after electrocochleography

Action mechanism

Authors' conclusion

No hearing loss but outer hair cells loss at 300 and 400 ppm in rats

Our conclusion

Ototoxic effect at 300 ppm in rats

Ethyl benzene [100-41-4]

Ethyl benzene
• TWAEV : 100 ppm | 434 mg/m³ D-TWAEV : 62 mg/kg/d

Population

Species : Rat Wag/Rij # : 8 Sex : Males
Age :

Exposure

Route : Inhalation
Duration : 8 h/d; 5 d
C/D reported : 550 ppm
CSU/DSU :
Ratio : 5.5
ASM :
BM :
Remarks :

Tests

Test type • Effects reported	Precisions on test • Remarks
Electrocochleography	Tone bursts at 1, 2, 4, 8, 12, 16 and 24 kHz
• Threshold increased by 2 - 30 dB in the 4- 24 kHz frequencies tested	• Test performed 4 - 8 weeks after the end of exposure
Light microscopy	
• Outer hair cells loss, especially in the mid-frequency region to an extent of 75 %	• Histology performed immediately after electrocochleography

Action mechanism

Authors' conclusion

Hearing loss at 550 ppm in rats due to outer hair cells loss

Our conclusion

Ototoxic effect at 550 ppm in rats

Ethyl benzene [100-41-4]

Ethyl benzene
• TWAEV : 100 ppm | 434 mg/m³ D-TWAEV : 62 mg/kg/d

Population

Species : Albino guinea pig # : 8 Sex : Females
Age :

Exposure

Route : Inhalation
Duration : 6 h/d; 5 d
C/D reported : 2500 ppm
CSU/DSU :
Ratio : 25
ASM :
BM :
Remarks :

Tests

Test type • Effects reported	Precisions on test • Remarks
Electrocochleography	Tone bursts at 1, 2, 4, 8, 12, 16 and 24 kHz
• No effect	• Test performed 4 - 8 weeks after the end of exposure
Light microscopy	
• No effect	• Histology performed immediately after electrocochleography

Action mechanism

Authors' conclusion

No hearing loss at 2500 ppm in guinea pigs due to low ethylbenzene concentration in blood in comparison with rats

Our conclusion

No ototoxic effect at 2500 ppm in guinea pigs

Ethyl benzene [100-41-4]

Ethyl benzene
• TWAEV : 100 ppm | 434 mg/m³ D-TWAEV : 62 mg/kg/d

Population

Species : Rat # : 6 Sex : Males

Age : 9 weeks

Exposure

Route : Gavage

Duration : 5 d/w; 2 w

C/D reported : 8.47 mmol/kg/d

CSU/DSU : 899 mg/kg/d

Ratio : 15

ASM :

BM :

Remarks :

Tests

Test type • Effects reported	Precisions on test • Remarks
Light and electron microscopy	Cochleogram
• - Almost complete loss in the three rows of outer hair cells in the medium and apical parts of the cochlea - About 50 % of the animals had losses in the basal part of the cochlea - Inner hair cell losses in some animals	• Histology performed 10 days after the end of exposure

Action mechanism

Authors' conclusion

High ototoxic effect of ethyl benzene in rats

Our conclusion

Ototoxic effect of ethyl benzene after exposure by oral way in rats

Ethyl benzene [100-41-4]

Ethyl benzene
• TWAEV : 100 ppm | 434 mg/m³ D-TWAEV : 62 mg/kg/d

Population

Species : Rat Sprague Dawley # : 14 Sex : Males

Age : 13 weeks

Exposure

Route : Inhalation

Duration : 6 h/d; 6 d/w; 13 w

C/D reported : 200, 400, 600, 800 ppm

CSU/DSU :

Ratio : 2 - 8

ASM :

BM :

Remarks : Background noise: < 66 dB SPL

Tests

Test type • Effects reported	Precisions on test • Remarks
Auditory brainstem responses	Clicks at 2, 4, 8 and 16 kHz
• - 200 ppm : no effect - 400 - 800 ppm : incresed thresholds at all frequencies from the 4 th week of exposure. - No recovery observed 8 weeks after the end of exposure	• Test performed at the end of 4 th, 8 th and 13 th week of exposure and 8 weeks after the end of exposure
Light and electron microscopy	
• - 200 ppm : up to 30% of outer hair cells (OHC) losses in the mid frequency region in 4 of the 8 animals - 400 ppm : considerable OHC losses, the highest in the 3rd row - 600 and 800 ppm : complete losses in the 3 rd row of the OHC and some inner hair cells losses	• Histology performed 8 weeks after the end of exposure

Action mechanism

Authors' conclusion

Ototoxic effect at 200 ppm ethyl benzene in rats exposed for 13 weeks. Hair cell loss is a more sensitive endpoint than auditory thresholds

Our conclusion

LOAEL of 200 ppm for ototoxicity of ethyl benzene in rats

BIBLIOGRAPHY

ATSDR 2007 Agency for Toxic Substances and Disease Registry (ATSDR) (2007). Toxicological profile for Ethylbenzene (Draft for Public Comment). Atlanta, GA, U.S. Department of Health and Human Services, Public Health Service.

Cappaert 1999 Cappaert, N.L., et al. (1999) The ototoxic effects of ethyl benzene in rats. Hear Res. 137(1-2): 91-102.

Cappaert 2000 Cappaert, N.L., et al. (2000) Ethyl benzene-induced ototoxicity in rats: a dose-dependent mid-frequency hearing loss. J Assoc Res Otolaryngol. 1(4): 292-9.

Cappaert 2001 Cappaert, N.L., et al. (2001) Simultaneous exposure to ethyl benzene and noise: synergistic effects on outer hair cells. Hear Res. 162(1-2): 67-79.

Cappaert 2002 Cappaert, N.L., et al. (2002) Differential susceptibility of rats and guinea pigs to the ototoxic effects of ethyl benzene. Neurotoxicol Teratol. 24(4): 503-10.

Gagnaire 2005 Gagnaire, F., et al. (2005) Relative ototoxicity of 21 aromatic solvents. Arch Toxicol. 79(6): 346-54.

Gagnaire 2007 Gagnaire, F., C. Langlais, et al. (2007). Ototoxicity in rats exposed to ethylbenzene and to two technical xylene vapours for 13 weeks. Arch Toxicol 81(2): 127-143.