Sudhakar K, Nageshwar M, Reddy KP. 2018. Abelmoschus moschatus extract reverses altered pain and neurohistology of a rat with developmental exposure of fluoride. *J Appl Pharm Sci* 8(6): 94-104.

Sudhakar K, Reddy KP. 2018. Protective effects of Abelmoschus moschatus seed extract on neurotransmitter system of developing brain of Wistar rats with gestational and post-natal exposure of sodium fluoride. *Int J Green Pharm* 12: S131-S139.

Sun ZR, Liu FZ, Wu LN, Lu Y, Yu DK. 2000. [Effects of high fluoride drinking water on the cerebral function of mice]. *Chin J Epidemiol* 19: 262-263.

Sun ZR, Liu FZ, Wu LN, Lu Y, Yu DK. 2008. Effects of high fluoride drinking water on the cerebral function of mice. *Fluoride* 41: 148-151.

Sun Z, Zhang Y, Xue X, Niu R, Wang J. 2018. Maternal fluoride exposure during gestation and lactation decreased learning and memory ability, and glutamate receptor mRNA expressions of mouse pups. *Hum Exp Toxicol* 37: 87-93.

Trivedi MH, Verma RJ, Chinoy NJ. 2007. Amelioration by black tea of sodium fluoride-induced changes in protein content of cerebral hemisphere, cerebellum and medulla oblongata in brain region of mice. *Acta Poloniae Pharm* 64: 221-225.

Trivedi MH, Verma RJ, Chinoy NJ. 2009. Mitigation of sodium fluoride induced toxicity in mice brain by black tea infusion. *Fluoride* 42: 29-33.

Trivedi MH, Verma RJ, Sangai NP, Chinoy NJ. 2011. Black tea extract mitigation of NaF-induced lipid peroxidation in different regions of mice brains. *Fluoride* 44: 243-254.

Trivedi MH, Verma RJ, Sangai NP, Chinoy NJ. 2012. Mitigation by black tea extract of sodium fluoride induced histopathological changes in brain of mice. *Fluoride* 45: 13-26.

Tsunoda M, Aizawa Y, Nakano K, Liu Y, Horiuchi T, Itai K, Tsunoda H. 2005. Changes in fluoride levels in the liver, kidney, and brain and in neurotransmitters of mice after subacute administration of fluoride. *Fluoride* 38: 284-292.

Varner JA, Jensen KF, Horvath W, Isaacson RL. 1998. Chronic administration of aluminum-fluoride or sodium-fluoride to rats in drinking water: Alterations in neuronal and cerebrovascular integrity. *Brain Res* 784(1-2): 284-298.

Verma RJ, Trivedi MH, Chinoy NJ. 2007. Black tea amelioration of sodium fluoride-induced alterations of DNA, RNA, and protein contents in the cerebral hemisphere, cerebellum, and medulla oblongata regions of mouse brain. *Fluoride* 40: 7-12.

Wang G, Li J, Zhu H, Zhu J. 2006. Effect of different doses of chronic exposure of fluoride on rat learning and memory behavior. *Studies of Trace Elements and Health* 23(2): 1-2.

Wang JD, Ge YM, Ning HM, Wang SL. 2004. Effects of high fluoride and low iodine on biochemical indexes of the brain and learning-memory of offspring rats. *Fluoride* 37: 201-208.

Wang J, Zhang Y, Guo Z, Li R, Xue X, Sun Z, Niu R. 2018. Effects of perinatal fluoride exposure on the expressions of miR-124 and miR-132 in hippocampus of mouse pups. *Chemosphere* 197: 117-122.

Wei N, Dong Y, Wang Y, Guan Z. 2014. [Effects of chronic fluorosis on neurobehavioral development in offspring of rats and antagonistic effect of vitamin E]. *Chin J Endemiol* 33: 125-128.

Whitford GM, Whitford JL, Hobbs SH. 2009. Appetitive-based learning in rats: Lack of effect of chronic exposure to fluoride. *Neurotoxicol Teratol* 31: 210-215.

Wu CX, Gu XL, Ge YM, Zhang JH, Wang JD. 2006. Effects of high fluoride and arsenic on brain biochemical indexes and learning-memory in rats. *Fluoride* 39: 274-279.

Wu NP, Zhao ZL, Gao WH, Li XQ. 1995. [Behavioral teratology in rats exposed to fluoride.] *Chin J Endemiol* 12(5): 271-273.

Wu NP, Zhao ZL, Gao WH, Li XQ. 2008. Behavioral teratology in rats exposed to fluoride. *Fluoride* 41: 129-133.

Xu X, Shen X, Zhang Z. 2001. Effect of fluorosis on mice learning and memory behaviors and brain SOD activity and MDA content *China Public Health* 17(1): 8-10.

Yang L, Jin P, Wang X, Zhou Q, Lin X, Xi S. 2018. Fluoride activates microglia, secretes inflammatory factors and influences synaptic neuron plasticity in the hippocampus of rats. *Neurotox* 69: 108-120.

Yu Q, Shao D, Zhang R, Ouyang W, Zhang Z. 2019. Effects of drinking water fluorosis on L-type calcium channel of hippocampal neurons in mice. *Chemosphere* 220: 169-175.

Yuan J, Li Q, Niu R, Wang J. 2019. Fluoride exposure decreased learning ability and the expressions of the insulin receptor in male mouse hippocampus and olfactory bulb. *Chemosphere* 224: 71-76.

Zhang C, Ren C, Chen H, Geng R, Fan H, Zhao H, Guo K, Geng D. 2013. The analog of Ginkgo biloba extract 761 is a protective factor of cognitive impairment induced by chronic fluorosis. *Biol Trace Elem Res* 153: 229-236.

Zhang KL, Lou DD, Guan ZZ. 2015. Activation of the AGE/RAGE system in the brains of rats and in SH-SY5Y cells exposed to high level of fluoride might connect to oxidative stress. *Neurotoxicol Teratol* 48: 49-55.

Zhang J, Zhu W, Zhang Z. 2009. [The effect of fluorine exposure of pregnant rats on the learning and memory capabilities of baby rats]. *Chinese Journal of Public Health* 25(11): 1347-1348.

Zhang J, Zhu WJ, Xu XH, Zhang ZG. 2011. Effect of fluoride on calcium ion concentration and expression of nuclear transcription factor kappa-B rho65 in rat hippocampus. *Exp Toxicol Pathol* 63: 407-411.

Zhang J, Zhang Z. 2013. Effects of chronic fluorosis on camkiiα, c-FOS, BAX, and BCL-2 channel signaling in the hippocampus of rats. *Fluoride* 46: 135-141.

Zhang Z, Shen X, Xu X. 2001. [Effects of selenium on the damage of learning-memory ability of mice induced by fluoride]. *J Hyg Res* 30: 144-146.

Zhang Z, Xu X, Shen X, Xua XH. 1999. [Effect of fluoride exposure on synaptic structure of brain areas related to learning-memory in mice]. *J Hyg Res* 28(4): 210-212.

Zhang Z, Xu X, Shen X, Xua XH. 2008. Effect of fluoride exposure on synaptic structure of brain areas related to learning-memory in mice. *Fluoride* 41: 139-143.

Zhao Q, Niu Q, Chen J, Xia T, Zhou G, Li P, Dong L, Xu C, Tian Z, Luo C, Liu L, Zhang S, Wang A. 2019. Roles of mitochondrial fission inhibition in developmental fluoride neurotoxicity: Mechanisms of action in vitro and associations with cognition in rats and children. *Arch Toxicol* 93(3): 709-726.

Zhao XL, Wu JH. 1998. Actions of sodium fluoride on acetylcholinesterase activities in rats. *Biomed Environ Sci* 11: 1-6.

Zheng X, Sun Y, Ke L, Ouyang W, Zhang Z. 2016. Molecular mechanism of brain impairment caused by drinking-acquired fluorosis and selenium intervention. *Environ Toxicol Pharmacol* 43: 134-139.

Zhu W, Zhang J, Zhang Z. 2011. Effects of fluoride on synaptic membrane fluidity and PSD-95 expression level in rat hippocampus. *Biol Trace Elem Res* 139: 197-203.

Zhu YL, Zheng YJ, LV XM, Ma Y, Zhang J. 2012. Effects of fluoride exposure on performance in water labyrinth and monoamine neurotransmitters of rats. *Journal of Xinjiang Medical University* 3: 014.

Zhu YP, Xi SH, Li MY, Ding TT, Liu N, Cao FY, Zeng Y, Liu XJ, Tong JW, Jiang SF. 2017. Fluoride and arsenic exposure affects spatial memory and activates the ERK/CREB signaling pathway in offspring rats. *Neurotox* 59: 56-64.

Studies Not Available in HAWC

Abdelaleem MM, El-Tahawy NFG, Abozaid SMM, Abdel-Hakim SA. 2018. Possible protective effect of curcumin on the thyroid gland changes induced by sodium fluoride in albino rats: Light and electron microscopic study. *Endocr Regul* 52: 59-68.

Abd-Elhakim YM, Mohammed AT, Ali HA. 2018. Impact of subchronic exposure to triclosan and/or fluoride on estrogenic activity in immature female rats: The expression pattern of calbindin-D9k and estrogen receptor alpha genes. *J Biochem Mol Toxicol* 32(2): 22027.

Abdumajidov OR. 2004. [Sex differences in lipid peroxidation and antioxidant defense of the brain tissue in intoxication with low doses of inorganic compounds]. *Uzbekiston Tibbiet Zhurnali*: 58-60.

Adebayo OL, Shallie PD, Salau BA, Ajani EO, Adenuga GA. 2013. Comparative study on the influence of fluoride on lipid peroxidation and antioxidants levels in the different brain regions of well-fed and protein undernourished rats. *J Trace Elem Med Biol* 27: 370-374.

Adedara IA, Ojuade TJD, Olabiyi BF, Idris UF, Onibiyo EM, Ajeigbe OF, Farombi EO. 2016. Taurine ameliorates renal oxidative damage and thyroid dysfunction in rats chronically exposed to fluoride. *Biol Trace Elem Res*: 1-8.

Ahmed SK, Kalleny NK, Attia AAEM, Elkateb LA. 2015. The possible protective role of chromium chloride against sodium fluoride-induced changes in the structure of the cerebellar cortex of the adult male albino rat. *Egypt J Histol* 38: 402-414.

Al Badawi MH, Mahmoud OM, Salem NA. 2016. Therapeutic potential of omega-3 against sodium fluoride toxicity on the cerebellar cortex of adult male albino rats: Histological and immunohistochemical study. *Egypt J Histol* 39: 170-178.

Alhayani A, Elshal EB, Aal IHA, Al-Shammeri E, Kabra H. 2013. Does vitamin E protect against sodium fluoride toxicity on the cerebellar cortex of albino rats? *Middle East J Sci Res* 16: 1019-1026.

Ameeramja J, Raghunath A, Perumal E. 2018. Tamarind seed coat extract restores fluoride-induced hematological and biochemical alterations in rats. *Environ Sci Pollut Res Int* 25(26): 26157-26166.

Antonyan OA. 1980. [Lipid per oxidation in fluorosis and the protective role of dietary factors]. *Zh Eksp Klin Med* 20: 381-388.

Ardelean I, Racoveanu N, Manescu S, Lupulescu A, Diaconescu M, Ghelerter L. 1964. Experimental investigations concerning the effect of fluorine on the thyroid gland. *Rum Med Rev* 8: 17-20.

Atmaca N, Atmaca HT, Kanici A, Anteplioglu T. 2014. Protective effect of resveratrol on sodium fluoride-induced oxidative stress, hepatotoxicity and neurotoxicity in rats. *Food Chem Toxicol* 70: 191-197.

Auskaps AM, Shaw JH. 1955. Hemoglobin concentration, thyroid weight and growth rate in rats during minimum fluoride ingestion. *J Nutr* 55: 611-621.

Bagmut I, Kolisnyk I, Titkova A, Petrenko T, Filipchenko S. 2018. Content of catecholamines in blood serum of rats under fluoride intoxication. *Georgian Med News* (280-281): 125-129.

Bakalyan PH, Antonyan OA. 1981. [Effect of fluorosis on glutathione peroxidase and glutathione reductase activities and sulfhydryl groups]. *Zh Eksp Klin Med* 21: 10-14.

Basha PM, Madhusudhan N. 2010. Pre and post natal exposure of fluoride induced oxidative macromolecular alterations in developing central nervous system of rat and amelioration by antioxidants. *Neurochem Res* 35: 1017-1028.

Basha PM, Madhusudhan N. 2011. Effect of maternal exposure of fluoride on oxidative stress markers and amelioration by selected antioxidants in developing central nervous system of rats. *Biologia* 66: 187-193.

Basha PM, Rai P, Begum S. 2011. Evaluation of fluoride-induced oxidative stress in rat brain: A multigeneration study. *Biol Trace Elem Res* 142: 623-637.

Basha PM, Sujitha NS. 2012. Combined influence of intermittent exercise and temperature stress on the modulation of fluoride toxicity. *Biol Trace Elem Res* 148: 69-75.

Basha PM, Saumya SM. 2013. Suppression of mitochondrial oxidative phosphorylation and TCA enzymes in discrete brain regions of mice exposed to high fluoride: Amelioration by panax ginseng (ginseng) and lagerstroemia speciosa (banaba) extracts. *Cell Mol Neurobiol* 33: 453-464.

Basha MP, Begum S, Madhusudhan N. 2014. Antioxidants in the management of fluoride induced neural oxidative stress in developing rats. *Int J Pharm Sci Res* 5: 201-206.

Benetato G, Giuran AM, Cirmaciu R, Cirje M, Petrescu A, Vacariu A. 1970. [Effect of fluorine in drinking water on the metabolism of Ca and Mg and on neuromuscular excitability: Experimental studies and clinical observations]. *Rev Roum Physiol* 7: 335-352.

Bharti VK, Srivastava RS. 2009. Fluoride-induced oxidative stress in rat's brain and its amelioration by buffalo (Bubalus bubalis) pineal proteins and melatonin. *Biol Trace Elem Res* 130: 131-140.

Bhatnagar M, Rao P, Saxena A, Bhatnagar R, Meena P, Barbar S, Chouhan A, Vimal S. 2006. Biochemical changes in brain and other tissues of young adult female mice from fluoride in their drinking water. *Fluoride* 39: 280-284.

Bilgili A, Akdogan M, Yildiz M, Eraslan G, Cetin N. 2004. The effects of fluoride on thyroid hormones in rabbits. *Indian Vet J* 81: 986-988.

Bobek S, Kahl S, Ewy Z. 1976. Effect of long-term fluoride administration on thyroid hormones level blood in rats. *Endocrinol Exp* 10: 289-295.

Bouaziz H, Ammar E, Ghorbel H, Ketata S, Jamoussi K, Ayadi F, Guermazi F, Zeghal N. 2004. Effect of fluoride ingested by lactating mice on the thyroid function and bone maturation of their suckling pups. *Fluoride* 37: 133-142.

Bouaziz H, Soussia L, Guermazi F, Zeghal N. 2005. Fluoride-induced thyroid proliferative changes and their reversal in female mice and their pups. *Fluoride* 38: 185-192.

Bouaziz HB, Amara I, Essefi M, Croute F, Zeghal N. 2010. Fluoride-induced brain damages in suckling mice. *Pestic Biochem Physiol* 96: 24-29.

Chauhan SS, Ojha S, Mahmood A. 2013. Effects of fluoride and ethanol administration on lipid peroxidation systems in rat brain. *Indian J Exp Biol* 51: 249-255.

Chen J, Chen X, Yang K, Xia T, Xie H. 2002. [Studies on DNA damage and apoptosis in rat brain induced by fluoride]. *Chin J Prev Med* 36: 222-224.

Chirumari K, Reddy PK. 2007. Dose-dependent effects of fluoride on neurochemical milieu in the hippocampus and neocortex of rat brain. *Fluoride* 40: 101-110.

Chouhan S, Yadav A, Kushwah P, Kaul RK, Flora SJS. 2011. Silymarin and quercetin abrogates fluoride induced oxidative stress and toxic effects in rats. *Mol Cell Toxicol* 7: 25-32.

Clay AB, Suttie JW. 1987. Effect of dietary fluoride on dairy cattle: Growth of young heifers. *J Dairy Sci* 70: 1241-1251.

Czechowicz K, Osada A, Slesak B. 1974. Histochemical studies on the effect of sodium fluoride on metabolism in Purkinje's cells. *Folia Histochem Cytochem* 12: 37-44.

Demole V, Lerch P. 1956. [Normality of fixation of radioactive iodine in the thyroid of rats during experimental fluorosis]. *Helv Physiol Pharmacol Acta* 14(4): 62-63.

Dhurvey V, Patil V, Thakare M. 2017. Effect of sodium fluoride on the structure and function of the thyroid and ovary in albino rats (rattus norvegicus). *Fluoride* 50: 235-246.

Domzalska E. 1966. [Influence of sodium fluoride on hypophysis, thyroid gland, parathyroid, and adrenal gland in the white rat]. *Czas Stomatol* 19: 839-844.

El-lethey HS, Kamel MM, Shaheed IB. 2011. Perinatal exposure to sodium fluoride with emphasis on territorial aggression, sexual behaviour and fertility in male rats. *Life Sci J* 8: 686-694.

Flora SJS, Mittal M, Mishra D. 2009. Co-exposure to arsenic and fluoride on oxidative stress, glutathione linked enzymes, biogenic amines and DNA damage in mouse brain. *J Neurol Sci* 285: 198-205.

Flora SJS, Mittal M, Pachauri V, Dwivedi N. 2012. A possible mechanism for combined arsenic and fluoride induced cellular and DNA damage in mice. *Metallomics* 4: 78-90.

Gabovich RD, Verzhikovskaia NV. 1958. [Effect of fluoride compounds on absorption of radioactive iodine by the thyroid gland in humans and in experimental conditions]. *Probl Endokrinol Gormonoter* 4: 49-54.

Galamini-Ligori M, Di Blasi F. 1961. [Action of sodium fluoride on the thyroid of hypophysectomized rats]. *Boll Soc Ital Biol Sper* 37: 1503-1506.

Galletti P, Held HR, Korrodi H, Wegmann T. 1956. [Investigations on the possible damaging effect of fluorine on the thyroid gland]. *Helv Med Acta* 23: 601-605.

Ge Y, Ning H, Feng C, Wang H, Yan X, Wang S, Wang J. 2006. Apoptosis in brain cells of offspring rats exposed to high fluoride and low iodine. *Fluoride* 39: 173-178.

Ge Y, Niu R, Zhang J, Wang J. 2011. Proteomic analysis of brain proteins of rats exposed to high fluoride and low iodine. *Arch Toxicol* 85: 27-33.

Ge YM, Ning HM, Wang SL, Wang JD. 2005. DNA damage in thyroid gland cells of rats exposed to long-term intake of high fluoride and low iodine. *Fluoride* 38: 318-323.

Ge YM, Ning HM, Wang SL, Wang JD. 2005. Comet assay of DNA damage in brain cells of adult rats exposed to high fluoride and low iodine. *Fluoride* 38: 209-214.

Ge YM, Ning HM, Wang SL, Wang JD. 2005. Effects of high fluoride and low iodine on brain histopathology in offspring rats. *Fluoride* 38: 127-132.

Ge YM, Ning HM, Gu XL, Yin M, Yang XF, Qi YH, Wang JD. 2013. Effects of high fluoride and low iodine on thyroid function in offspring rats. *J Integr Agric* 12: 502-508.

Guan ZZ. 1986. [Morphology of the brain of the offspring of rats with chronic fluorosis]. *Chin J Pathol* 15: 297-299.

Guan Z, Wang Y, Xiao K. 1997. [Influence of experimental fluorosis on phospholipid content and fatty acid composition in rat brain]. *Chin Med J* 77: 592-596.

Guan Z-Z, Wang Y-N, Xiao K-Q, Dai D-Y, Chen Y-H, Liu J-L, Sindelar P, Dallner G. 1998. Influence of chronic fluorosis on membrane lipids in rat brain. *Neurotoxicol Teratol* 20: 537-542.

Guan ZZ, Shan KR, Wang YN, Dallner G. 2006. [Changes of lipids and nicotinic receptors in rat brains and pheochromocytoma with fluorosis]. *Chin J Endemiol* 25: 121-124.

Gushchin SK. 1951. [Effect of sodium fluoride on iodine metabolism in rabbit tissue organs; on the etiology of endemic goiter]. *Gig Sanit* 2: 45-48.

Hamza RZ, Al-Harbi MS. 2014. Sodium fluoride induced neurotoxicity and possible antioxidant role of selenium and curcumin in male mice. *Biosci Biotechnol Res Asia* 11: 81-87.

Hamza RZ, El-Shenawy NS, Ismail HAA. 2015. Protective effects of blackberry and quercetin on sodium fluoride-induced oxidative stress and histological changes in the hepatic, renal, testis and brain tissue of male rat. *J Basic Clin Physiol Pharmacol* 26: 237-251.

Haojun Z, Yaoling W, Ke Z, Jin L, Junling W. 2012. Effects of NaF on the expression of intracellular Ca2+ fluxes and apoptosis and the antagonism of taurine in murine neuron. *Toxicol Mech Methods* 22: 305-308.

Hara K. 1980. Studies on fluorosis especially effects of fluoride on thyroid metabolism. *J Dent Health* 30: 42-57.

Harris NO, Hayes RL. 1955. A tracer study of the effect of acute and chronic exposure to sodium fluoride on the thyroid iodine metabolism of rats. *J Dent Res* 34: 470-477.

Hassan HA, Abdel-Aziz AF. 2010. Evaluation of free radical-scavenging and anti-oxidant properties of black berry against fluoride toxicity in rats. *Food Chem Toxicol* 48: 1999-2004.

Hoogstratten B, Leone NCLG, Shupe J, Greenwood DA, Lieberman J. 1965. Effect of fluorides on hematopoietic system, liver, and thyroid gland in cattle. *J Amer Med Assoc* 192: 26-32.

Inkielewicz I, Rogowska M, Krechniak J. 2006. Lipid peroxidation and antioxidant enzyme activity in rats exposed to fluoride and ethanol. *Fluoride* 39: 53-59.

Inkielewicz I, Czarnowski W. 2008. Oxidative stress parameters in rats exposed to fluoride and aspirin. *Fluoride* 41: 76-82.

Inkielewicz-Stepniak I, Czarnowski W. 2010. Oxidative stress parameters in rats exposed to fluoride and caffeine. *Food Chem Toxicol* 48: 1607-1611.

Jiang P, Li G, Zhou X, Wang C, Qiao Y, Liao D, Shi D. 2018. Chronic fluoride exposure induces neuronal apoptosis and impairs neurogenesis and synaptic plasticity: Role of GSK-3beta/beta-catenin pathway. *Chemosphere* 214: 430-435.

Jiang SF, Xi SH, Yao SQ, Tong JW, Zhang YS, Wang Q, Su J, Li MY. 2013. [Effects of fluoride, arsenic and co-exposure on expression of Bcl-2 and Bax in hippocampus and cerebral cortex of rats]. *Chin J Endemiol* 32: 365-369.

Jiang Y, Guo X, Sun Q, Shan Z, Teng W. 2016. Effects of excess fluoride and iodide on thyroid function and morphology. *Biol Trace Elem Res* 170: 382-389.

Jin TX, Guan ZZ, Zhang H. 2011. [The effect of fluoride on α subunit of calcium/calmodulin-dependent protein kinase-II mRNA and protein expression in central nervous system]. *Chin J Endemiol* 30: 247-250.

Jonderko G, Kita K, Pietrzak J, Primus-Slowinska B, Ruranska B, Zylka-Wloszczyk M, Straszecka J. 1983. [Effect of subchronic sodium fluoride poisoning on the thyroid gland of rabbits with normal and increased supply of iodine]. *Endokrynol Pol* 34: 195-203.

Kahl S, Bobek S. 1975. [Effect of fluoride administration on radiothyroxine turnower in rats]. *Endokrynol Pol* 26: 391-396.

Kahl S, Ewy Z. 1975. Effect of single and long term sodium fluoride administration on biosynthesis of the thyroid hormone in rats. *Fluoride* 8: 191-198.

Kapoor V, Prasad T, Paliwal VK. 2001. Blood biochemical constituents in calves following subclinical levels of fluoride toxicosis. *Fluoride* 34: 126-131.

Karawya FS, Zahran NM, Azzam EZ. 2015. Is water fluoridation a hidden cause of obesity? Histological study on thyroid follicular cells of albino rats. *Egypt J Histol* 38: 547-557.

Kaur T, Bijarnia RK, Nehru B. 2009. Effect of concurrent chronic exposure of fluoride and aluminum on rat brain. *Drug Chem Toxicol* 32: 215-221.

Kelimu A, Liu KT, Lian J, Hu HH, Zheng YJ, Wang TM. 2008. [Effects of vitamin C and E on the ultrastructure in liver, kidney and brain of fluorosis rats]. *Chin J Endemiol* 27: 378-381.

Kinawy AA. 2019. Synergistic oxidative impact of aluminum chloride and sodium fluoride exposure during early stages of brain development in the rat. *Environ Sci Pollut Res Int* 26(11): 10951-10960.

Knizhnikov VA. 1959. [Effect of potable water with high fluoride concentration on thyroid function]. *Gig Sanit* 24: 20-25.

Knizhnikov VA, Tsypin AB, Shcherbova EN, Bugryshev PF. 1963. [The effect of drinking water with an increased fluorine content on the bioelectrical activity of the brain and heart under experimental conditions]. *Gig Sanit* 28: 16-19.

Kondo T, Yoshida M, Kasahara K. 1976. [Acute fluorosis in female rats: Time of inhibition and recovery of cholinesterase in serum and salivary glands]. *Jpn J Dent Health* 26: 187-192.

Kowalewska M. 1974. [Biopotentials of the organ of hearing in chronic sodium fluoride poisoning]. *J Pol Otolaryngol* 28: 417-424.

Krechniak J, Inkielewicz I. 2005. Correlations between fluoride concentrations and free radical parameters in soft tissues of rats. *Fluoride* 38: 293-296.

Leonard BE. 1972. Effect of phentolamine on the increase in brain glycolysis following the intraventricular administration of dibutyryl-3,5-cyclic adenosine monophosphate and sodium fluoride to mice. *Biochem Pharmacol* 21: 115-117.

Liu G, Zhang W, Jiang P, Li X, Liu C, Chai C. 2012. Role of nitric oxide and vascular endothelial growth factor in fluoride-induced goitrogenesis in rats. *Environ Toxicol Pharmacol* 34: 209-217.

Li H, Cai Q, Wang D. 2012. [Effect of fluoride on the expression of rat thyroid peroxidase mRNA]. *Chin J Endemiol* 31: 515-517.

Li H, Cai Q, Wang D. 2012. [Effects of fluoride on rat thyroid morphology, thyroid peroxidase activity and the expression of thyroid peroxidase protein]. *Chin J Endemiol* 31: 271-274.

Liu H, Hou C, Zeng Q, Zhao L, Cui Y, Yu L, Wang L, Zhao Y, Nie J, Zhang B, Wang A. 2016. Role of endoplasmic reticulum stress-induced apoptosis in rat thyroid toxicity caused by excess fluoride and/or iodide. *Environ Toxicol Pharmacol* 46: 277-285.

Liu YJ, Gao Q, Wu CX, Guan ZZ. 2010. [Changes of the c-Jun N-terminal kinase in the brains of rats with chronic fluorosis]. *Chin J Endemiol* 29: 608-612.

Liu YJ, Guan ZZ, Gao Q, Pei JJ. 2011. Increased level of apoptosis in rat brains and SH-SY5Y cells exposed to excessive fluoride-A mechanism connected with activating JNK phosphorylation. *Toxicol Lett* 204: 183-189.

Lohakare J, Pattanaik AK. 2013. Effects of addition of fluorine in diets differing in protein content on urinary fluoride excretion, clinical chemistry and thyroid hormones in calves. *Brazilian J Anim Sci* 42: 751-758.

Long YG, Wang YN, Chen J, Jiang SF, Nordberg A, Guan ZZ. 2002. Chronic fluoride toxicity decreases the number of nicotinic acetylcholine receptors in rat brain. *Neurotoxicol Teratol* 24: 751-757.

Lou DD, Liu YF, Zhang KL, Yu YN, Guan ZZ. 2011. [Changes of reactive oxygen species level and mitochondria fission-fusion in cortical neurons of rats with chronic fluorosis]. *Chin J Endemiol* 30: 256-260.

Lou DD, Liu YF, Qin SL, Zhang KL, Yu YN, Guan ZZ. 2012. [Changed transcription level of mitochondrial fission and fusion gene loci in cortical neurons of rats with chronic fluorosis]. *Chin J Endemiol* 31: 125-129.

Lou DD, Zhang KL, Qin SL, Liu YF, Yu YN, Guan ZZ. 2012. [Alteration of mitochondrial distribution and gene expression of fission 1 protein in cortical neurons of rats with chronic fluorosis]. *Chin J Pathol* 41: 243-247.

Lou DD, Pan JG, Zhang KL, Qin SL, Liu YF, Yu YN, Guan ZZ. 2013. [Changed expression of mito-fusion 1 and mitochondrial fragmentation in the cortical neurons of rats with chronic fluorosis]. *Chin J Prev Med* 47: 170-174.

Lou DD, Zhang KL, Pan JG, Qin SL, Liu YF, Yu YN, Guan ZZ. 2013. [Influence of chronic fluorosis on the expression of mitochondrial fission protein dynamin-related 1 in the cortical neurons of rats]. *Chin J Prev Med* 47: 561-564.

Lou DD, Zhang KL, Qin SL, Liu YF, Liu YJ, Guan ZZ. 2013. [Effects of chronic fluorosis on 4.8 kb mitochondrial DNA in liver, kidney and brain of rats]. *Chin J Endemiol* 32: 121-124.

Lou DD, Guan ZZ, Pei JJ. 2014. Alterations of apoptosis and expressions of Bax and Bcl-2 in the cerebral cortices of rats with chronic fluorosis. *Fluoride* 47: 199-207.

Luo GY, Niu RY, Sun ZL, Zhang JH, Wang JM, Wang C, Wang JD. 2011. Reduction of CaMKII expression in the hippocampus of rats from ingestion of fluoride and/or lead. *Fluoride* 44: 63-69.

Ma T, Liu D, Song K. 1999. Cytochemical study of neuron enzyme at anterior horn of spinal cord in rats with experimental fluorosis. *J Chin Med Univ* 28: 81-82.

Ma TX, Yu HT, Song KQ. 2008. [Expression of c-fos and Caspase 8 in cerebral cortex of rats with experimental fluorosis]. *Chin J Endemiol* 27: 131-133.

Mach Z, Zygulska-Machowa H. 1959. O wplywie fluoru na przemiane J131 [Russian and English summ.]. *Endokrynol Pol* 10: 157-162.

Machida H. 1989. [A study on the rabbit thermoregulatory system effects of high dose sodium fluoride]. *Dent Sci Rep* 89: 607-626.

Madan J, Puri JP, Singh JK. 2009. Growth, feed efficiency and blood profile of buffalo calves consuming high levels of fluoride. *Trop Anim Health Prod* 41: 295-298.

Madhusudhan N, Basha PM, Begum S, Ahmed F. 2009. Fluoride-induced neuronal oxidative stress and its amelioration by antioxidants in developing rats. *Fluoride* 42: 179-187.

Madhusudhan N, Basha PM, Rai P, Ahmed F, Prasad GR. 2010. Effect of maternal fluoride exposure on developing CNS of rats: Protective role of Aloe vera, Curcuma longa and Ocimum sanctum. *Indian J Exp Biol* 48: 830-836.

Manocha SL, Warner H, Olkowski ZL. 1975. Cytochemical response of kidney, liver and nervous system of fluoride ions in drinking water. *Histochem J* 7: 343-355.

Mansour HH, Tawfik SS. 2012. Efficacy of lycopene against fluoride toxicity in rats. *Pharm Biol* 50: 707-711.

Mietkiewski K, Walczak M, Trojanowicz R. 1966. [Effect of sodium fluoride on the neurosecretory system in guinea pigs]. *Endokrynol Pol* 17: 121-131.

Mohamed NE. 2016. The role of calcium in ameliorating the oxidative stress of fluoride in rats. *Biol Trace Elem Res* 170: 128-144.

Muhlemann HR, Schneider R. 1956. [Mitotic activity of rat thyroid epithelium after administration of fluoridated drinking water]. *Schweiz Med Wochenschr* 86: 625-627.

Nabavi SF, Eslami S, Moghaddam AH, Nabavi SM. 2011. Protective effects of curcumin against fluoride-induced oxidative stress in the rat brain. *Neurophysiology* 43: 287-291.

Nabavi SF, Moghaddam AH, Nabavi SM, Eslami S. 2011. Protective effect of curcumin and quercetin on thyroid function in sodium fluoride intoxicated rats. *Fluoride* 44: 147-152.

Nabavi SF, Habtemariam S, Jafari M, Sureda A, Nabavi SM. 2012. Protective role of gallic acid on sodium fluoride induced oxidative stress in rat brain. *Bull Environ Contam Toxicol* 89: 73-77.

Nabavi SF, Nabavi SM, Latifi AM, Mirzaei M, Habtemariam S, Moghaddam AH. 2012. Mitigating role of quercetin against sodium fluoride-induced oxidative stress in the rat brain. *Pharm Biol* 50: 1380-1383.

Nabavi SF, Nabavi SM, Habtemariam S, Moghaddam AH, Sureda A, Mirzaei M. 2013. Neuroprotective effects of methyl-3-O-methyl gallate against sodium fluoride-induced oxidative stress in the brain of rats. *Cell Mol Neurobiol* 33: 261-267.

Nabavi SM, Sureda A, Nabavi SF, Latifi AM, Moghaddam AH, Hellio C. 2012. Neuroprotective effects of silymarin on sodium fluoride-induced oxidative stress. *J Fluor Chem* 142: 79-82.

Narayanaswamy M, Piler MB. 2010. Effect of maternal exposure of fluoride on biometals and oxidative stress parameters in developing CNS of rat. *Biol Trace Elem Res* 133: 71-82.

Narbutt B, Romer TE, Grabski J, Szymik N. 1971. [Influence of natrium fluoride on the structure of the rat thyroid]. *Endokrynol Pol* 22: 445-451.

Narbutt B, Romer TE, Grabski J, Szymik N. 1971. [The influence of sodium fluoride on the morphology of the thyroid gland in rats]. *Endokrynol Pol* 22: 361-365.

Niu RY, Sun ZL, Cheng ZT, Liu HT, Chen HC, Wang JD. 2008. Effects of fluoride and lead on N-methyl-D-aspartate receptor 1 expression in the hippocampus of offspring rat pups. *Fluoride* 41: 101-110.

Niu R, Wang J, Sun Z, Xue X, Yan X, Zhang J. 2015. Transcriptional regulatory dynamics of the hypothalamic-pituitary-testicular axis in male mice exposed to fluoride. *Environ Toxicol Pharmacol* 40: 557-562.

Niu R, Zhang Y, Liu S, Liu F, Sun Z, Wang J. 2015. Proteome alterations in cortex of mice exposed to fluoride and lead. *Biol Trace Elem Res* 164: 99-105.

Ogilvie AL. 1952. Histological findings in the kidney, liver, pancreas, adrenal and thyroid gland of the rat following sodium fluoride administration. *J Dent Res* 31: 598-598.

Okayasu I, Tsuchida M, Yanagisawa F. 1985. Hyperplastic nodules of thyroid parafollicular cells (C cells) in rats induced by prolonged low dose ingestion of NaF. *Fluoride* 18: 111-117.

Pal S, Sarkar C. 2014. Protective effect of resveratrol on fluoride induced alteration in protein and nucleic acid metabolism, DNA damage and biogenic amines in rat brain. *Environ Toxicol Pharmacol* 38: 684-699.

Pan Y, Lu P, Yin L, Chen K, He Y. 2015. Effect of fluoride on the proteomic profile of the hippocampus in rats. *Z Naturforsch C* 70: 151-157.

Phillips PH, Lamb AR. 1934. Histology of certain organs and teeth in chronic toxicosis due to fluorin. *Arch Path* 17: 169-176.

Portela ML. 1972. [Biochemical effects in the prolonged ingestion of fluorides in rats]. *Arch Latinoam Nutr* 22: 291-308.

Prestes DS, Filappi A, Schossler DR, Duarte FA, Dressler VL, Flores EMM, Cecim M. 2009. Functional and histological evaluations of thyroid of sheep submitted to sodium fluoride administration. *Arq Bras Med Vet Zootec* 61: 293-298.

Puentes F, Cremer HD. 1966. Experiments on fluoride-iodine antagonism in the thyroid gland. *Adv Fluorine Res* 4: 213-220.

Qian W, Miao K, Li T, Zhang Z. 2013. Effect of selenium on fluoride-induced changes in synaptic plasticity in rat hippocampus. *Biol Trace Elem Res* 155: 253-260.

Qing-Feng S, Ying-Peng X, Tian-Tong X. 2019. Matrix metalloproteinase-9 and p53 involved in chronic fluorosis induced blood-brain barrier damage and neurocyte changes. *Arch Med Sci* 15(2): 457-466.

Qiu YH, Kong DM, Yang Q, Zhao N. 2010. [Influence of high-fluoride on thyroid function and brain damage in rats]. *Chin J Endemiol* 29: 146-149.

Raghavendra M, Ravindra RK, Raghuveer YP, Narasimha JK, Uma MRV, Navakishor P. 2016. Alleviatory effects of hydroalcoholic extract of cauliflower (brassica oleracea var. botrytis) on thyroid function in fluoride intoxicated rats. *Fluoride* 49: 84-90.

Rakhov GM. 1964. [Effect of calcium and fluorine in drinking water on the iodine metabolism and status of the thyroid gland in iodine insufficiency in food]. *Gig Sanit* 29: 12-17.

Ranpariya VL, Parmar SK, Sheth NR, Chandrashekhar VM. 2011. Neuroprotective activity of matricaria recutita against fluoride-induced stress in rats. *Pharm Biol* 49: 696-701.

Reddy KP, Sailaja G, Krishnaiah C. 2009. Protective effects of selenium on fluoride induced alterations in certain enzymes in brain of mice. *J Environ Biol* 30: 859-864.

Rogalska A, Kuter K, Zelazko A, Glogowska-Gruszka A, Swietochowska E, Nowak P. 2017. Fluoride alteration of [3H]glucose uptake in Wistar rat brain and peripheral tissues. *Neurotoxicol Res* 31: 436-443.

Saka O, Hallac P, Urgancioğlu I. 1965. The effect of fluoride on the thyroid of the rat. *New Istanbul Contrib Clin Sci* 8: 87-90.

Samanta A, Chanda S, Bandyopadhyay B, Das N. 2016. Establishment of drug delivery system nanocapsulated with an antioxidant (+)-catechin hydrate and sodium meta borate chelator against sodium fluoride induced oxidative stress in rats. *J Trace Elem Med Biol* 33: 54-67.

Sarkar C, Das N, Pal S, Dinda B. 2014. Oxidative stress induced alteration of protein and nucleic acid metabolism in fluoride-intoxicated rat brain: Protection by 3α-hydroxy olean-12-en-27-oic acid isolated from neanotis wightiana. *Int J Pharm Sci Res* 5: 3047-3066.

Sarkar C, Pal S. 2014. Ameliorative effect of resveratrol against fluoride-induced alteration of thyroid function in male Wistar rats. *Biol Trace Elem Res* 162: 278-287.

Sarkar C, Pal S, Das N, Dinda B. 2014. Ameliorative effects of oleanolic acid on fluoride induced metabolic and oxidative dysfunctions in rat brain: Experimental and biochemical studies. *Food Chem Toxicol* 66: 224-236.

Seffner W, Teubener W, Runde H, Wiedner H, Vogt J, Otto G, Zschau E, Geinitz D, Franke J. 1990. Boron as an antidote to fluorosis? II. Studies on various organs of pigs. *Fluoride* 23: 68-79.

Selim AOA, El-Haleem MR, Ibrahim IH. 2012. Effect of sodium fluoride on the thyroid gland of growing male albino rats: Histological and biochemical study. *Egypt J Histol* 35: 470-482.

Shao Q, Wang Yn, Guan Z. 2000. [Influence of free radical inducer on the level of oxidative stress in brain of rats with fluorosis]. *Chin J Prev Med* 34: 330-332.

Sharma C, Suhalka P, Sukhwal P, Jaiswal N, Bhatnagar M. 2014. Curcumin attenuates neurotoxicity induced by fluoride: An in vivo evidence. *Pharmacogn Mag* 10: 61-65.

Shashi A. 1992. Studies on alterations in brain lipid metabolism following experimental fluorosis. *Fluoride* 25: 77-84.

Shashi A. 1993. Nucleic acid levels in thyroid gland in acute and chronic fluoride intoxication. *Fluoride* 26: 191-196.

Shashi A, Singh JP, Thapar SP. 1994. Effect of long-term administration of fluoride on levels of protein, free amino acids and RNA in rabbit brain. *Fluoride* 27: 155-159.

Shashi A. 2003. Histopathological investigation of fluoride-induced neurotoxicity in rabbits. *Fluoride* 36: 95-105.

Shashi A, Neetika S, Bhardwaj M. 2009. Neuronal DNA damage and apoptosis in brain of rat exposed to fluoride. *Asian J Microbiol Biotechnol Environ Sci* 11: 629-632.

Shayiq RM, Raza H, Kidwai AM. 1986. Fluoride and lipid peroxidation a comparative study in different rat tissues. *Bull Environ Contam Toxicol* 37: 70-76.

Shen QF, Li HN, Xu TT, Xia YP. 2012. [Damage of blood brain barrier of spinal cord in rats with chronic fluorosis]. *Chin Med J* 92: 2357-2361.

Shen Q, Tian R, Li H, Xu T, Xia Y. 2014. [White matter injury of spinal cord in rats with chronic fluorosis and recovery after defluoridation]. *Chin Med J* 94: 1189-1192.

Shen X, Zhang Z, Xu X. 2004. [Influence of combined iodine and fluoride on phospholipid and fatty acid composition in brain cells of rats]. *J Hyg Res* 33: 158-161.

Shivarajashankara YM, Shivashankara AR, Bhat PG, Rao SH. 2001. Effect of fluoride intoxication on lipid peroxidation and antioxidant systems in rats. *Fluoride* 34: 108-113.

Shivarajashankara YM, Shivashankara AR, Bhat PG, Rao SH. 2002. Brain lipid peroxidation and antioxidant systems of young rats in chronic fluoride intoxication. *Fluoride* 35: 197-203.

Shivarajashankara YM, Shivashankara AR, Bhat PG, Rao SM, Rao SH. 2002. Histological changes in the brain of young fluoride-intoxicated rats. *Fluoride* 35: 12-21.

Siebenhuner L, Miloni E, Burgi H. 1984. [Effects of fluoride on thyroid hormone biosynthesis: Studies in a highly sensitive test system]. *Klin Wochenschr* 62: 859-861.

Singh R, Srivastava AK, Gangwar NK. 2017. Clinico-pathological studies on the co-exposure of cypermethrin and fluoride in experimental rats with ameliorative action of Vitamin E. *Vet Pract* 18(2): 207-210.

Soni KK, Shrivastava VK. 2007. Sodium fluoride induced histopathological changes in thyroid gland of male mus musculus. *Biochem Cell Arch* 7: 317-320.

Stee EW. 1968. *Effect of sodium fluoride and AMOX (NF3O) on growth and thyroid function in the rat*. No. AMRL-TR-67-189. Wright-Patterson Air Force Base, OH: pp. 67.

Štolc V, Podoba J. 1960. Effect of fluoride on the biogenesis of thyroid hormones. *Nature* 188: 855-856.

Sugivama Y. 1967. [The effect of sodium fluoride administration on the parathyroid glands]. *Hirosaki Med J* 19: 520-529.

Sun Y, Ke L, Zheng X, Li T, Ouyang W, Zhang Z. 2016. Effects of different levels of calcium intake on brain cell apoptosis in fluorosis rat offspring and its molecular mechanism. *Biol Trace Elem Res*: 1-12.

Takata H. 1958. The effect of fluorine upon the uptake of I131 by the thyroid glands. *Folia Pharmacol Jpn* 54: 230-236.

Teng Y, Zhang J, Zhang Z, Feng J. 2017. The effect of chronic fluorosis on calcium ions and CaMKIIα, and c-fos expression in the rat hippocampus. *Biol Trace Elem Res*: 295-302.

Trabelsi M, Guermazi F, Zeghal N. 2001. Effect of fluoride on thyroid function and cerebellar development in mice. *Fluoride* 34: 165-173.

Tsuchida M, Okayasu I, Kohyama Y, Kurihara H, Tanaka H, Yanagisawa F, Date C, Hayashi M, Mui K, Asada M. 1986. Effects of long term, low dose ingestion of fluoride on the thyroid gland in rats. *Stud Environ Sci* 27: 307-312.

Vani ML, Reddy KP. 2000. Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice. *Fluoride* 33: 17-26.

Wang C, Liang C, Ma J, Manthari RK, Niu R, Wang J, Wang J, Zhang J. 2018. Co-exposure to fluoride and sulfur dioxide on histological alteration and DNA damage in rat brain. *J Biochem Mol Toxicol* 32.

Wang H, Yang Z, Zhou B, Gao H, Yan X, Wang J. 2009. Fluoride-induced thyroid dysfunction in rats: Roles of dietary protein and calcium level. *Toxicol Ind Health* 25: 49-57.

Wang J, Niu R, Sun Z, Lv L, Smith GW. 2008. Effects of protein and calcium supplementation on bone metabolism and thyroid function in protein and calcium deficient rabbits exposed to fluoride. *Fluoride* 41: 283-291.

Wang JD, Ge YM, Ning HM, Wang SL. 2004. Effects of high fluoride and low iodine on oxidative stress and antioxidant defense of the brain in offspring rats. *Fluoride* 37: 264-270.

Wang JL. 2007. [Effect of fluoride on the intracellular Ca2+ in neurons of mice]. *Chin J Endemiol* 26: 505-507.

Wang Y, Guan Z, Xiao K. 1997. [Changes of coenzyme Q content in brain tissues of rats with fluorosis]. *Chin J Prev Med* 31: 330-333.

Wang Y, Dong Y, Wei N, Guan Z. 2015. [Influence of chronic fluorosis on expression of quinone oxidoreductase-1 and heme oxygenase-1 in rat brains]. *Chin J Endemiol* 34: 250-253.

Wedzisz A, Cieciura J. 1988. Effect of small sodium fluoride feed supplements on the serum thyroid hormone content of rats. *Bromatol Chem Toksykol* 21: 174-175.

Wei N, Dong YT, Deng J, Wang Y, Qi XL, Yu WF, Xiao Y, Zhou JJ, Guan ZZ. 2018. Changed expressions of N-methyl-d-aspartate receptors in the brains of rats and primary neurons exposed to high level of fluoride. *J Trace Elem Med Biol* 45: 31-40.

Yan N, Liu Y, Liu S, Cao S, Wang F, Wang Z, Xi S. 2016. Fluoride-induced neuron apoptosis and expressions of inflammatory factors by activating microglia in rat brain. *Mol Neurobiol* 53: 4449-4460.

Yang H, Xing R, Liu S, Yu H, Li P. 2016. Gamma-Aminobutyric acid ameliorates fluoride-induced hypothyroidism in male Kunming mice. *Life Sci* 146: 1-7.

Yang H, Xing R, Liu S, Yu H, Li P. 2019. Analysis of the protective effects of gamma-aminobutyric acid during fluoride-induced hypothyroidism in male Kunming mice. *Pharm Biol* 57(1): 29-37.

Yang M, Ren Z, Zhou B, Guan Z, Yu W. 2017. [Expression of endonuclease G in the brain tissue of rats with chronic fluorosis]. *Chin J Endemiol* 36: 327-332.

Yuan SD, Xie QW, Lu FY. 1993. Changes of serotonin content and turnover rate in hypothalamus of female rat during fluorosis. *Fluoride* 26: 57-60.

Zhai JX, Guo ZY, Hu CL, Wang QN, Zhu QX. 2003. [Studies on fluoride concentration and cholinesterase activity in rat hippocampus]. *Chin J Ind Hyg Occup Dis* 21: 102-104.

Zhan CW, Huo DJ. 1988. Ultrastructural findings in liver, kidneys, thyroid-gland and cardiac-muscle of rabbits following sodium-fluoride administration. *Fluoride* 21: 32-38.

Zhan XA, Xu ZR, Li JX, Wang M. 2005. Effects of fluorosis on lipid peroxidation and antioxidant systems in young pigs. *Fluoride* 38: 157-161.

Zhan XA, Li JX, Wang M, Xu ZR. 2006. Effects of fluoride on growth and thyroid function in young pigs. *Fluoride* 39: 95-100.

Zhang KL, Lou DD, Liu YF, Qin SL, Guan ZZ. 2012. [Changes of P-glycoprotein and nuclear factor κB in the cerebral cortex of rat with chronic fluorosis]. *Chin J Endemiol* 31: 613-616.

Zhang KL, Lou DD, Guan ZZ. 2013. [Expression of receptor for advanced glycation endproducts and nuclear factor κB in brain hippocampus of rat with chronic fluorosis]. *Chin J Endemiol* 32: 625-628.

Zhang WD, Zhang Y, Liu GY, Jiang P, Chai CY. 2008. [Effects of fluoride on ultrastructure of thyroids in rats]. *Chin J Endemiol* 27: 622-624.

Zhang ZG, Wang XY, Nian WW, Liao QX, Zhang R, Ouyang W. 2017. Effects of calcium on drinking fluorosis-induced hippocampal syntaptic plasticity impairment in the offspring of rats. *Transl Neurosci* 8: 191-200.

Zhao W, Zhu H, Yu Z, Aoki K, Misumi J, Zhang X. 1998. Long-term effects of various iodine and fluorine doses on the thyroid and fluorosis in mice. *Endocr Regul* 32: 63-70.

Zhao WY. 1988. [A preliminary study of the interaction of iodine and fluoride in experimental iodine goiter and fluorosis]. *Chin J Prev Med* 22: 146-148.

Zhao XL, Gao WH, Zhao ZL. 1994. [Effects of sodium fluoride on the activity of Ca2+Mg(2+)-ATPase in synaptic membrane in rat brain]. *Chin J Prev Med* 28: 264-266.

Zhavoronkov AA, Polyakova GA. 1973. Morphological and functional state of the hypothalamo-hypophyseal neurosecretory system in experimental fluorosis. *Bull Exp Biol Med* 75: 194-196.

Zhou B, Luo G, Wang C, Niu R, Wang J. 2014. Effects of fluoride on expression of cytokines in the hippocampus of adult rats. *Fluoride* 47: 191-198.

In Vitro Experimental Studies

As described in **Figure 2**, 60 in vitro experimental studies were included; however, data extraction was not conducted on in vitro studies. Therefore, in vitro experimental studies are not available in HAWC with the exception of in vitro studies that also reported in vivo non-human animal data that meet the relevant criteria for being made available in HAWC. The following lists of references are organized as studies that are available in HAWC (n = 6) followed by studies that are not available in HAWC (n = 54).

Studies Available in HAWC

Chen J, Niu Q, Xia T, Zhou G, Li P, Zhao Q, Xu C, Dong L, Zhang S, Wang A. 2018. ERK1/2-mediated disruption of BDNF-TrkB signaling causes synaptic impairment contributing to fluoride-induced developmental neurotoxicity. *Toxicology* 410: 222-230.

Niu Q, Chen J, Xia T, Li P, Zhou G, Xu C, Zhao Q, Dong L, Zhang S, Wang A. 2018. Excessive ER stress and the resulting autophagic flux dysfunction contribute to fluoride-induced neurotoxicity. *Environ Pollut* 233: 889-899.

Shan KR, Qi XL, Long YG, Nordberg A, Guan ZZ. 2004. Decreased nicotinic receptors in PC12 cells and rat brains influenced by fluoride toxicity: A mechanism relating to a damage at the level in post-transcription of the receptor genes. *Toxicology* 200: 169-177.

Zhang KL, Lou DD, Guan ZZ. 2015. Activation of the AGE/RAGE system in the brains of rats and in SH-SY5Y cells exposed to high level of fluoride might connect to oxidative stress. *Neurotoxicol Teratol* 48: 49-55.

Zhao Q, Niu Q, Chen J, Xia T, Zhou G, Li P, Dong L, Xu C, Tian Z, Luo C, Liu L, Zhang S, Wang A. 2019. Roles of mitochondrial fission inhibition in developmental fluoride neurotoxicity: Mechanisms of action in vitro and associations with cognition in rats and children. *Arch Toxicol* 93(3): 709-726.

Zhao XL, Wu JH. 1998. Actions of sodium fluoride on acetylcholinesterase activities in rats. *Biomed Environ Sci* 11: 1-6.

Studies Not Available in HAWC

Ardelean I, Racoveanu N, Manescu S, Lupulescu A, Diaconescu M, Ghelerter L. 1964. Experimental investigations concerning the effect of fluorine on the thyroid gland. *Rum Med Rev* 8: 17-20.

Chen J, Chen X, Yang K. 2000. [Effects of selenium and zinc on the DNA damage caused by fluoride in pallium neural cells of rats]. *J Hyg Res* 29: 216-217.

Chen L, Ning H, Yin Z, Song X, Feng Y, Qin H, Li Y, Wang J, Ge Y, Wang W. 2017. The effects of fluoride on neuronal function occurs via cytoskeleton damage and decreased signal transmission. *Chemosphere* 185: 589-594.

Chen R, Zhao LD, Liu H, Li HH, Ren C, Zhang P, Guo KT, Zhang HX, Geng DQ, Zhang CY. 2017. Fluoride induces neuroinflammation and alters Wnt signaling pathway in BV2 microglial cells. *Inflammation* 40: 1123-1130.

Cheng TJ, Chen TM, Chen CH, Lai YK. 1998. Induction of stress response and differential expression of 70 kDa stress proteins by sodium fluoride in HeLa and rat brain tumor 9L cells. *J Cell Biochem* 69: 221-231.

Deng MF, Zhu D, Liu YP, He WW, Gui CZ, Guan ZZ. 2018. Attenuation by 7-nitroindazole of fluoride-induced toxicity in SH-SY5Y cells exposed to high fluoride: Effects on nitric oxide, nitric oxide synthetase activity, nNOS, and apoptosis. *Fluoride* 51(4): 328-339.

Flores-Mendez M, Ramirez D, Alamillo N, Hernandez-Kelly LC, Del Razo LM, Ortega A. 2014. Fluoride exposure regulates the elongation phase of protein synthesis in cultured Bergmann glia cells. *Toxicol Lett* 229: 126-133.

Gao Q, Liu YH, Guan ZZ. 2008. Oxidative stress might be a mechanism connected with the decreased alpha 7 nicotinic receptor influenced by high-concentration of fluoride in SH-SY5Y neuroblastoma cells. *Toxicol In Vitro* 22: 837-843.

Goschorska M, Gutowska I, Baranowska-Bosiacka I, Piotrowska K, Metryka E, Safranow K, Chlubek D. 2018. Influence of acetylcholinesterase inhibitors used in Alzheimer's Disease treatment on the activity of antioxidant enzymes and the concentration of glutathione in THP-1 macrophages under fluoride-induced oxidative stress. *Int J Environ Res Pub Health* 16(1).

Guan ZZ, Shan KR, Xiu J, Long YG. 2005. [Fluorosis on expression of nicotinic acetylcholine receptors in protein and gene levels in human SH-SY5Y neuroblastoma cells]. *Chin J Prev Med* 39: 26-29.

Guan ZZ, Shan KR, Wang YN, Dallner G. 2006. [Changes of lipids and nicotinic receptors in rat brains and pheochromocytoma with fluorosis]. *Chin J Endemiol* 25: 121-124.

Haojun Z, Yaoling W, Ke Z, Jin L, Junling W. 2012. Effects of NaF on the expression of intracellular Ca2+ fluxes and apoptosis and the antagonism of taurine in murine neuron. *Toxicol Mech Methods* 22: 305-308.

Hong-Liang L, Qiang Z, Yu-Shan C, Lei Z, Gang F, Chang-Chun H, Liang Z, Aiguo W. 2014. Fluoride-induced thyroid cell apoptosis. *Fluoride* 47: 161-169.

Inkielewicz-Stepniak I, Radomski MW, Wozniak M. 2012. Fisetin prevents fluoride- and dexamethasone-induced oxidative damage in osteoblast and hippocampal cells. *Food Chem Toxicol* 50: 583-589.

Jin TX, Guan ZZ, Zhang H. 2011. [The effect of fluoride on α subunit of calcium/calmodulin-dependent protein kinase-II mRNA and protein expression in central nervous system]. *Chin J Endemiol* 30: 247-250.

Kariya T, Kotani M, Field JB. 1974. Effects of sodium fluoride and other metabolic inhibitors on basal and TSH stimulated cyclic AMP and thyroid metabolism. *Metab Clin Exper* 23: 967-973.

Ke L, Zheng X, Sun Y, Ouyang W, Zhang Z. 2016. Effects of sodium fluoride on lipid peroxidation and PARP, XBP-1 expression in PC12 cell. *Biol Trace Elem Res* 173: 161-167.

Lee J, Han YE, Favorov O, Tommerdahl M, Whitsel B, Lee CJ. 2016. Fluoride induces a volume reduction in CA1 hippocampal slices via MAP kinase pathway through volume regulated anion channels. *Exp Neurobiol* 25: 72-78.

Levesque L, Mizzen CA, McLachlan DR, Fraser PE. 2000. Ligand specific effects on aluminum incorporation and toxicity in neurons and astrocytes. *Brain Res* 877: 191-202.

Li H, Gao MT, Xu KY, Wang CY. 2007. Effect of sodium fluoride on the primary porcine thyroid cells and thyroid peroxidase activity. *J Clin Rehabil Tissue Eng Res* 11: 7425-7428.

Li H, Gao MT, Xu KY, Cui MY, Dai X. 2008. [Effect of fluoride on thyroid functioning in primary porcine thyrocyte]. *Chin J Endemiol* 27: 38-40.

Li H, Huang H, Xu Y, Gao Y, Liu Z. 2010. [Toxic effects of fluoride on rat cerebral cortex astrocytes in vitro]. *J Hyg Res* 39: 86-88.

Liu H, Zeng Q, Cui Y, Yu L, Zhao L, Hou C, Zhang S, Zhang L, Fu G, Liu Y, Jiang C, Chen X, Wang A. 2014. The effects and underlying mechanism of excessive iodide on excessive fluoride-induced thyroid cytotoxicity. *Environ Toxicol Pharmacol* 38: 332-340.

Liu HL, Zeng Q, Cui YS, Zhao L, Zhang L, Fu G, Hou CC, Zhang S, Yu LY, Jiang CY, Wang ZL, Chen XM, Wang AG. 2014. The role of the IRE1 pathway in excessive iodide- and/or fluoride-induced apoptosis in Nthy-ori 3-1 cells in vitro. *Toxicol Lett* 224: 341-348.

Liu YJ, Guan ZZ, Gao Q, Pei JJ. 2011. Increased level of apoptosis in rat brains and SH-SY5Y cells exposed to excessive fluoride-A mechanism connected with activating JNK phosphorylation. *Toxicol Lett* 204: 183-189.

Liu Y, Gao Q, Tang Z, Zhang X, Guan Z. 2015. [The expression and correlation between neural nicotinic acetylcholine receptor subunit α3 and mitogen-activated protein kinase cell signaling transduction pathway in human neuroblastoma cell line SH-SY5Y overexposed to fluoride]. *Chin J Endemiol* 34: 553-558.

Madaoui S, Rappaport L, Nunez J. 1974. Prostaglandins and in vitro TSH-dependent iodide binding by rat thyroid glands. *Biochimie* 56: 109-113.

Nakagawa-Yagi Y, Saito Y, Kitoh N, Ogane N, Fujisawa E, Nakamura H. 1993. Fluoride causes suppression of neurite outgrowth in human neuroblastoma via an influx of extracellular calcium. *Biochem Biophys Res Commun* 191: 727-736.

Ong J, Kerr DIB. 1995. Interactions of N-ethylmaleimide and aluminium fluoride with GABA(B) receptor function in rat neocortical slices. *Eur J Pharmacol* 287: 197-200.

Pastan I, Macchia V, Katzen R. 1968. Effect of fluoride on the metabolic activity of thyroid slices. *Endocrinology* 83: 157-160.

Rubakhova VM. 1977. [Effect of serotonin and sodium fluoride on visceral nerve conductors]. *Vyestsi Akademii Navuk BSSR Syeryya Biyalahichnykh Navuk* 1: 117-119.

Shayiq RM, Raza H, Kidwai AM. 1986. Fluoride and lipid peroxidation a comparative study in different rat tissues. *Bull Environ Contam Toxicol* 37: 70-76.

Shuhua X, Ziyou L, Ling Y, Fei W, Sun G. 2012. A role of fluoride on free radical generation and oxidative stress in BV-2 microglia cells. *Mediators Inflamm* 2012: 1-8.

Singh P, Das TK. 2019. Ultrastructural localization of 4-hydroxynonenal adducts in fluoride-exposed cells: Protective role of dietary antioxidants. *Fluoride* 52(1): 49-58.

Taylor P. 1972. Comparison of the effects of various agents on thyroidal adenyl cyclase activity with their effects on thyroid hormone release. *J Endocrinol* 54: 137-145.

Tu W, Zhang Q, Liu Y, Han LY, Wang Q, Chen PP, Zhang S, Wang AG, Zhou X. 2018. Fluoride induces apoptosis via inhibiting SIRT1 activity to activate mitochondrial p53 pathway in human neuroblastoma SH-SY5Y cells. *Toxicol Appl Pharmacol* 347: 60-69.

van der Voet GB, Schijns O, de Wolff FA. 1999. Fluoride enhances the effect of aluminium chloride on interconnections between aggregates of hippocampal neurons. *Arch Physiol Biochem* 107: 15-21.

Wang JL. 2007. [Effect of fluoride on the intracellular Ca2+ in neurons of mice]. *Chin J Endemiol* 26: 505-507.

Wang J, Gao Y, Cheng X, Yang J, Zhao Y, Xu H, Zhu Y, Yan Z, Manthari RK, Mehdi OM, Wang J. 2019. GSTO1 acts as a mediator in sodium fluoride-induced alterations of learning and memory related factors expressions in the hippocampus cell line. *Chemosphere* 226: 201-209.

Wei N, Dong YT, Deng J, Wang Y, Qi XL, Yu WF, Xiao Y, Zhou JJ, Guan ZZ. 2018. Changed expressions of N-methyl-d-aspartate receptors in the brains of rats and primary neurons exposed to high level of fluoride. *J Trace Elem Med Biol* 45: 31-40.

Willems CB-V, Sande J, Dumont JE. 1972. Inhibition of thyroid secretion by sodium fluoride in vitro. *Biochim Biophys Acta* 264: 197-204.

Woodward JJ, Harms J. 1992. Potentiation of N-methyl-D-aspartate-stimulated dopamine release from rat brain slices by aluminum fluoride and carbachol. *J Neurochem* 58: 1547-1554.

Wu J, Cheng M, Liu Q, Yang J, Wu S, Lu X, Jin C, Ma H, Cai Y. 2015. Protective role of tert-butylhydroquinone against sodium fluoride-induced oxidative stress and apoptosis in PC12 cells. *Cell Mol Neurobiol* 35: 1017-1025.

Xia T, Zhang M, He WH, He P, Wang AG. 2007. [Effects of fluoride on neural cell adhesion molecules mRNA and protein expression levels in primary rat hippocampal neurons]. *Chin J Prev Med* 41: 475-478.

Xu B, Xu Z, Xia T, He P, Gao P, He W, Zhang M, Guo L, Niu Q, Wang A. 2011. Effects of the Fas/Fas-L pathway on fluoride-induced apoptosis in SH-SY5Y cells. *Environ Toxicol* 26: 86-92.

Xu Z, Xu B, Xia T, He W, Gao P, Guo L, Wang Z, Niu Q, Wang A. 2013. Relationship between intracellular Ca2+ and ROS during fluoride-induced injury in SH-SY5Y cells. *Environ Toxicol* 28: 307-312.

Yamashita K, Field JB. 1972. Elevation of cyclic guanosine 3,5; monophosphate levels in dog thyroid slices caused by acetylcholine and sodium fluoride. *J Biol Chem* 247: 7062-7066.

Yan L, Liu S, Wang C, Wang F, Song Y, Yan N, Xi S, Liu Z, Sun G. 2013. JNK and NADPH oxidase involved in fluoride-induced oxidative stress in BV-2 microglia cells. *Mediators Inflamm* 2013: 895-975.

Zhang CY, Chen R, Wang F, Ren C, Zhang P, Li Q, Li HH, Guo KT, Geng DQ, Liu CF. 2016. EGb-761 attenuates the anti-proliferative activity of fluoride via DDK1 in PC-12 cells. *Neurochem Res* 42(2): 606-614.

Zhang M, Wang A, He W, He P, Xu B, Xia T, Chen X, Yang K. 2007. Effects of fluoride on the expression of NCAM, oxidative stress, and apoptosis in primary cultured hippocampal neurons. *Toxicology* 236: 208-216.

Zhang M, Wang A, Xia T, He P. 2008. Effects of fluoride on DNA damage, S-phase cell-cycle arrest and the expression of NF-kappaB in primary cultured rat hippocampal neurons. *Toxicol Lett* 179: 1-5.

Zhang S, Zheng X, Sun Y, Wang Y, Zhang Z. 2015. Alterations in oxidative stress and apoptosis in cultured PC12 cells exposed to fluoride. *Fluoride* 48: 213-222.

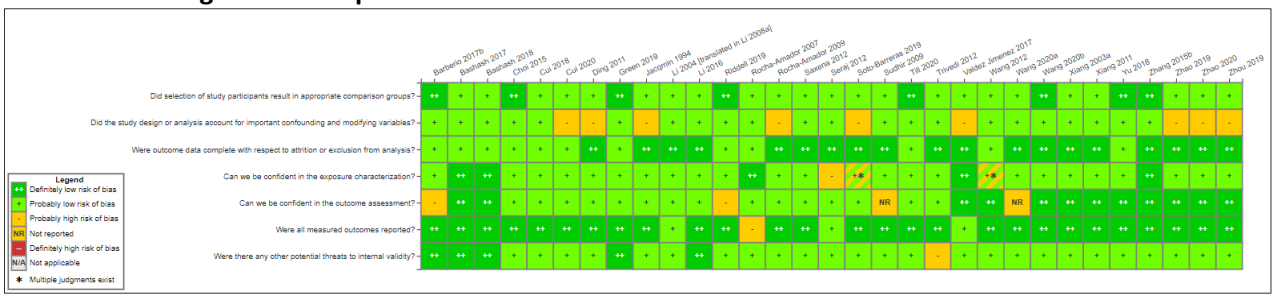
Zhao L, Xiao Y, Deng CM, Tan LC, Guan ZZ. 2016. Protective effect of lovastatin on neurotoxicity of excessive fluoride in primary hippocampal neurons. *Fluoride* 49: 36-46.

Zhao XL, Gao WH, Zhao ZL. 1994. [Effects of sodium fluoride on the activity of Ca2+Mg(2+)-ATPase in synaptic membrane in rat brain]. *Chin J Prev Med* 28: 264-266.

Appendix 3. Risk-of-bias Figures

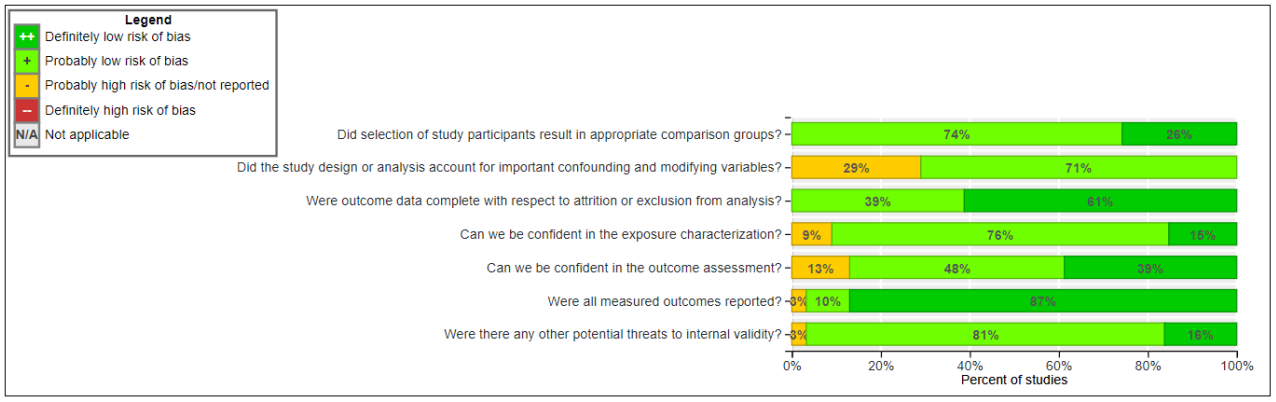
Studies in Humans

Figure A3-1. Risk-of-bias Heatmap for Low Risk-of-bias Human Neurodevelopmental or Cognitive Studies Following Fluoride Exposure



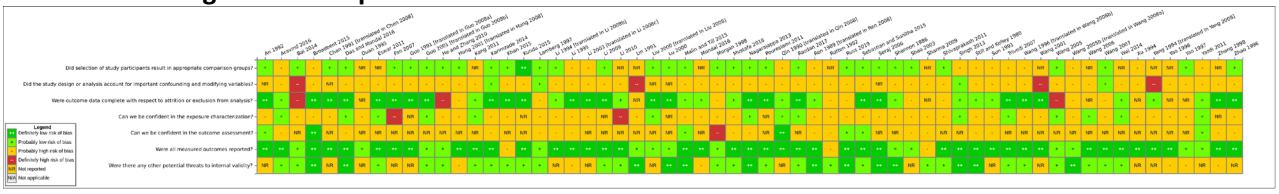
Interactive figure and additional study details in HAWC here.

Figure A3-2. Risk-of-bias Bar Chart for Low Risk-of-bias Human Neurodevelopmental or Cognitive Studies Following Fluoride Exposure



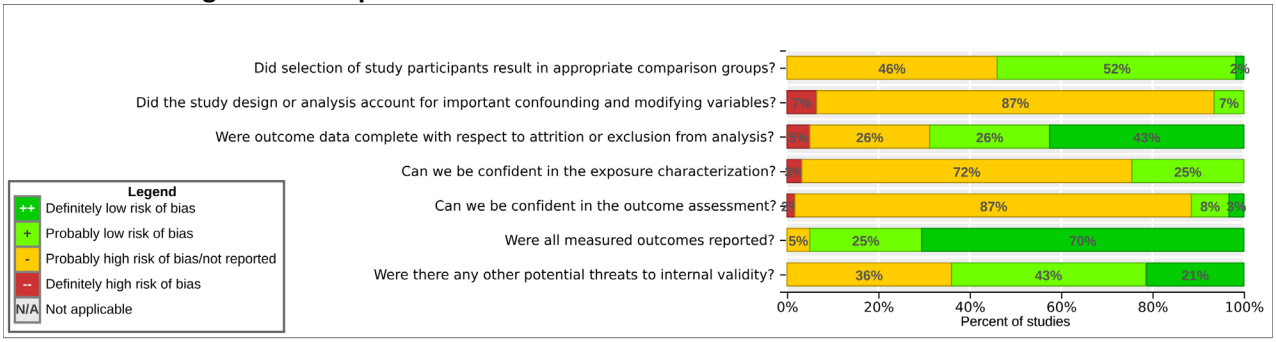
Interactive figure and additional study details in HAWC here.

Figure A3-3. Risk-of-bias Heatmap for High Risk-of-bias Human Neurodevelopmental or Cognitive Studies Following Fluoride Exposure



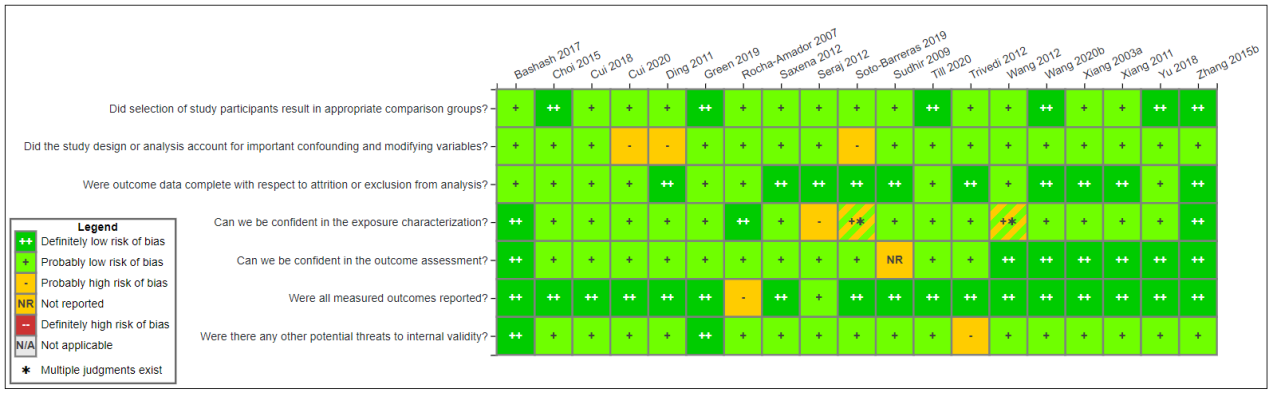
Interactive figure and additional study details in HAWC here.

Figure A3-4. Risk-of-bias Bar Chart for High Risk-of-bias Human Neurodevelopmental or Cognitive Studies Following Fluoride Exposure



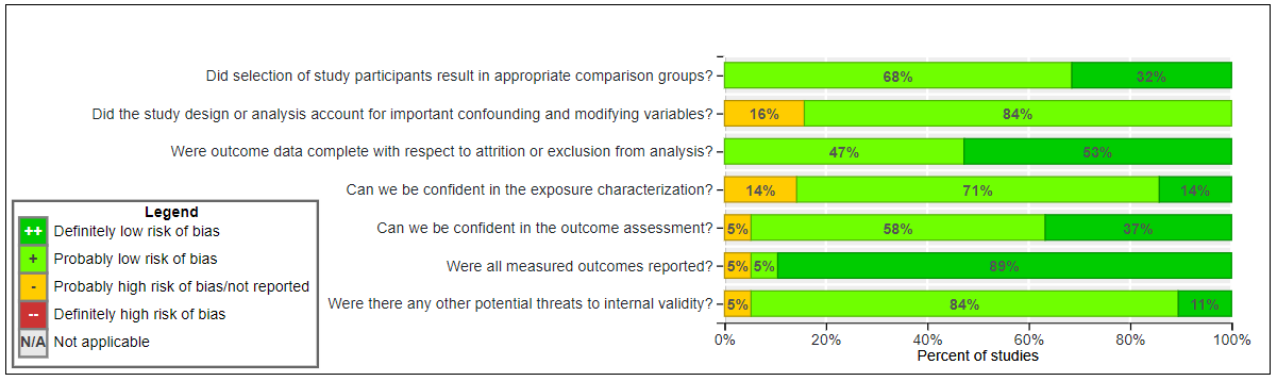
Interactive figure and additional study details in HAWC here.

Figure A3-5. Risk-of-bias Heatmap for Low Risk-of-bias Children's IQ Studies Following Fluoride Exposure



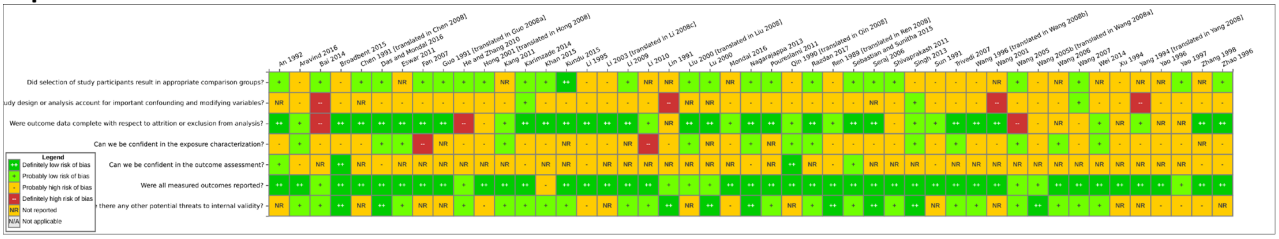
Interactive figure and additional study details in HAWC here.

Figure A3-6. Risk-of-bias Bar Chart for Low Risk-of-bias Children's IQ Studies Following Fluoride Exposure



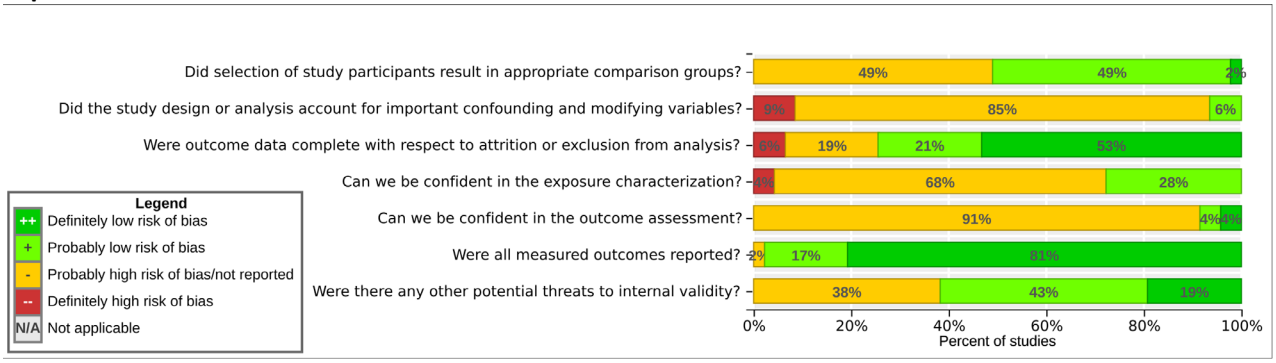
Interactive figure and additional study details in HAWC here.

Figure A3-7. Risk-of-bias Heatmap for High Risk-of-bias Children's IQ Studies Following Fluoride Exposure



Interactive figure and additional study details in HAWC here.

Figure A3-8. Risk-of-bias Bar Chart for High Risk-of-bias Children's IQ Studies Following Fluoride Exposure

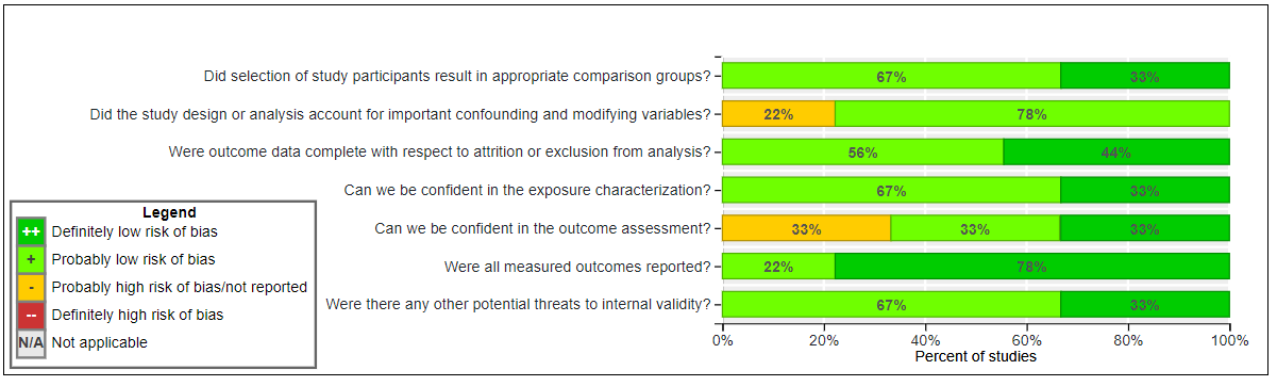


Interactive figure and additional study details in HAWC here.

Figure A3-9. Risk-of-bias Heatmap for Low Risk-of-bias Children's Other Neurodevelopmental Effect Studies Following Fluoride Exposure

Interactive figure and additional study details in HAWC here.

Figure A3-10. Risk-of-bias Bar Chart for Low Risk-of-bias Children's Other Neurodevelopmental Effect Studies Following Fluoride Exposure

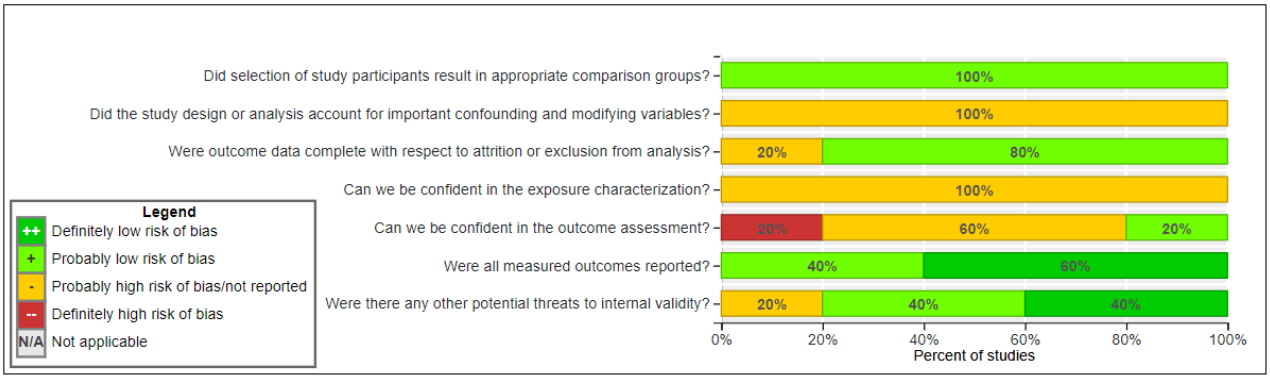


Interactive figure and additional study details in HAWC here.

Figure A3-11. Risk-of-bias Heatmap for High Risk-of-bias Children's Other Neurodevelopmental Effect Studies Following Fluoride Exposure

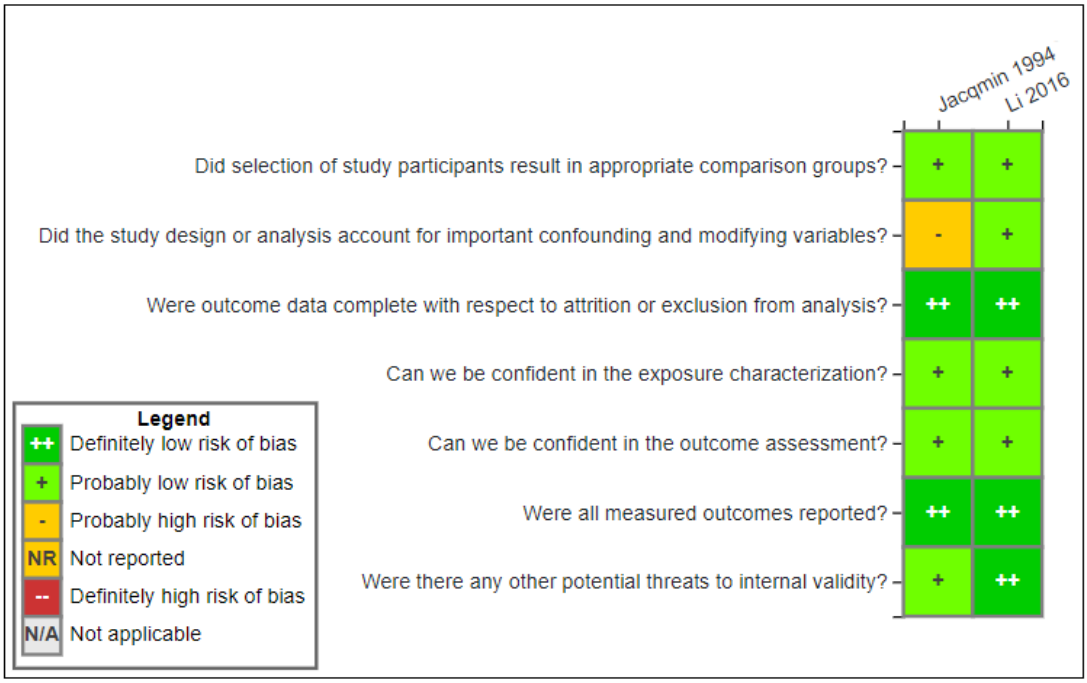
Interactive figure and additional study details in HAWC here.

Figure A3-12. Risk-of-bias Bar Chart for High Risk-of-bias Children's Other Neurodevelopmental Effect Studies Following Fluoride Exposure



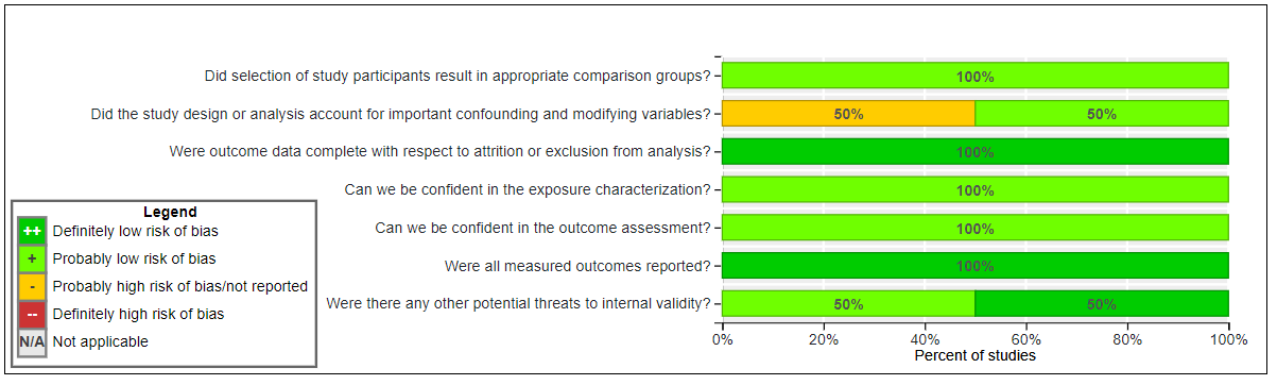
Interactive figure and additional study details in HAWC here.

Figure A3-13. Risk-of-bias Heatmap for Low Risk-of-bias Adult Cognitive Studies Following Fluoride Exposure



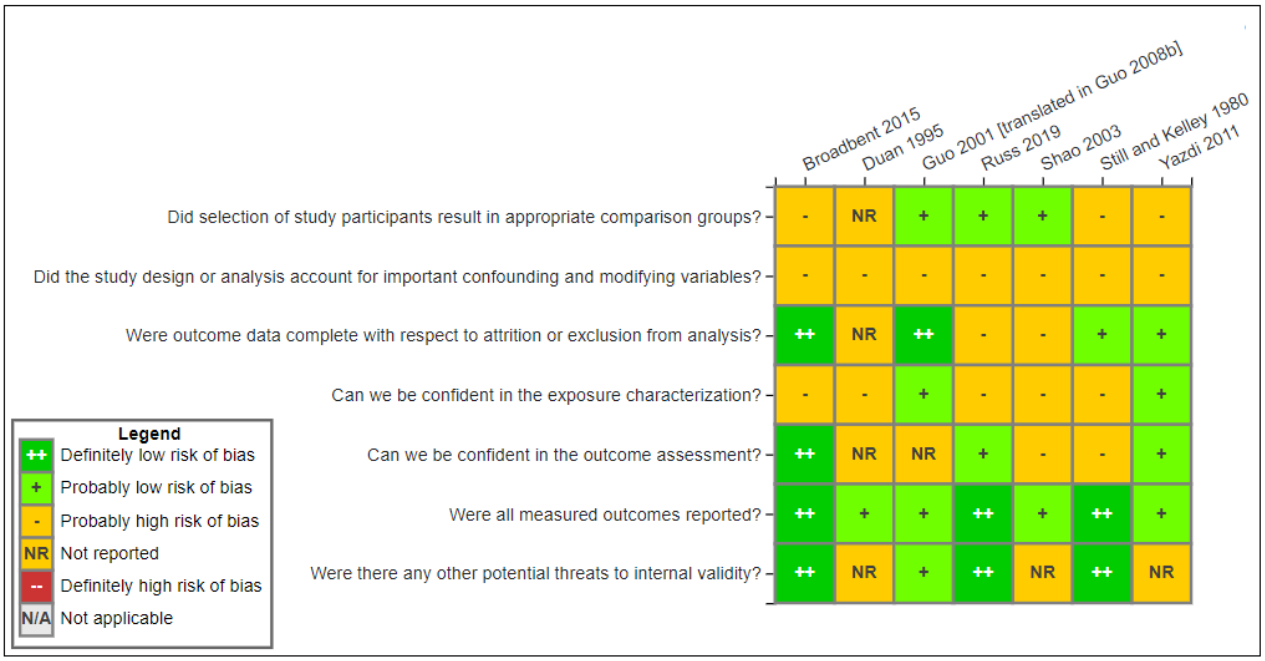
Interactive figure and additional study details in HAWC here.

Figure A3-14. Risk-of-bias Bar Chart for Low Risk-of-bias Adult Cognitive Studies Following Fluoride Exposure



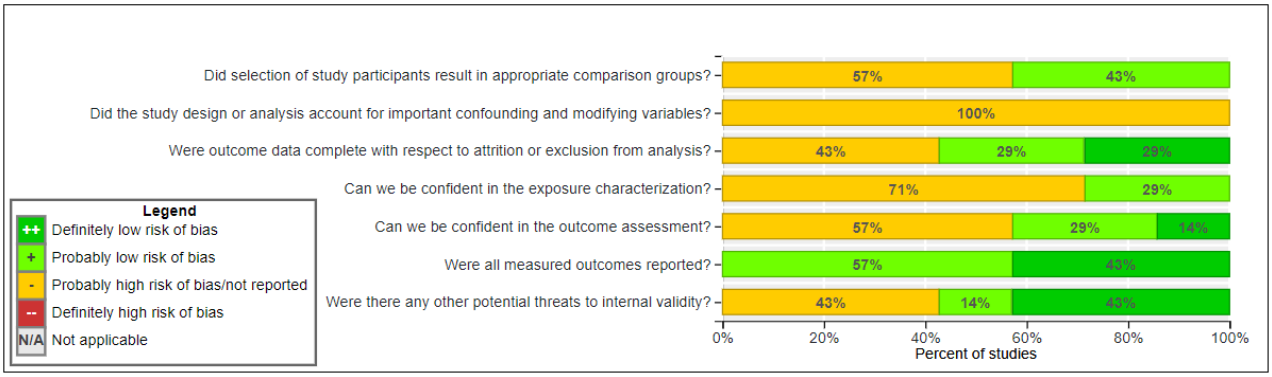
Interactive figure and additional study details in HAWC here.

Figure A3-15. Risk-of-bias Heatmap for High Risk-of-bias Adult Cognitive Studies Following Fluoride Exposure



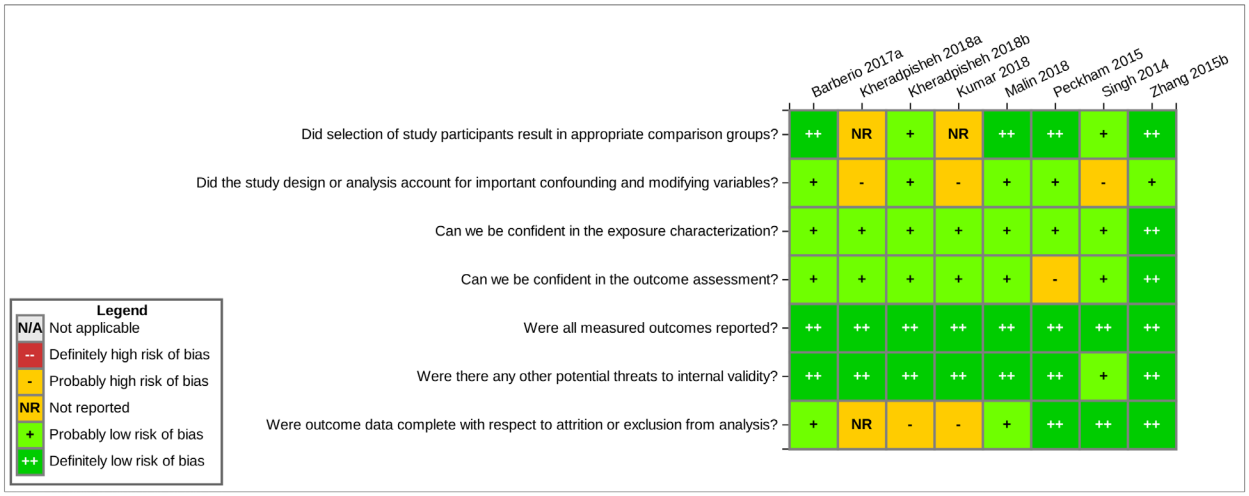
Interactive figure and additional study details in HAWC here.

Figure A3-16. Risk-of-bias Bar Chart for High Risk-of-bias Adult Cognitive Studies Following Fluoride Exposure



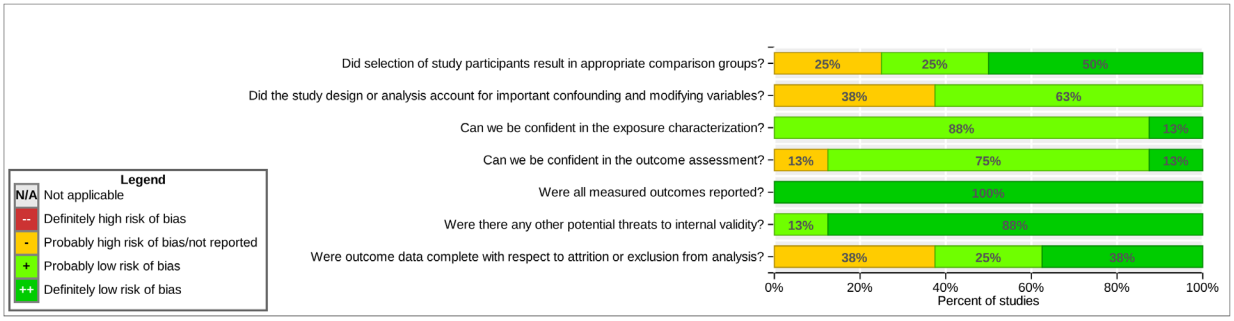
Interactive figure and additional study details in HAWC here.

Figure A3-17. Risk-of-bias Heatmap for Low Risk-of-bias Human Mechanistic Studies Following Fluoride Exposure



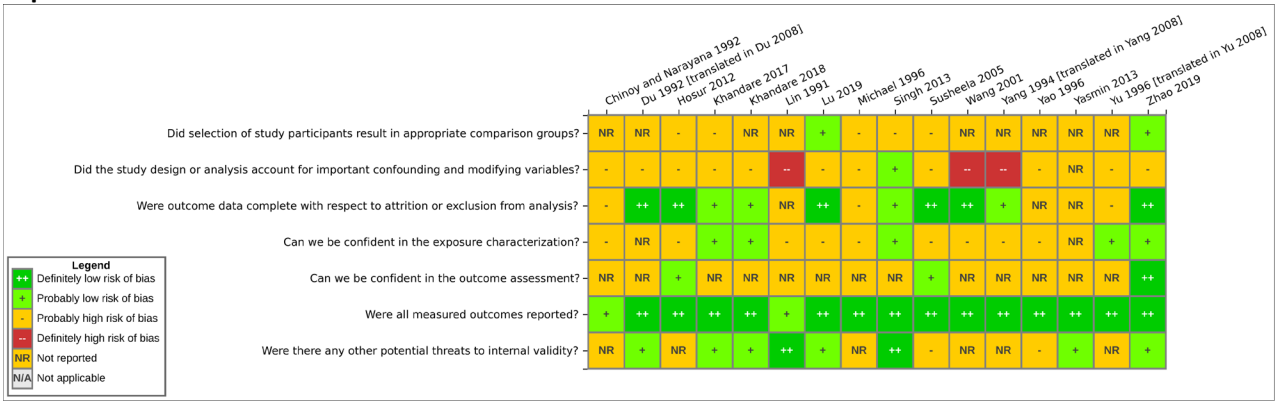
Interactive figure and additional study details in HAWC here.

Figure A3-18. Risk-of-bias Bar Chart for Low Risk-of-bias Human Mechanistic Studies Following Fluoride Exposure



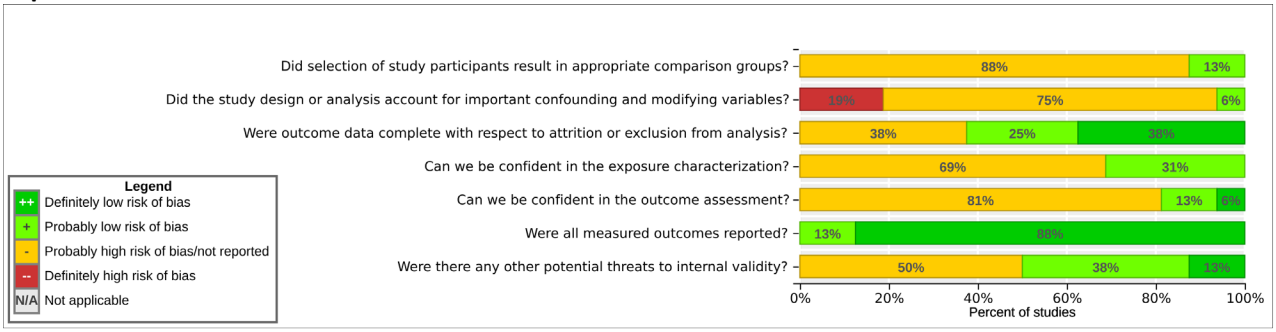
Interactive figure and additional study details in HAWC here.

Figure A3-19. Risk-of-bias Heatmap for High Risk-of-bias Human Mechanistic Studies Following Fluoride Exposure



Interactive figure and additional study details in HAWC here.

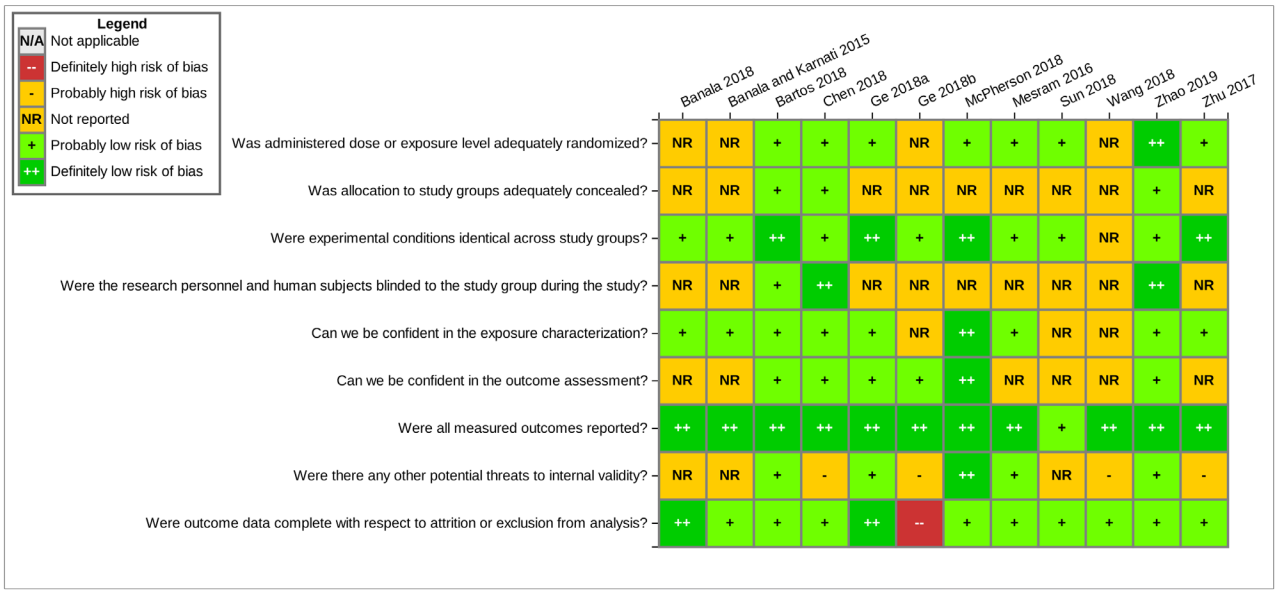
Figure A3-20. Risk-of-bias Bar Chart for High Risk-of-bias Human Mechanistic Studies Following Fluoride Exposure



Interactive figure and additional study details in HAWC here.

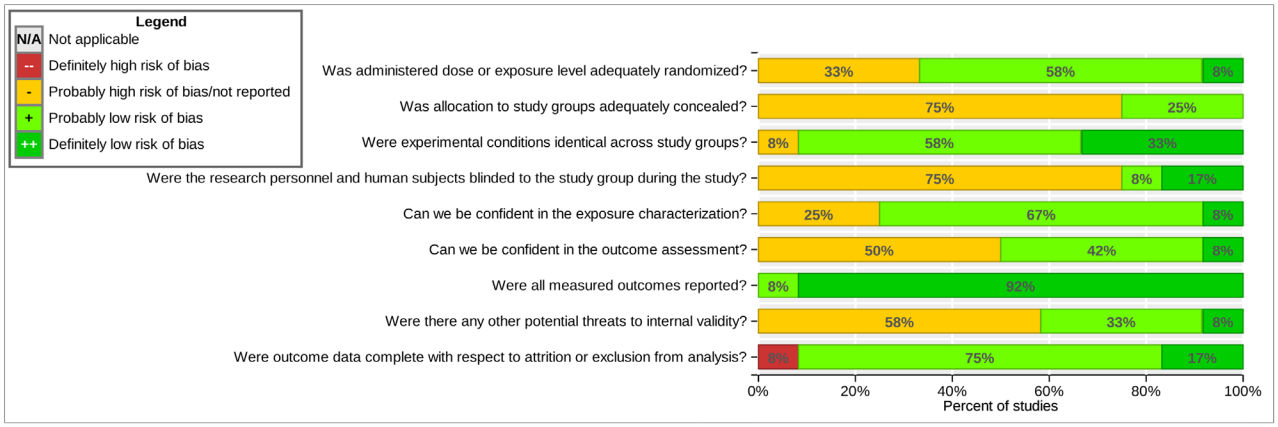
Studies in Non-human Animals

Figure A3-21. Risk-of-bias Heatmap for New Developmental Animal Learning and Memory Studies Following Fluoride Exposure



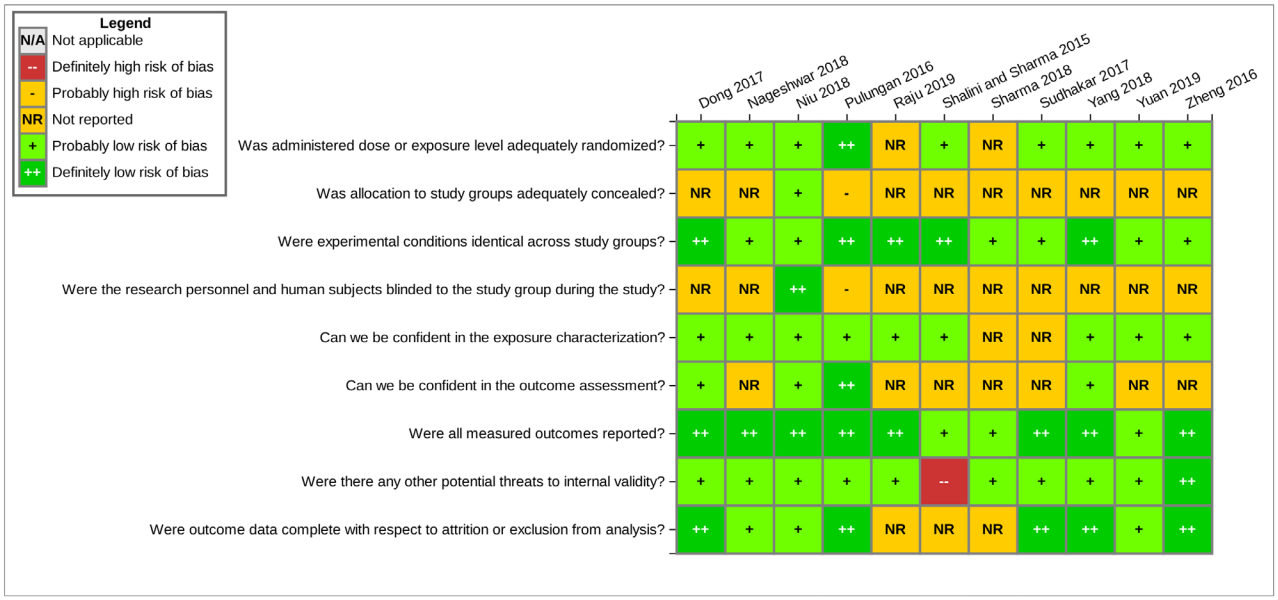
Interactive figure and additional study details in HAWC here.

Figure A3-22. Risk-of-bias Bar Chart for New Developmental Animal Learning and Memory Studies Following Fluoride Exposure



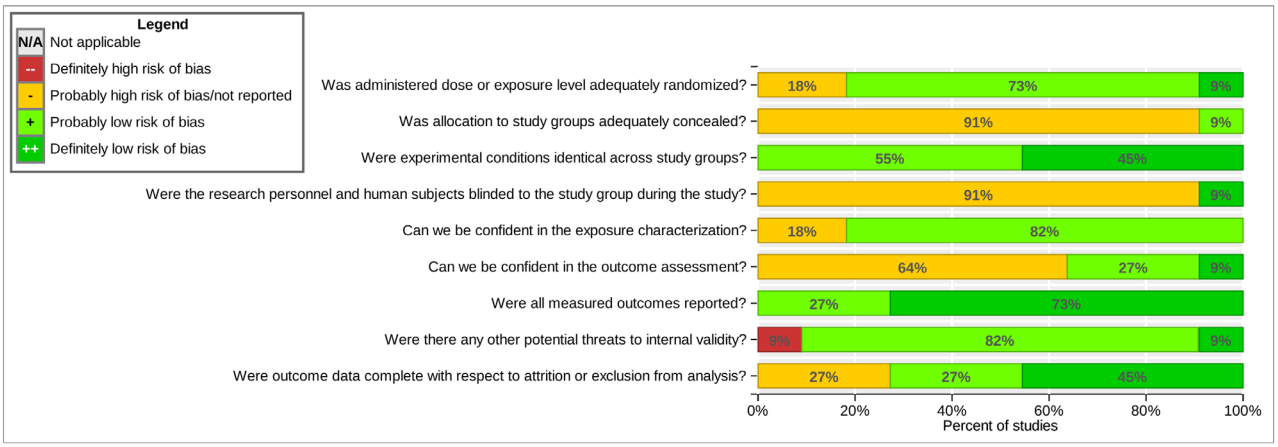
Interactive figure and additional study details in HAWC here.

Figure A3-23. Risk-of-bias Heatmap for New Adult Animal Learning and Memory Studies Following Fluoride Exposure



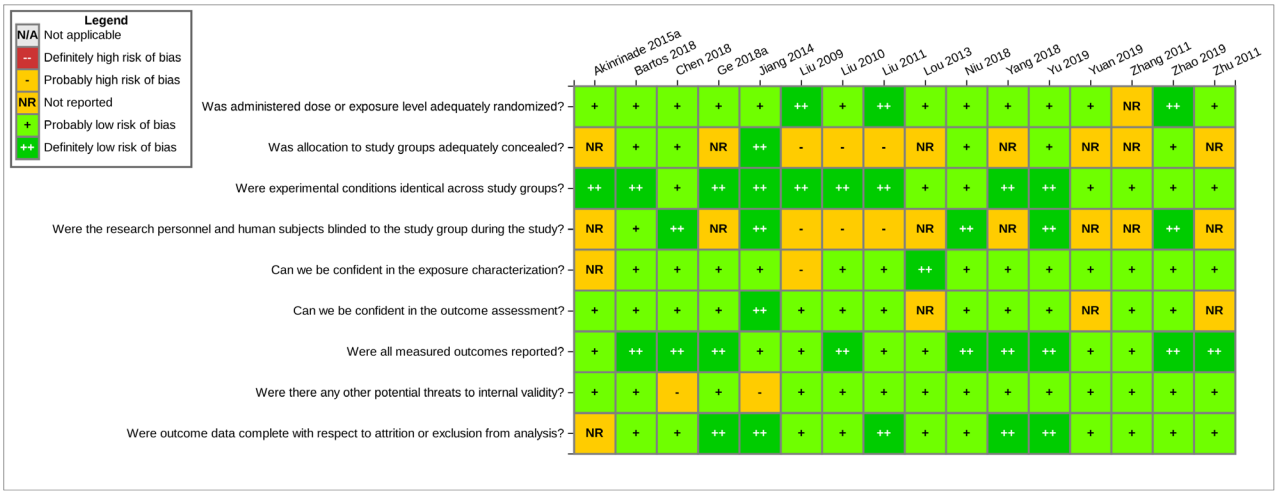
Interactive figure and additional study details in HAWC here.

Figure A3-24. Risk-of-bias Bar Chart for New Adult Animal Learning and Memory Studies Following Fluoride Exposure



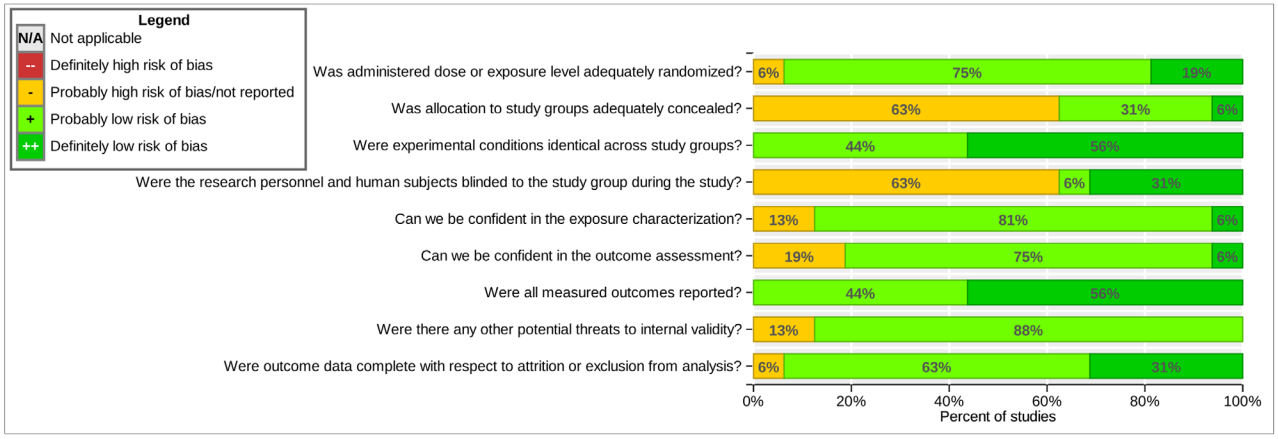
Interactive figure and additional study details in HAWC here.

Figure A3-25. Risk-of-bias Heatmap for Low Risk-of-bias Animal Biochemical Studies Following Fluoride Exposure



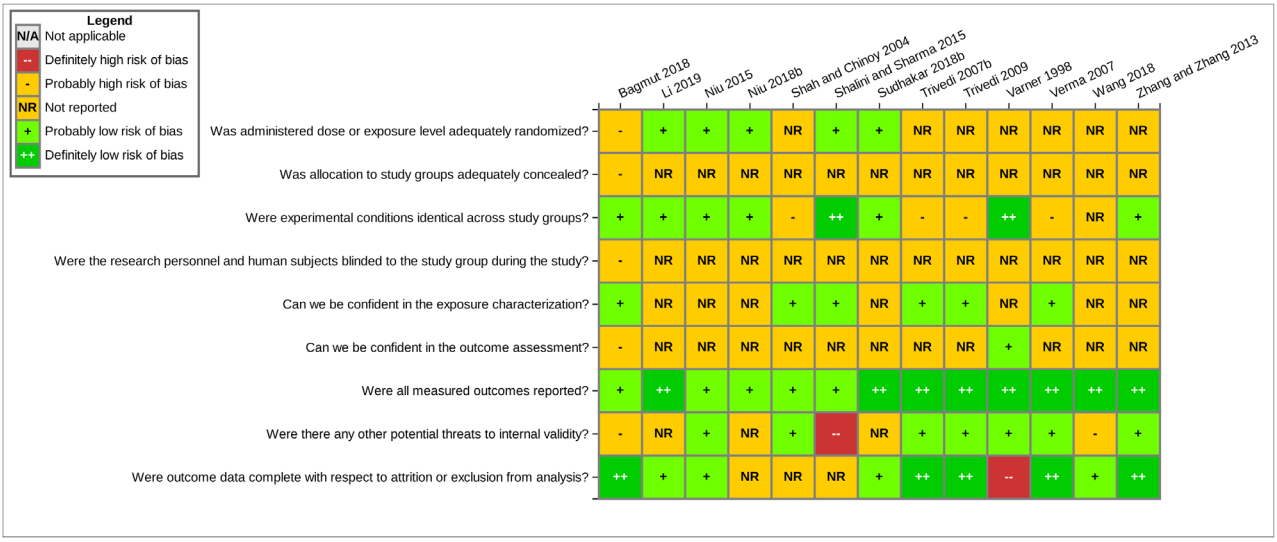
Interactive figure and additional study details in HAWC here.

Figure A3-26. Risk-of-bias Bar Chart for Low Risk-of-bias Animal Biochemical Studies Following Fluoride Exposure



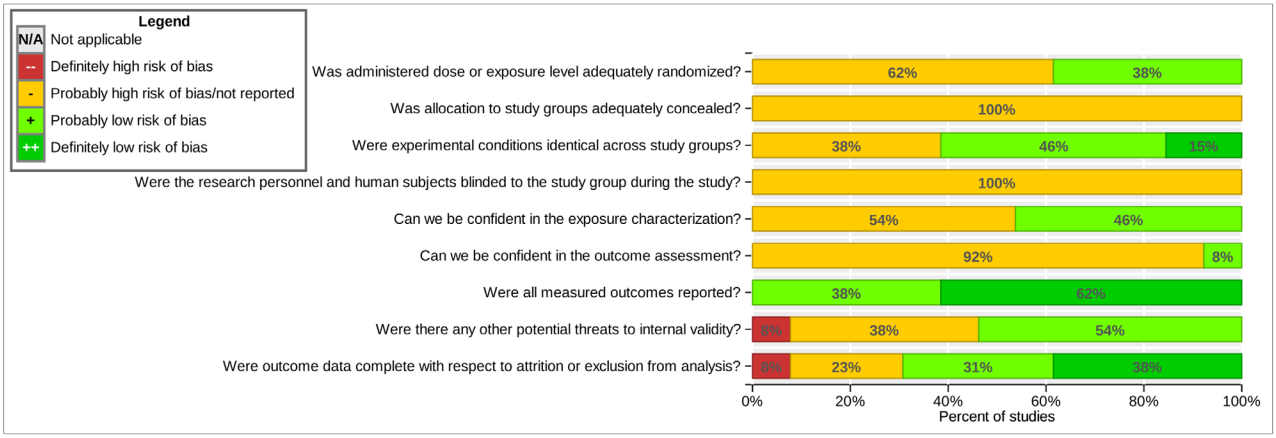
Interactive figure and additional study details in HAWC here.

Figure A3-27. Risk-of-bias Heatmap for High Risk-of-bias Animal Biochemical Studies Following Fluoride Exposure



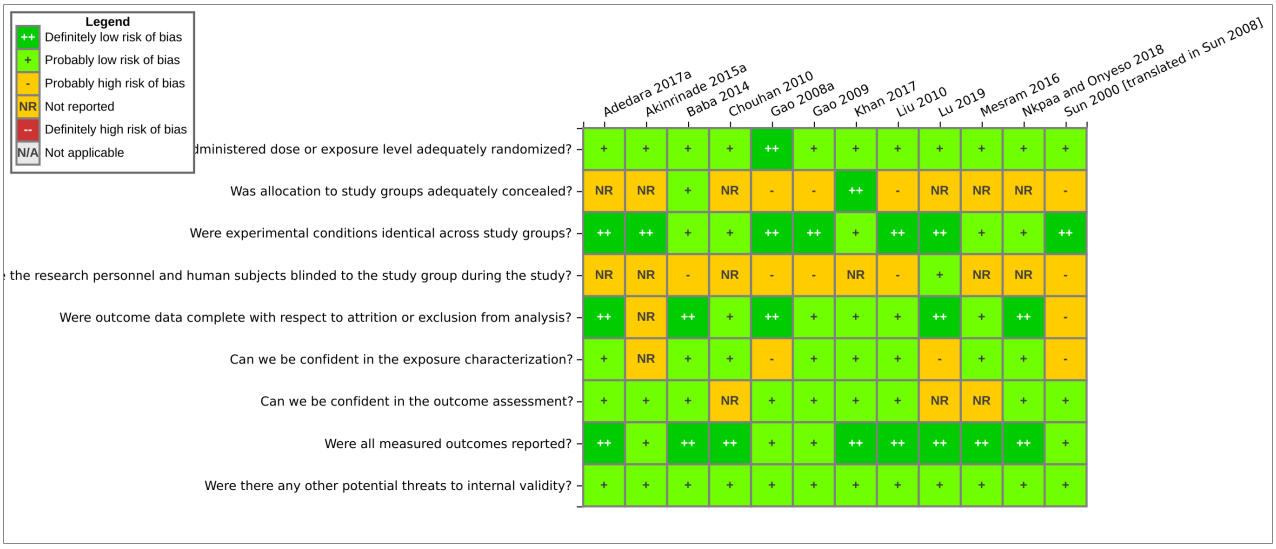
Interactive figure and additional study details in HAWC here.

Figure A3-28. Risk-of-bias Bar Chart for High Risk-of-bias Animal Biochemical Studies Following Fluoride Exposure



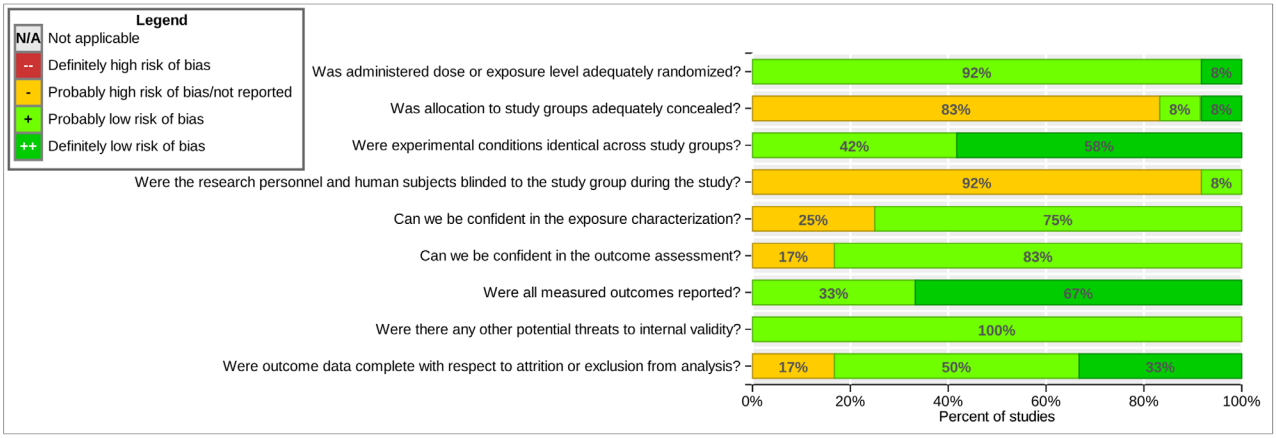
Interactive figure and additional study details in HAWC here.

Figure A3-29. Risk-of-bias Heatmap for Low Risk-of-bias Animal Neurotransmission Studies Following Fluoride Exposure



Interactive figure and additional study details in HAWC here.

Figure A3-30. Risk-of-bias Bar Chart for Low Risk-of-bias Animal Neurotransmission Studies Following Fluoride Exposure

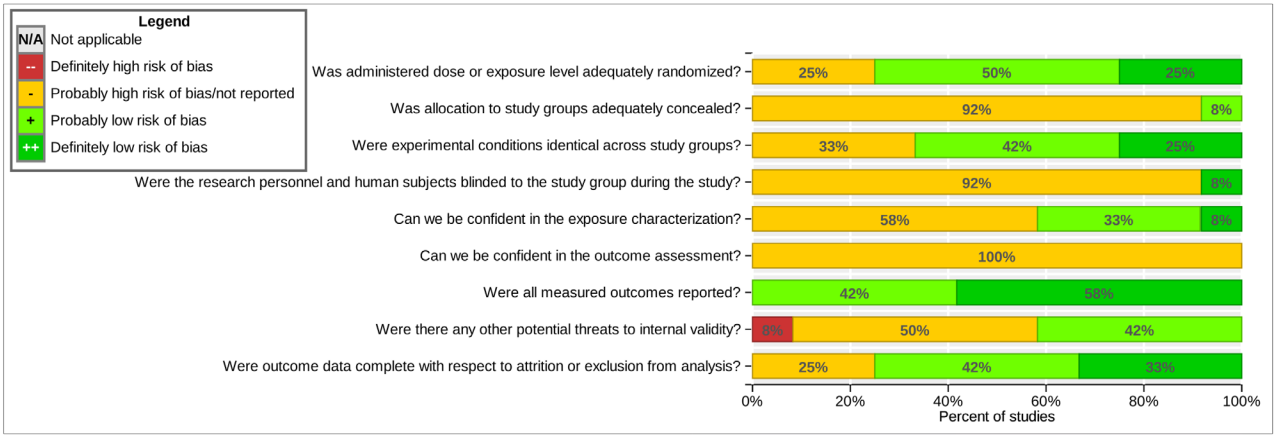


Interactive figure and additional study details in HAWC here.

Figure A3-31. Risk-of-bias Heatmap for High Risk-of-bias Animal Neurotransmission Studies Following Fluoride Exposure

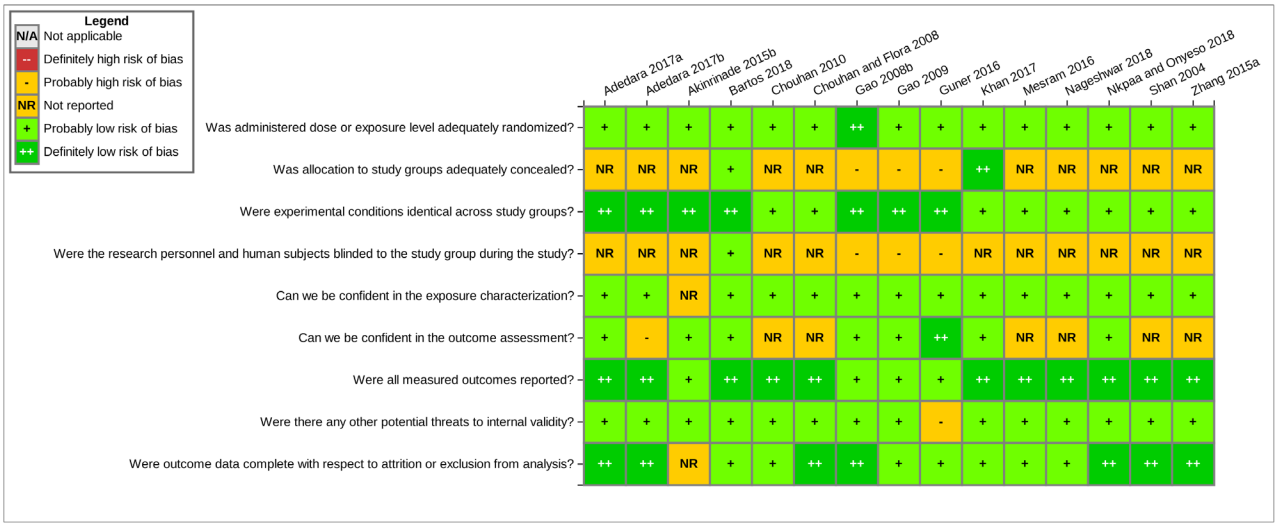
Interactive figure and additional study details in HAWC here.

Figure A3-32. Risk-of-bias Bar Chart for High Risk-of-bias Animal Neurotransmission Studies Following Fluoride Exposure



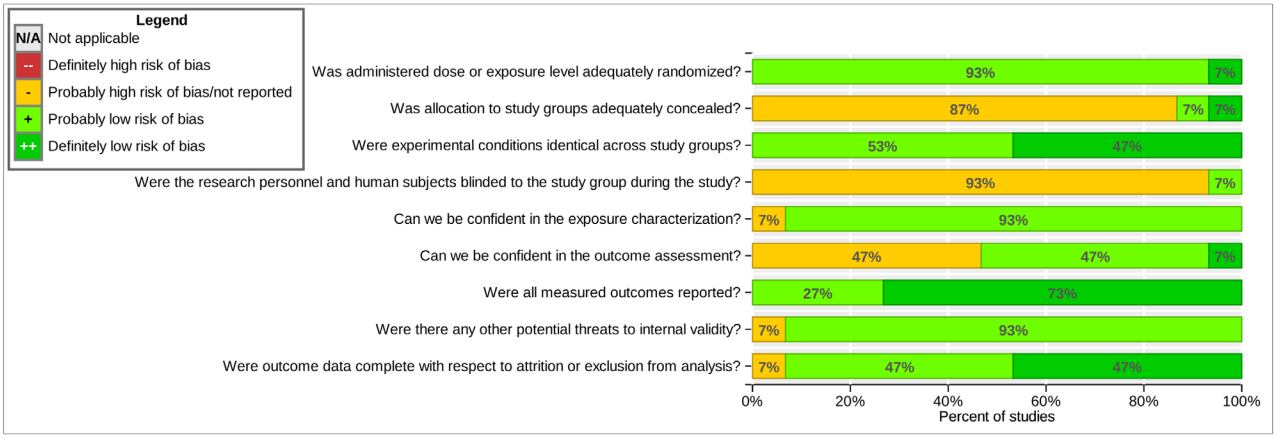
Interactive figure and additional study details in HAWC here.

Figure A3-33. Risk-of-bias Heatmap for Low Risk-of-bias Animal Oxidative Stress Studies Following Fluoride Exposure



Interactive figure and additional study details in HAWC here.

Figure A3-34. Risk-of-bias Bar Chart for Low Risk-of-bias Animal Oxidative Stress Studies Following Fluoride Exposure

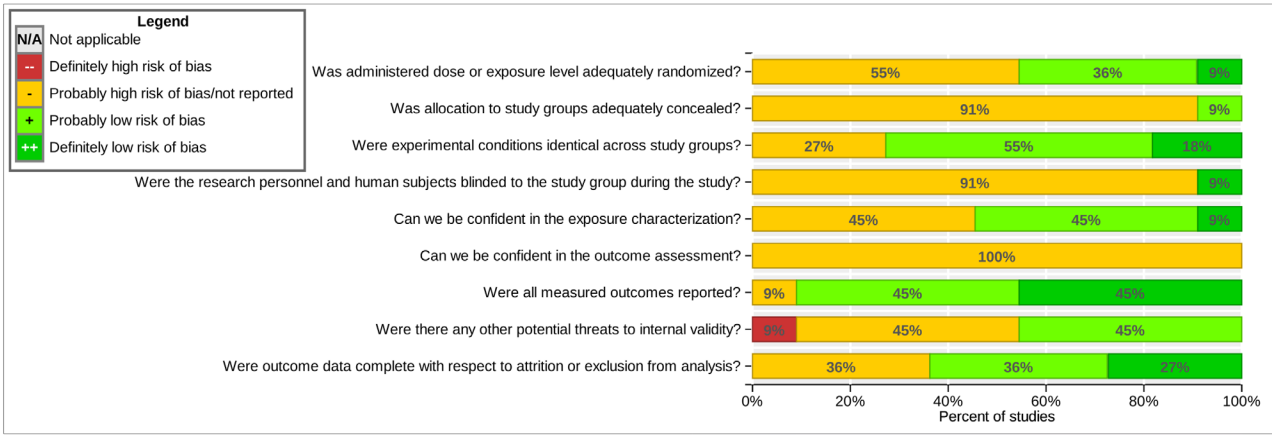


Interactive figure and additional study details in HAWC here.

Figure A3-35. Risk-of-bias Heatmap for High Risk-of-bias Animal Oxidative Stress Studies Following Fluoride Exposure

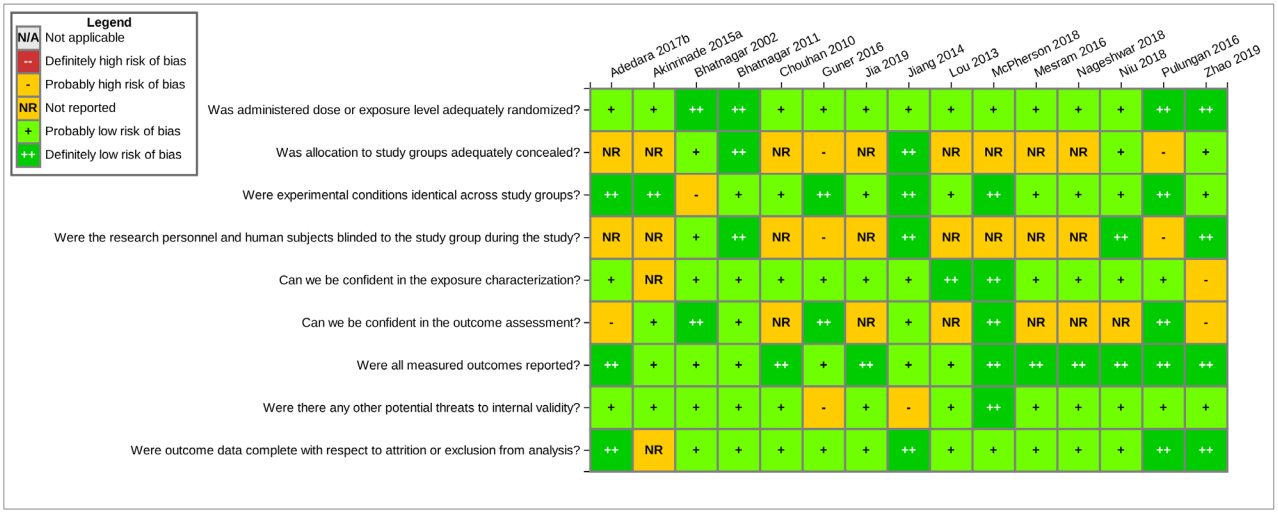
Interactive figure and additional study details in HAWC here.

Figure A3-36. Risk-of-bias Bar Chart for High Risk-of-bias Animal Oxidative Stress Studies Following Fluoride Exposure



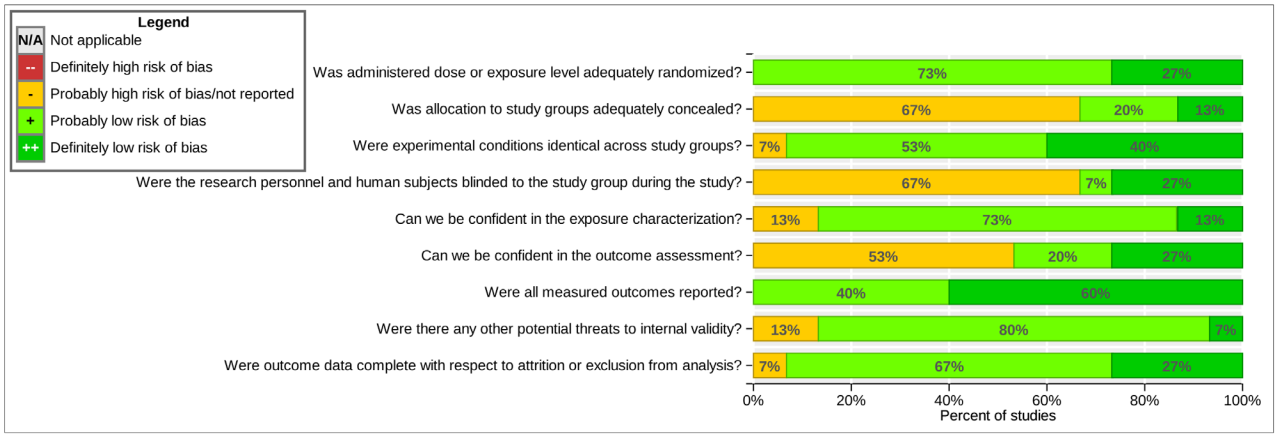
Interactive figure and additional study details in HAWC here.

Figure A3-37. Risk-of-bias Heatmap for Low Risk-of-bias Animal Histopathology Studies Following Fluoride Exposure



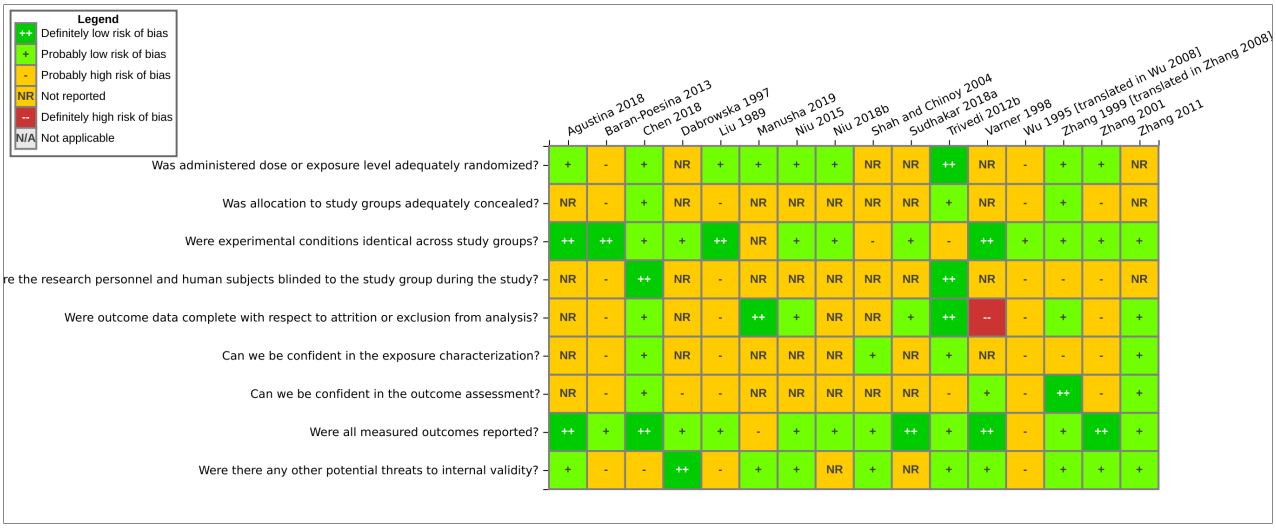
Interactive figure and additional study details in HAWC here.

Figure A3-38. Risk-of-bias Bar Chart for Low Risk-of-bias Animal Histopathology Studies Following Fluoride Exposure



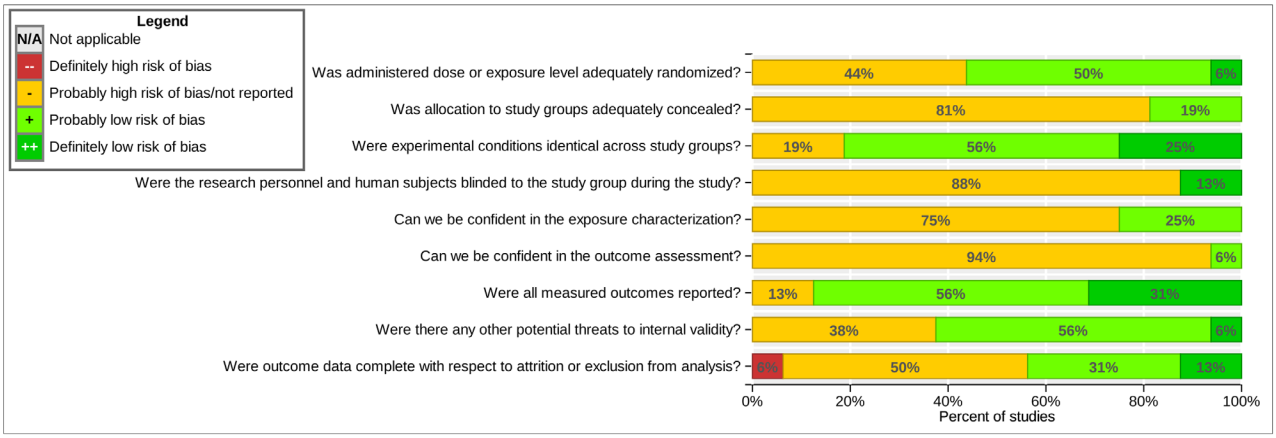
Interactive figure and additional study details in HAWC here.

Figure A3-39. Risk-of-bias Heatmap for High Risk-of-bias Animal Histopathology Studies Following Fluoride Exposure



Interactive figure and additional study details in HAWC here.

Figure A3-40. Risk-of-bias Bar Chart for High Risk-of-bias Animal Histopathology Studies Following Fluoride Exposure



Interactive figure and additional study details in HAWC here.

Appendix 4. Details for Low Risk-of-bias Studies

IQ studies

Bashash *et al.* (2017)

Study Details:

- ***Study design*:** Prospective cohort
- ***Population*:** Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) participants (pregnant mothers and their children aged 4 or 6–12 years).
- ***Study area:*** Mexico City, Mexico
- ***Sample size:*** 299 mother–child pairs, of whom 211 had data for the IQ analyses.
- ***Data relevant to the review:*** Adjusted and unadjusted associations between IQ scores and maternal or child's urinary fluoride concentrations.
- ***Reported association with fluoride exposure:*** Yes: Significant association between maternal urinary fluoride and IQ score (adjusted β = −2.50; 95% CI: −4.12, −0.59). No significant associations with children's urinary fluoride.

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Study participants were selected from two different cohorts from three hospitals in Mexico City that serve low-to-moderate income populations. One cohort was from an observational study of prenatal lead exposure and neurodevelopment outcomes and the other was from a randomized trial of the effect of calcium on maternal blood lead levels. The authors state that participants had no history of psychiatric disorders, high-risk pregnancies, gestational diabetes, illegal drug use, or continuous prescription drugs, but they do not include any information on smoking habits. Study populations appear to be similar, but there may be some differences because subjects were selected from two different cohorts that were recruited from slightly different time periods.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the exposure groups were similar despite the subjects coming from different original study populations where different methods were used for recruitment.
- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Data were collected via questionnaire on maternal age, education, marital status at first prenatal visit, birth order, birth weight, gestational age at delivery, maternal smoking, maternal IQ, and HOME scores. All models were adjusted for gestational age at birth, child's sex, birth weight, birth order, child's age at testing, maternal marital status, smoking history, age at delivery, maternal IQ, education, and cohort, with additional testing for children's urinary fluoride, mercury, lead, and calcium. Sensitivity analyses additionally adjusted for HOME score. Confounders not considered included BMI, iodine deficiency, arsenic, and maternal mental health and

nutrition. Arsenic is assumed not to be a potential co-exposure in this population as the study authors did not discuss it as an issue but did discuss other co-exposures. Arsenic is included in the water quality control program in Mexico City and is not considered a concern in this population.

- *Potentially important study-specific confounders:* All key confounders were addressed.
  - *Direction/magnitude of effect*: Not applicable.
- *Basis for rating:* Probably low risk of bias based on direct evidence that key confounders including other potential co-exposures were addressed and indirect evidence that the methods used to collect the information were valid and reliable and that arsenic is not likely to be an issue in this study population.

- ***Attrition:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Although there was a large amount of attrition, the study authors clearly describe all reasons for attrition and also provide characteristics to compare those participants included to those excluded. There were some slight differences between those included and those excluded, but there is nothing to indicate that the attrition would potentially bias the results.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
- ***Exposure:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Urinary fluoride concentrations were determined in spot urine samples (2nd morning void) collected from mothers (during at least one trimester) and children ages 6–12 years. Fluoride content was measured using ion-selective electrode-based assays. QC methods were described including between laboratory correlations. All samples were measured in duplicate. Extreme outliers were excluded. Urinary dilution was addressed by using creatinine-adjusted levels.
    - *Direction/magnitude of effect*: Not applicable.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that exposure was consistently assessed using well-established methods that directly measured exposure.
- ***Outcome:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Outcome was assessed using the McCarthy Scales of Children's Abilities (MSCA) in 4-year-old children (translated into Spanish) and the Wechsler Abbreviated Scale of Intelligence (WASI) in 6–12-year-olds. The WASI is a well-established test and the validity of both tests is well documented by the authors. Inter-examiner reliability was evaluated and reported with a correlation of 0.99 (++ for methods). The study report stated that psychologists were blind to the children's fluoride exposure (++ for blinding). Overall rating for methods and blinding = ++.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
  - *Rating:* Definitely low risk of bias (++)

- *Summary:* All outcomes outlined in the abstract, introduction, and methods are reported in sufficient detail.
- *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.

- ***Other potential threats:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:*
    - *Statistical analyses:* Statistical analyses used were appropriate for the study. Statistical tests of bivariate associations [using Chi-square tests for categorical variables and analysis of variance (ANOVA)] were used to compare the means of the outcomes or exposure within groups based on the distribution of each covariate. Generalized additive models (GAMs) were used to estimate the adjusted association between fluoride exposure and measures of children's intelligence. Residual diagnostics were used to examine model assumptions and identify any potentially influential observations. Results are reported as adjusted effects and 95% CIs. In sensitivity analyses, regression models accounted for clustering at the cohort level by using cohort as a fixed effect in the models. Although using cohort as a random effect would be more appropriate, using individual-level exposure data and accounting for numerous potential confounders in the models likely captured the cohort effect. Additional models with cohort as a random effect were also subsequently made available via personal communication with the study authors and showed similar results to the main model.
    - *Other potential concerns:* None identified.
  - *Basis for rating:* Definitely low risk if bias based on direct evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Definitely or probably low risk-of-bias ratings in confounding, exposure, and outcome. Study strengths include individual exposure measurements, outcome blindly assessed, and the prospective cohort study design.

Choi *et al.* (2015)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** First grade children (ages 6–8 years)
- ***Study area:*** Mianning County in southern Sichuan, China
- ***Sample size:*** 51 first grade children
- ***Data relevant to the review:*** Associations between IQ (digit span for auditory span and working memory and block design for visual organization and reasoning components of WISC-IV only) with continuous urine or drinking water fluoride levels. Study also had information based on dental fluorosis score.
- ***Reported association with fluoride exposure:*** Yes: Compared to the normal/questionable dental fluorosis, the moderate/severe dental fluorosis group was associated with significantly lower total (adjusted β = −4.28; 95% CI: −8.22, −0.33) and backward (adjusted β = −2.13; 95% CI: −4.24,

−0.02) digit span scores. Linear correlations between total digit span and fluoride in urine (adjusted β = −1.67; 95% CI: −5.46, 2.12) and in drinking water (adjusted β = −1.39; 95% CI: −6.76, 3.98) were observed but not significant. Other outcomes not significantly associated with fluoride exposure.

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Subjects were selected during the same time frame using the same methods. Fifty-one first-grade children residing in Mianning County in southern Sichuan, China were included in this pilot study. It is not specified if the 51 children represented all the first-grade children from this area or if some refused to participate. Children who did not speak Chinese, were not students at the Primary School of Sunshui Village in Mianning County, or those with chronic or acute disease that might affect neurobehavioral function tests were excluded. Demographic characteristics are presented in Table 1 of the study, which indicates that subjects were similar. Potential confounders are adjusted for in the statistical analyses.
  - *Basis for Rating:* Definitely low risk of bias based on direct evidence that the exposure groups were similar and were recruited within the same time frame using the same methods with no evidence of differences in participation/response rates.
- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* The parents or guardians completed a questionnaire on demographic and personal characteristics of the children (sex, age at testing, parity, illnesses before age 3, and past medical history) and caretakers (age, parity, education and occupational histories, residential history, and household income). A 20-µL capillary blood sample was collected at the school by a Mianning County Center for Disease Control (CDC) health practitioner and tested for possible iron deficiency which could be used as a covariate of neurodevelopmental performance. Confounders that were not assessed include: maternal BMI, parental mental health, maternal smoking status, maternal reproductive factors, parental IQ, and HOME score. However, the study authors noted that confounding bias appeared to be limited due to the minimal diversity in the social characteristics of the subjects. The study authors indicated that CDC records documented that levels of other contaminants including arsenic and lead were very low in the area. Iodine differences were not specifically addressed, but there is no indication from the information provided that this might be a concern.
  - *Potentially important study-specific confounders:* All key confounders were considered in this study.
    - *Direction/magnitude of effect*: Not applicable.
  - *Basis for rating:* Probably low risk of bias because there is direct evidence that the key confounders are taken into account and indirect evidence that co-exposure to arsenic is likely not an issue in this area and that methods used for collecting the information were valid and reliable.

- ***Attrition:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* The majority of results were reported for the 51 children stated to be included in the pilot study. In Table 5 of the study, the N for each dental fluorosis category only totals 43, but the text indicates 8 children did not have a Dean Index because permanent teeth had not erupted.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
- ***Exposure:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* The study used three different measurements of fluoride exposure: well water fluoride concentrations from the residence during pregnancy and onwards, fluoride concentrations from children's first morning urine samples, and degree of children's dental fluorosis. Fluoride concentrations in community well water were measured and recorded by Mianning County CDC; specific methods were not reported, but they likely used standard methods as they were conducted by the CDC and were likely the same as those used to measure the fluoride in urine. Migration of subjects was noted to be limited. Well water fluoride concentrations of the mother's residence during pregnancy and onward were used to characterize a child's lifetime exposure. To provide a measure of the accumulated body burden, each child was given a 330-mL (11.2-oz) bottle of Robust© distilled water (free from fluoride and other contaminants) to drink the night before the clinical examinations, after emptying the bladder and before bedtime. The first urine sample the following morning was collected at home, and the fluoride concentration was determined on a 5-mL sample using an ion-specific electrode at the Mianning CDC. There is no indication that urinary fluoride levels accounted for dilution nor was it clear that the method of administering water to the children and collection methods sufficiently controlled for differences in dilution. One of the investigators, a dentist, performed a blinded dental examination on each child's permanent teeth to rate the degree of dental fluorosis using the Dean Index. The Dean Index is a commonly used index in epidemiological studies and remains the gold standard in the dentistry armamentarium. The Index has the following classifications: normal, questionable, very mild, mild, moderate, and severe. Quality control (QC) procedures are not reported but were likely appropriate.
    - *Direction/magnitude of effect***:** Current levels were used to assess lifetime exposure. This is likely to be a non-differential exposure misclassification and direction of bias is unknown. Because subject migration appears to be limited, it is likely that the current fluoride levels are adequate reflections of past exposure. Dental fluorosis would be an indicator that exposure occurred in the past and there was a fair correlation between degree of dental fluorosis and current urine and water fluoride levels, with both increasing with increasing levels of dental fluorosis.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using well-established methods that directly measure exposure.

- ***Outcome:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* The study authors adopted culture-independent tests considered feasible for children aged 6 to 8 years. The Wide Range Assessment of Memory and Learning (WRAML) was used for the assessment of memory and learning. Three subtests were also used. The Finger Windows subtest assesses sequential visual memory. The Design Memory subtest assesses the ability to reproduce designs from memory following a brief exposure. The Visual Learning subtest assesses the ability to learn the locations of pictured objects over repeated exposures. The Wechsler Intelligence Scale for Children-Revised (WISC-IV) included digit span for auditory span and working memory and block design for visual organization and reasoning. The grooved pegboard test assesses manual dexterity. The tests used have been validated on a western population. Although there is no information provided to indicate that they were validated on the study population, the study authors indicated that the tests were culture-independent (+ for methods). Blinding of the outcome assessors or steps to minimize potential bias was not reported. However, it is unlikely that the assessors had knowledge of the individual exposure as children all came from the same area, and water and urine levels were tested at the CDC. (+ for blinding). Overall = +.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that all outcomes were assessed blindly using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* All outcomes outlined in the abstract, introduction, and methods are reported in sufficient details.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:*
    - *Statistical analyses:* Statistical analyses were appropriate. Multiple regression models evaluate the associations between exposure indicators and test scores after adjusting for potential confounders. Specific regression models are not described or refenced, just stated to be “standard regression analysis with confounder adjustment.” The distributions of fluoride concentrations in urine and water were skewed and were log10-transformed to approximate a Gaussian distribution (test not specified). Results are reported as adjusted effects and 95% CIs. There is no evidence that residual diagnostics were used to examine model assumptions; however, the impact on the effect estimates is expected to be minimal.
    - *Other potential concerns:* It should be noted that this study was a pilot study and, therefore, had a relatively small sample size (i.e., 51 children).
  - *Basis for rating:* Probably low risk if bias based on indirect evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.

- ***Basis for classification as low risk-of-bias study overall: Probably low risk-of-bias ratings in the*** confounding, exposure, and outcome risk-of-bias domains. Study strengths include individual fluoride measurements with blinding at outcome assessment likely. All key confounders and many other confounders were taken into account in the study design or analysis.

Cui *et al.* (2018)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** School children aged 7–12 years from four schools in two districts in China with different fluoride levels
- ***Study area:*** Jinghai and Dagang in Tianjin City, China
- ***Sample size:*** 323 school children
- ***Data relevant to the review:*** IQ scores by urine fluoride levels.
- ***Reported association with fluoride exposure:*** Yes: Significant correlation between IQ score and urinary fluoride (adjusted β = −2.47; 95% CI: −4.93, −0.01).

Risk of Bias:

- **Author contacts:**
  - Authors were contacted in June 2019 to obtain additional information for risk-of-bias evaluation.
- ***Population selection:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Four schools were selected from the same district in China. The schools were selected based on levels of fluoride in the local drinking water and the degree of school cooperation. No details were provided on the number of schools in given areas or the difficulty in getting school cooperation. It was noted that the residents in the four areas had similar living habits, economic situations, and educational standards. Although authors do not provide the specific data to support this, fluoride levels and IQ scores were provided by different subject characteristics. The areas were classified as historically endemic fluorosis and non-fluorosis. Cluster sampling was used to select the grades in each school according to previously set child ages, and classroom was randomly selected with all students within a selected classroom included. Reasons for exclusion do not appear to be related to exposure or outcome.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the exposure groups were similar and recruited within the same time frame using the same methods, with no evidence of differences in participation/response rates.
- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* The measurements of all covariates were obtained by structured questionnaires that were completed by children with the help of their parents. Confounders that were assessed include: child's gender, child's ethnicity, child's age, child's BMI, birth (normal vs abnormal), mother's age at delivery, mother's education, income per family member, mother's smoking/alcohol during pregnancy, family member smoking, environmental noise, iodine region (non-endemic vs iodine-excess-

endemic area), factory within 30 m of residence, iodine salt, diet supplements, seafood/pickled food/tea consumption, surface water consumption, physical activity, stress, anger, anxiety/depression, trauma, having a cold 5 times a year, thyroid disease in relatives, mental retardation in relatives, and cancer in relatives. Covariates not considered include parity, maternal and paternal IQ, and quantity and quality of caregiving environment (e.g., HOME score). The authors report that there are no other environmentally toxic substances that may affect intelligence, such as high arsenic or iodine deficiency according to the Tianjin Centers for Disease Prevention and Control.

- *Potentially important study-specific confounders:* All key confounders were considered in this study.
  - *Direction/magnitude of effect*: Not applicable.
- *Basis for rating:* Probably low risk of bias because there is indirect evidence that the key confounders are considered, methods for collecting the information are valid and reliable, and co-exposure to arsenic is likely not an issue in this area.

- ***Attrition:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Of the 400 children enrolled, 35 were excluded because they did not have informed consent signed by a guardian or they moved out of the area. Forty-two children were excluded because they did not have a DRD2 genotyping measurement. It is unclear if these children were from the same schools or if they were evenly distributed throughout the study area. It was also unclear if the excluded subjects were similar to those included in the study. In the study, some analyses had fewer than the 323 subjects, but this seems reasonable given the subgroups that were being evaluated.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
- ***Exposure:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Although children were selected based on area fluoride levels, fluoride in the urine was used in the analysis. Urine was collected from each child the morning of enrollment and analyzed within a week. Fluoride levels were measured using an ion-selective electrode according to the China standard. A brief description of the method was provided, but no QC methods were reported. The study authors did not account for urinary dilution in the spot samples.
    - *Direction/magnitude of effect*: Not accounting for dilution could cause there to be some exposure misclassification. The direction and magnitude would depend on where the differences occurred.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using acceptable methods that provide individual levels of exposure.
- ***Outcome:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* IQ was measured by professionals using the Combined Raven's Test-The Rural in China method, which is the appropriate test for the study population (++ for methods). Blinding or other methods to reduce bias were not reported. Although it is

unlikely that the outcome assessor would have knowledge of the child's urine fluoride levels, there is potential that they would know if the child was from an endemic or non-endemic area if the IQ tests were conducted at the child's school, and there was no information provided on how the IQ tests were administered. Correspondence with the study author noted the cross-sectional nature of the study with outcome and exposure assessed at the same time making the outcome assessors blind to the exposure. However, there is still potential for knowledge of the area (+ for blinding).

- *Basis for rating:* Probably low risk of bias based on indirect evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.

- ***Selective Reporting:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* All outcomes in the abstract, introduction, and methods are reported in sufficient details.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:*
    - *Statistical analyses:* Statistical analyses were appropriate. Multiple linear regression models were applied to evaluate the relationship between urine fluoride levels and IQ scores, accounting for numerous potential confounders. The urinary fluoride levels were log-transformed due to a skewed distribution. Residual diagnostics were used to examine model assumptions. Model robustness was tested through bootstrap, sensitivity analysis after excluding potential outliers, and cross-validation techniques. Results are reported as adjusted effects and 95% CIs. The analysis did not account for clustering at the school level or at the grade level (students were from four schools in grades selected via a clustered sampling method). There is no evidence that the sampling strategy was otherwise accounted for via sampling weights. The impact of these factors on the effect estimates is expected to be minimal given the use of individual-level data and adjustment for several potential confounders.
    - *Other potential concerns:* None identified.
  - *Basis for rating:* Probably low risk if bias based on indirect evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Probably low risk-of-bias ratings in confounding, exposure, and outcome. Study strengths include individual exposure measurements but is limited by the cross-sectional study design and lack of accounting for urine dilution. All key confounders were accounted for in the study design or analysis.

Cui *et al.* (2020)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** School children aged 7–12 years
- ***Study area:*** Tianjin City, China (one randomly selected school from each district based on iodine levels in the water), presumably was an expansion of the Cui *et al.* (2018)
- ***Sample size:*** 498 school children
- ***Data relevant to the review:*** IQ scores by urine fluoride levels.
- ***Reported association with fluoride exposure:*** Yes: A 2-point decrease in IQ was observed in the highest urinary fluoride group compared to the lowest urinary fluoride group (i.e., 110.00 in ≥2.5-mg/L group versus 112.16 in <1.6-mg/L group); however, the results did not achieve statistical significance based on a one-way ANOVA comparing the three different urinary fluoride categories only.

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for the 2020 publication. Authors were contacted in June 2019 for additional information on the Cui *et al.* (2018) publication. Information obtained from that correspondence may have been used for additional information in the 2020 publication.
- ***Population selection:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Subjects were recruited from 2014 to 2018. One school was selected from each district where water concentrations of water iodine were <10, 10–100, 100–150, 150–300 and >300 µg/L. In each school, classes were randomly sampled for the appropriate age group of 7–12 years old. A table of subject characteristics was provided by IQ. A total of 620 children were recruited, and 122 children who did not have complete information or enough blood sample were excluded. Reasons for exclusion do not appear to be related to exposure or outcome. The characteristics of the 498 included children are presented.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the exposure groups were similar and were recruited within the same time frame using the same methods, with no evidence of differences in participation/response rates.
- ***Confounding:***
  - *Rating:* Probably high risk of bias (-)
  - *Summary:* It was noted by the study authors that there were no other environmental poisons except water fluoride. Other studies also conducted in this area of China noted specifically that arsenic was not a concern. Iodine was addressed as that was one of the main points of the study. Twenty-one factors (provided in Table 1 of the study) were selected as confounders, and a homemade questionnaire of unspecified validity was used for obtaining the information. It was noted that child age, stress, and anger were significantly associated with IQ although it is unclear if these varied by fluoride level. However, Cui *et al.* (2018) indicates that stress and anger were not significantly

associated with fluoride, and it is assumed that results would be similar for this study even though more children were included in the current study.

- *Potentially important study-specific confounders:* Age (children 7–12 years old)
  - *Direction/magnitude of effect*: Age is a potential confounder for IQ, even in the narrow age range evaluated in this study. The direction of effects may depend on the number of children in each age group within the different urinary fluoride categories; however, these data were not provided. In general, there were fewer subjects ≤ 9 years of age (i.e., 111) compared to > 9 years of age (i.e., 387) with a significantly higher IQ in the ≤9-year-old age group. Therefore, if exposure were higher in the older subjects, this could bias away from the null.
- *Basis for rating:* Probably high risk of bias because there is indirect evidence that age was not addressed as a confounder and it may be related to both IQ and exposure.

- ***Attrition:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Of the 620 (20%) children recruited, 122 were excluded due to incomplete information or inadequate blood sample. No information was provided to indicate if there were similarities or differences in the children included versus the children excluded, but exclusion is unlikely to be related to either outcome or exposure.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
- ***Exposure:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Children's morning urine was collected with a clean polyethylene tube and fluoride was measured using a fluoride ion-selective electrode following Chinese standard WS/T 89-2015. A brief description was provided, but no QC methods were reported. The study authors do not account for urinary dilution in the spot samples.
    - *Direction/magnitude of effect*: Not accounting for dilution could cause there to be some exposure misclassification. The direction and magnitude would depend on where the differences occurred.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using acceptable methods that provide individual levels of exposure.
- ***Outcome:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* IQ was measured using the Combined Raven's Test, which is an appropriate test for the study population (++ for methods). Blinding was not mentioned; however, the outcome assessors would not likely have knowledge of the child's urinary fluoride. Subjects appear to have been recruited based on iodine levels and it is, therefore, unlikely that there would be any knowledge of potential fluoride exposure. Correspondence with the study authors for the Cui *et al.* (2018) study also indicated that the outcome assessors would have been blind.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.

- ***Selective Reporting:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* All outcomes in the abstract, introduction, and methods are reported in sufficient details.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:*
    - *Statistical analyses:* One-way ANOVA was used to make comparisons between mean IQ by urinary fluoride levels. Consideration of heterogeneity of variances was not reported. There is no adjustment for potential confounders or for clustering of children at the school level. There is no evidence that the sampling strategy was otherwise accounted for (i.e., via sampling weights. The impact of these factors on the effect estimates is expected to be minimal given the use of individual-level data. The primary focus of the study was to evaluate associations between IQ and thyroid hormone or dopamine levels (not between IQ and fluoride levels). It should also be noted that more advanced analyses used for thyroid hormone- and dopamine-IQ associations still lacked adjustment for school and accounting for clustering of children from the same school.
    - *Other potential concerns:* None identified.
  - *Basis for rating:* Probably low risk if bias based on indirect evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Probably low risk-of-bias ratings in exposure and outcome. Study strengths include individual exposure measurements, but the study is limited by the cross-sectional study design, lack of accounting for urine dilution, and by not addressing age as a potential confounder.

Ding *et al.* (2011)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** Elementary school children aged 7–14 years old
- ***Study area:*** Hulunbuir City, Inner Mongolia, China
- ***Sample size:*** 331 school children
- ***Data relevant to the review:*** IQ mean difference based on 10 categories of urine fluoride.
- ***Reported association with fluoride exposure:*** Yes: Significant association between urinary fluoride and IQ score (each 1 mg/L increase in urinary fluoride was associated with a lower IQ score of 0.59 points; 95% CI: −1.09, −0.08).

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for additional information because it was not necessary.

- ***Population selection:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* The study randomly selected 340 7–14-year-olds from four nearby elementary schools in Hulunbuir. Authors stated that the four elementary schools appeared to be very similar in teaching quality. The study authors noted that they followed the principles of matching social and natural factors like economic situation, educational standards, and geological environments as much as possible; however, how this was done is unclear and no table of study subject characteristics by group was provided.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the exposure groups were similar and were recruited within the same time frame using the same methods, with no evidence of differences in participation/response rates.
- ***Confounding:***
  - *Rating:* Probably high risk of bias (-)
  - *Summary:* It was noted that none of the four sites had other potential neurotoxins including arsenic in their drinking water. Although they did not provide the specifics, they did provide a reference. In addition, iodine deficiency was noted as not being issue in any of the four areas. Age was the only confounder adjusted in the model. Although dental fluorosis severity by % female was reported, not enough data were provided to determine if it was a confounder that should have been considered in the regression. The study authors note that future studies will include covariates such as parents' educational attainment, mother's age at delivery, and household income.
  - *Potentially important study-specific confounders:* Gender
    - *Direction/magnitude of effect***:** There is not enough information to determine if there is an effect from gender. There were some differences in dental fluorosis level by gender, but it is unclear how this might impact the results or if the distribution of gender differed by age.
  - *Basis for rating:* Probably high risk of bias based on indirect evidence that there were differences in gender that were not considered in the study design or analyses.
- ***Attrition:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Data were relatively complete (i.e., <5% loss). Of the 340 subjects selected for inclusion, 5 were excluded because they lived in the area for less than a year with an additional 4 not consenting to participate.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that exclusion of subjects from analysis was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
- ***Exposure:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Spot urine samples were collected and measured using China CDC standards. All samples were analyzed twice using a fluoride ion-selective electrode. Recovery rates were specified as 95–105% with an LOD of 0.05 mg/L. Water samples were collected from small-scale central water supply systems and tube wells with handy pumps and were processed using standard methods similar to the urine samples. Quality assurance validation was reported. A blind professional examiner evaluated the children for dental

fluorosis using the Dean's Index. All urine and water samples were above the LOD. Urine levels were the primary exposure measure used in the analysis. The study authors did not account for urinary dilution in the spot samples. The mean urine fluoride concentration was correlated with the dental fluorosis levels.

- *Direction/magnitude of effect*: Spot urine samples that did not account for dilution could have exposure misclassification. The misclassification is likely non-differential and potential direction of bias is unknown.

- *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using well-established methods that directly measure exposure.

- ***Outcome:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* IQ was determined using the Combined Raven's Test-The Rural in China (CRT-RC3) (++ for methods). Although blinding was not reported, it is unlikely that the IQ assessors had knowledge of the children's urine levels or even of the water levels from the four sites as these were sent to a separate lab for testing (+ for blinding). Overall rating for methods and blinding = +.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* All outcomes outlined in the abstract, introduction, and methods are reported in sufficient details.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:*
    - *Statistical analyses:* Statistical analyses were reasonable (ANOVA and multiple linear regression), but consideration of homogeneity of variance was not reported. The NASEM committee review (NASEM 2021) pointed out a potential concern for the lack of accounting for clustering at the school-level because children were selected from four elementary schools. However, as pointed out in the ***Selection*** domain, the authors stated that they followed the principles of matching social and natural factors like economic situation, educational standards, and geological environments to the extent possible and that the four elementary schools appeared to be very similar in teaching quality. There is no evidence that the sampling strategy was otherwise accounted for (I.e., via sampling weights). The impact of these factors on the effect estimates is expected to be minimal given the use of individual-level data and adjustment for age as a potential confounder.
    - *Other potential concerns:* None identified.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the statistical analyses were appropriate and that there were no other potential threats to risk of bias.

- ***Basis for classification as low risk-of-bias study overall:*** Probably low risk-of-bias ratings in exposure and outcome. Study strengths include individual exposure measurements, but the study is limited by the cross-sectional study design, lack of accounting for urine dilution, and by not addressing gender as a potential confounder.

Green *et al.* (2019)

Study Details:

- ***Study design*:** Prospective cohort
- ***Population*:** Maternal-Infant Research on Environmental Chemicals (MIREC) participants (pregnant mothers and their children aged 3–4 years)
- ***Study area:*** 10 cities, Canada
- ***Sample size:*** 512 mother−child pairs (238 from non-fluoridated areas, 162 from fluoridated areas; 264 females, 248 males)
- ***Data relevant to the review:*** Adjusted linear regression models evaluating associations between IQ in both genders together and separate, with maternal urinary fluoride across all three trimesters, or with estimated maternal fluoride intake.
- ***Reported association with fluoride exposure:*** Yes: Significantly lower full-scale IQ per 1-mg/L increase in maternal urinary fluoride in boys (adjusted β = −4.49), but not girls (adjusted β = 2.40) and not in both genders combined (adjusted β = −1.95); significantly lower full-scale IQ per 1-mg increases in maternal intake in both genders combined (adjusted β = −3.66 [no sex interaction]); significantly lower full-scale IQ per 1-mg/L increase in drinking water fluoride in both genders combined (adjusted β = −5.29 [no sex interaction]).

Risk of Bias:

- **Author contacts:**
  - Authors were contacted in June 2019 for additional information for the risk if bias evaluation.
- ***Population selection:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Pregnant women were recruited from the same population, during the same timeframe, and using the same methods as the MIREC program. Methods were reported in detail.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that the exposed groups were similar and were recruited with the same methods during the same time frame.
- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* The study considered several possible covariates including maternal age, pre-pregnancy BMI, marriage status, birth country, race, maternal education, employment, income, HOME score, smoking during pregnancy, secondhand smoke in the home, alcohol consumption during pregnancy, parity, child's gender, child's age at testing, gestational age, birth weight, time of void, and time since last void. The study also conducted secondary analyses to test for lead, mercury, arsenic, and PFOA. There is no indication of any other potential co-exposures in this study population. Iodine deficiency

or excess could not be assessed but is not expected to differentially occur. The study was not able to assess parental IQ or mental health disorders. Methods used to obtain the information included questionnaires and laboratory tests.

- *Potentially important study-specific confounders:* All key confounders were addressed.
  - *Direction/magnitude of effect:* Not applicable.
- *Basis for rating:* Probably low risk of bias based on indirect evidence that the methods used to collect the information were valid and reliable and direct evidence that key confounders including potential co-exposures were addressed.

- ***Attrition:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Of the 610 recruited children, 601 (98.5%) completed testing. Of the 601 mother-child pairs, 512 (85.2%) had all three maternal urine samples and complete covariate data, and 400 (66.6%) had data available to estimate fluoride intake.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
- ***Exposure:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Spot urine samples from all three trimesters of pregnancy were evaluated using appropriate methods, and results were adjusted for creatinine and specific gravity. Fluoride intake was estimated based on fluoride water levels and information on consumption of tap water and other water-based beverages (e.g., tea, coffee) was obtained via questionnaire.
    - *Direction/magnitude of effect***:** There is not any specific direction or magnitude of bias expected. Urinary fluoride levels are reflective of a recent exposure. Having measurements from all three trimesters of pregnancy provides a better representation of actual exposure than a single measurement although the potential for missed high exposure is possible. However, the possibility of the occurrence of missed high exposure would be similar in all females and would be non-differential. For the fluoride intake, exposure was based on the fluoride levels in the water at the residence. If women worked outside the home and the majority of intake occurred from areas outside the home (and were different from levels in the home), there is potential to bias toward the null.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using well-established methods that directly measured exposure.
- ***Outcome:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* The Wechsler Preschool and Primary Scale of Intelligence was normalized for ages 2.5–<4.0 and child sex using the U.S population-based norms. Blinding was not reported, but it is unlikely that the outcome assessors had knowledge of the maternal fluoride level or were aware if the city had fluoridated water.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.

- ***Selective Reporting:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* All outcomes were reported.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:*
    - *Statistical analyses:* Multivariate linear regression analyses were used to evaluate the associations between maternal urinary fluoride and fluoride intake and children's IQ scores. Regression diagnostics were used to test assumptions for linearity, normality, and homogeneity. There were no potential influential observations (based on Cook's distance). Sensitivity analyses showed that the effects of maternal urinary fluoride (MUF), fluoride intake, and water fluoride were robust to the exclusion of two very low IQ scores in males (<70). City was accounted for as a covariate in the regression models published. Additional models with city as a random effect were also subsequently made publicly available and showed similar results to the main model.
    - *Other potential concerns:* None identified.
  - *Basis for rating:* Definitely low risk if bias based on direct evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Probably low risk-of-bias ratings in confounding, exposure, and outcome. Study strengths include individual exposure measurements, prospective cohort design, and addressing potential key confounders.

Rocha-Amador *et al.* (2007)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** Children aged 6–10 years
- ***Study area:*** Moctezuma (low fluoride, low arsenic) and Salitral (high fluoride, high arsenic) of San Luis Potosí State and 5 de Febrero (high fluoride, high arsenic) of Durango State, Mexico
- ***Sample size:*** 132 children
- ***Data relevant to the review:*** Associations between full-scale IQ, performance IQ, verbal IQ and child's urine or water fluoride levels.
- ***Reported association with fluoride exposure:*** Yes: Significant associations between fluoride and IQ scores (full-scale IQ adjusted βs of –10.2 [water] and −16.9 [urine]; CIs not reported); arsenic also present, but the effect was smaller (full-scale IQ adjusted βs of –6.15 [water] and –5.72 [urine]; CIs not reported).

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***

- *Rating:* Probably low risk of bias (+)
- *Summary:* All children in 1st through 3rd grades in three rural areas in Mexico (n = 480) were screened for study eligibility including age, time at residence, and address. Authors report that the three selected communities were similar in population and general demographic characteristics. Children who had lived in the area since birth and were 6–10 years old were eligible to participate (n = 308). Of the 308 children, 155 were randomly selected and the response rate was 85%, but participation was not reported by area. It was noted, however, that no significant differences in age, gender, or time of residence were observed between participants and non-participants. Timeframe for selection was not mentioned but appears to be similar. Sociodemographic characteristics of subjects was provided in Table 1 of the study. There was a significant difference in SES and transferrin saturation, but these were taken into account in the analysis.
- *Basis for rating:* Probably low risk of bias based on indirect evidence that the populations were similar and differences were noted and addressed in the analysis.

- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* The study design or analysis accounted for child's age, sex, SES, transferrin saturation, weight, height, blood lead levels, and mother's education. Arsenic levels were highly correlated with fluoride levels; however, arsenic and fluoride were evaluated alone, and arsenic was found to have less of an effect on IQ than fluoride. This provides evidence that arsenic has been addressed as a co-exposure and cannot explain the association between fluoride exposure and decreased IQ. Smoking was not addressed and methods for measuring many of the confounders were not reported.
  - *Potentially important study-specific confounders:* Arsenic
    - *Direction/magnitude of effect*: The presence of arsenic in this study, which also demonstrated an association, would bias the effect away from the null. Although arsenic may contribute to some of the magnitude of the observed effect of fluoride (the exact impact of arsenic on the magnitude cannot be assessed), the presence of arsenic does not fully explain the observed association between fluoride and IQ. The presence of arsenic may affect the magnitude of the association between IQ and fluoride, but it has no impact on the direction of the effect.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the methods used to collect the information were valid and reliable and direct evidence that key confounders were addressed.
- ***Attrition:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Of 155 children randomly selected for study participation, 85% responded to enroll. According to the authors, there were no significant differences in age, gender, or time of residence between responders and non-responders. However, no data are provided to support this, and no breakdown of responders/non-responders by region is provided. Data were provided for the 132 children agreeing to participate.

- *Basis for rating:* Probably low risk of bias based on indirect evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.

- ***Exposure:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Urine samples were collected on the same day as psychological evaluations and were analyzed for fluoride according to NIOSH Method 8308 (Fluoride in Urine). For QC, a reference standard was also used (NIST SRM 2671a). Urine samples were also analyzed for arsenic by using the Atomic Absorption Spectrophotometer with hydride system and used a reference standard for QC. Levels were adjusted for urinary creatinine levels to account for dilution in the spot samples. Tap water samples were collected from each child's home on the day of biological monitoring. Fluoride was measured with a sensitive, specific ion electrode. Detailed methods are provided including internal quality controls. It was noted that in the high fluoride group it was common to drink bottled water low in fluoride and to only use the tap water for cooking; therefore, urine was considered the most appropriate measure of exposure. Only children who had lived at the same residence since birth were included.
    - *Direction/magnitude of effect***:** Not applicable.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that exposure was consistently assessed using well-established methods that directly measured exposure.
- ***Outcome:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Neuropsychological profiles were assessed through the WISC-RM (revised for Mexico). This is a well-established test appropriately adjusted for the study population. However, no additional validation is provided (+ for methods). The study report stated that the test assessors were masked to both arsenic and fluoride water levels (++ for blinding). Overall rating for methods and blinding = +.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
  - *Rating:* Probably high risk of bias (-)
  - *Summary:* It was reported that an interaction between fluoride and arsenic was measured, but it was only noted in the discussion that the study design precluded testing statistical interaction between fluoride and arsenic. This provides indirect evidence of selective reporting.
  - *Basis for rating:* Probably high risk of bias based on indirect evidence that there was selective reporting.
- ***Other potential threats:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:*
  - *Statistical analyses:*
    - *Statistical analyses:* Statistical analyses used were appropriate for the study. Multivariate linear analyses were used to evaluate the associations between fluoride in water and urine and children's IQ scores Exposures were natural

log-transformed, but rationale was not provided. Regression diagnostics were not used to test model assumptions for linearity, normality, and homogeneity. The analyses did not account for clustering at the community level. The three selected communities were similar in population and general demographic characteristics. Although the analysis used individual-level exposures rather than area-level exposures, if the exposure levels within a certain area are highly correlated (which might be expected), then the results might still be biased. However, the overall impact on the effect estimates is expected to be minimal given the use of individual-level data and adjustment for multiple potential confounders.

        - *Other potential concerns:* None identified.
    - *Basis for rating:* Probably low risk if bias based on indirect evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Definitely or probably low risk-of-bias ratings in confounding, exposure, and outcome. Study strengths include individual exposure measurements and outcomes blindly assessed, but it is limited by the cross-sectional study design and not being able to completely rule out the influence of arsenic in the results.

Saxena *et al.* (2012)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** Children aged 12 years
- ***Study area:*** Madhya Pradesh, India
- ***Sample size:*** 170 children
- ***Data relevant to the review:*** Mean IQ grade (not standard scores; higher IQ grades are associated with lower intelligence) by water fluoride quartiles, continuous water fluoride, or continuous urinary fluoride.
- ***Reported association with fluoride exposure:*** Yes: Significant correlations between IQ score and water (r = 0.534) and urinary (r = 0.542) fluoride levels. Significant increase in mean IQ grade (i.e., increase in proportion of children with intellectual impairment) with increasing water fluoride quartile.

Risk of Bias:

- **Author contacts:**
    - Authors were contacted in August of 2017 to obtain additional information for risk-of-bias evaluation.
- ***Population selection:***
    - *Rating:* Probably low risk of bias (+)
    - *Summary:* There was indirect evidence that subjects were similar and were recruited using the same methods during the same time frame. The study participants were selected from a stratified cluster of geographic areas based on fluoride concentration in groundwater. According to the authors, the selected villages were similar in population and demographic characteristics. Data are provided to show the breakdown in SES,

parental education, height/age, and weight/height and no significant differences were noted. Participation was stated to be voluntary, but participation rates were not provided. It is unclear if the 170 subjects were selected with 100% participation or if the 170 subjects were all that were asked to participate, but it appears that all subjects participated. Timing of the recruitment was not provided but is assumed to occur during the same time frame.

- *Basis for rating:* Probably low risk of bias based on indirect evidence that subjects were similar and recruited using the same methods during the same time frame.

- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* There was indirect evidence that key confounders including potential co-exposures were addressed using reasonable methods. A questionnaire, completed with the assistance of parents, was used to collect information on child characteristics (age, sex, height, weight), residential history, medical history (including illness affecting nervous system and head trauma), educational level of the head of the family (in years), and SES of the family. The SES was recorded according to the Pareek and Trivedi classification. The nutritional status of the children was calculated using the Waterlow's classification, which defines two groups for malnutrition using height for age ratio (chronic condition) and weight for height ratio (acute condition). Within both groups, it categorizes the malnutrition as normal, mildly impaired, moderately impaired, or severely impaired. Urinary lead and arsenic were analyzed using the atomic absorption spectrophotometer. Urinary iodine was measured using the Dunn method. Authors do not report which covariates were included in the multivariate regression models; however, there was no difference in reported demographic characteristics. All subjects were the same age, and there was no difference in iodine, lead, or arsenic between the groups. Mean urinary arsenic levels did increase with increasing fluoride even though there was no significant difference by group.
  - *Potentially important study-specific confounders:* All key confounders were considered in this study.
    - *Direction/magnitude of effect***:** Not applicable.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the methods used to collect the information were valid and reliable and that key confounders including potential co-exposures were addressed.
- ***Attrition:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Results were provided for all 170 children stated to be included in the study.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence of no attrition.
- ***Exposure:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* A sample of 200 mL of drinking water was collected at each child's home. The fluoride levels were analyzed by a fluoride ion-selective electrode. Each subject was also asked to collect a sample of their first morning urine. The fluoride content in the urine was determined using a fluoride ion-selective electrode. QA/QC and LOD were not reported and urinary dilution was not assessed. Although only current levels were measured, children who had changed water source since birth were excluded.

        - *Direction/magnitude of effect*: Spot urine samples that did not account for dilution could have exposure misclassification. The misclassification is likely non-differential and not likely to bias in any specific direction. Children who had changed water since birth were excluded, but it was not specifically noted that the fluoride in the water source was stable over the years.
    - *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using well-established methods that directly measured exposure.
- ***Outcome:***
    - *Rating:* Probably low risk of bias (+)
    - *Summary:* Intelligence is assessed using the Raven's Standard Progressive Matrices and categorized into five grade levels. Although it was not noted that the test was validated to the study population, the test is visual and would be applicable to most populations (+ for methods). There is no mention of blinding by test administrators or evaluators and the exposure groups come from different geographic areas. It was also not reported who measured the levels of fluoride from the home or urine samples. Correspondence with the study authors indicated that the outcome assessors were blind to the children's fluoride status (++ for blinding). Overall rating for methods and blinding = +.
    - *Basis for rating:* Probably low risk of bias based on indirect evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
    - *Rating:* Definitely low risk of bias (++)
    - *Summary:* All outcomes outlined in the abstract, introduction, and methods are reported in sufficient detail.
    - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
    - *Rating:* Probably low risk of bias (+)
    - *Summary:*
        - *Statistical analyses:* One way analysis of variance (ANOVA), simple linear regression, and multiple linear regression were used to compare mean intelligence grades by water fluoride levels and to assess the association between grades and urinary fluoride. Consideration of heterogeneity of variance (for ANOVA) was not reported. Regression diagnostics were not used to test model assumptions for linearity, normality, and homogeneity. Given the ordinal nature of the intelligence grade variable (score from 1 to 5), ordinal logistic regression would have been a more appropriate method. There was no adjustment for area-level clustering in multivariate analyses (although subjects were selected via stratified cluster sampling from two areas). Although the analysis used individual-level exposures rather than area-level exposures, if the exposure levels within a certain area are highly correlated (which might be expected), then the results might still be biased. However, the overall impact on the effect estimates is expected to be minimal given the use of individual-level data and adjustment for potential confounders.
        - *Other potential concerns:* None identified.

  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the statistical analyses were appropriate, and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Probably low risk-of-bias ratings in confounding, exposure, and outcome. Study strengths include individual exposure measurements and the addressing of potential key confounders, but it was limited by the cross-sectional study design and lack of addressing dilution in the urine samples.

Seraj *et al.* (2012)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** Children aged 6–11 years
- ***Study area:*** five villages, Makoo, Iran
- ***Sample size:*** 293 children
- ***Data relevant to the review:*** IQ (mean and distribution) assessed by Raven's Colored Progressive Matrices and presented by fluoride area; beta was also provided for water fluoride.
- ***Reported association with fluoride exposure:*** Yes: Significant association between water fluoride and IQ score (adjusted β = −3.865; CIs not reported); significantly higher IQ score in normal area (97.77 ± 18.91) compared with medium (89.03 ± 12.99) and high (88.58 ± 16.01) areas.

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Subjects were selected from five villages in Makoo. The villages were stated to all be rural with similar general demographic and geographic characteristics and were comparable in terms of SES and parental occupations. Children were 6–11 years old. Age, gender, and education were taken into account in the analysis. No other characteristics were provided or discussed. Participation rates were not reported. There is indirect evidence that the populations were similar, and some possible differences were addressed.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that subjects were similar and recruited using the same methods during the same time frame.
- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Age, gender, dental fluorosis intensity, and educational levels (child's and parents') were evaluated as potential confounders. Other potential confounders such as smoking were not discussed. Information was obtained from a detailed questionnaire. Lead was measured, but only found in low levels in the drinking water throughout the study regions. Iodine in the water was also stated to be measured and residents were receiving iodine-enriched salt. Arsenic was not addressed, but there is no evidence that

arsenic levels would vary across villages in this area. Based on water quality maps, co-exposure to arsenic is likely not a major concern in this area.

- *Potentially important study-specific confounders:* Arsenic.
  - *Direction/magnitude of effect*: Conceptually, if there were differential amounts of arsenic in the different villages, co-exposure to arsenic could bias the results with the direction of the bias dependent on where the arsenic was present; however, arsenic is not expected to be a major concern in this study area based on water quality maps.
- *Basis for rating:* Probably low risk of bias based on indirect evidence that the methods used to collect the information were valid and that key confounders including potential co-exposures were addressed or were not likely to be an issue in the study area.

- ***Attrition:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Attrition was low if it occurred. It was noted that 293 out of 314 children living in the villages were recruited. It is not clear if 21 children were excluded based on exclusion criteria or if they refused to participate; however, this accounts for less than 10% of the population and results were available for all 293 subjects.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
- ***Exposure:***
  - *Rating:* Probably high risk of bias (-)
  - *Summary:* Exposure was primarily based on area of residence. Fluoride in the groundwater was analyzed by the SPADNS (Sulfophenylazo dihydroxynaphthalene-disulfonate) method, utilizing 4000 UV-Vis spectrophotometer in the environmental health engineering laboratory of the Public Health School of Tehran University of Medical Sciences. Specific details were not provided on methods of collection, samples locations, or if these locations represented the primary sources of drinking water for the subjects. Villages were categorized into normal (0.5–1 ppm), moderate (3.1±0.9 ppm), and high (5.2±1.1 ppm) fluoride based on the mean fluoride content of all seasons presumably for the stated 12-year time period. Subjects were stated to be long-life residents of the village. Dental fluorosis was also measured and increased in severity with fluoride levels; however, all areas had some degree of dental fluorosis. Although authors used an average fluoride level in varying seasons over presumably 12 years, they used a less-established method without reporting reliability or validity, nor did they provide data to indicate that the mean was truly representative of the fluoride levels over time and throughout the village. Although dental fluorosis severity increased with increasing fluoride levels, the data could also indicate potential exposure misclassification.
    - *Direction/magnitude of effect*: The presence of dental fluorosis in all groups indicates that there may have been different exposure in some children at a younger age. Although there were only about 20 children in the “normal” fluoride group with very mild to mild dental fluorosis, this could bias the results toward the null because those children may have experienced a higher level of

fluoride at some point. The other two fluoride groups were exposed to fluoride levels that likely exceeded those in the "normal" fluoride group.

- *Basis for rating:* Probably high risk of bias based on indirect evidence that exposure was assessed using insensitive methods.

- ***Outcome:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Intelligence was evaluated using the Raven's Color Progressive Matrices. This is a well-established method. Although the study authors did not provide data to indicate that the methods were valid in this study population, the test is designed to be culturally diverse. (+ for methods). The study report stated that test administrators were blinded. (++ for blinding). Overall rating for methods and blinding = +.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that outcomes were blindly assessed using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* All outcomes outlined in the abstract, introduction, and methods were reported. However, because they did not report the method for obtaining the betas in Table 4 of the study, it is not clear if these were adjusted or unadjusted betas.
  - *Basis for rating:* Probably low risk of bias based on direct evidence that all the study's measured outcomes were reported, but the results were not sufficiently reported.
- ***Other potential threats:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:*
    - *Statistical analyses:* Statistical methods for comparisons of IQ level by exposure groups were reasonable (ANOVA, post hoc test and Kruskal-Wallis test), but consideration of heterogeneity of variance was not reported. Clustering at the village levels was not accounted for in multivariate analyses which used area-level water fluoride levels. Because the exposure levels within a certain area are highly correlated (which might be expected), the results are likely to be biased. There was adjustment for some potential individual-level confounders, and the children were from five rural areas with similar general demographic and geographic characteristics and were comparable in terms of SES and parental occupations. These factors are expected to mitigate some of the impact of lack of accounting for clustering, and the overall impact on the effect estimates is expected to be minimal.
    - *Other potential concerns:* None identified.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Probably low risk-of-bias ratings in confounding and outcome. Study strengths include addressing potential key confounders, but it was limited by the cross-sectional study design and the group-level exposure data.

Soto-Barreras *et al.* (2019)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** Children aged 9–10 years
- ***Study area:*** Chihuahua, Mexico
- ***Sample size:*** 161 children
- ***Data relevant to the review:*** Water fluoride, urinary fluoride, exposure dose, and dental fluorosis index by IQ grade.
- ***Reported association with fluoride exposure:*** No: Results were not presented to evaluate an association between fluoride exposure and IQ, but rather to compare fluoride levels within IQ grades. For this reason, the results for this study are not comparable to other studies that evaluated IQ scores by fluoride exposure levels. No significant differences in measured fluoride levels across IQ grades were observed.

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Subjects were selected using a multistage cluster sampling. During the first stage, 13 public elementary schools were randomly selected from a pool of 73 using a cluster sample design. Secondly, only fourth grade students were included. Authors stated that they wanted to keep the same grade level, but they were not specific as to why fourth graders were selected as opposed to any other grade. Lastly, only children whose parents or guardians attended and responded to the survey were included. There is no information provided on how the 13 schools selected may be similar or different from the 60 schools not selected. There is no information provided on the number of children in the fourth grade to know participant rates. It was only noted that 245 children were examined, but 161 were included after the exclusion rules were applied. Inclusion and exclusion criteria are presented. Reasons for exclusion do not appear to be related to exposure or outcome. Characteristics of participants and non-participants are not compared; however, characteristics of the 161 included children were provided and any differences were taken into account in the analysis.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the exposed groups were similar and were recruited using similar methods during the same time frame.
- ***Confounding:***
  - *Rating:* Probably high risk of bias (-)
  - *Summary:* No confounders were considered when evaluating fluoride associations with intelligence; they were only applied when evaluating fluoride levels and dental caries. Based on Table 4 of the study, there was no significant association between IQ grade and child's age, sex, parental education, or SES status. No other information was reported or considered. There is no information on potential co-exposures. Based on

water quality maps, the arsenic prediction indicates a greater than 50% probability of exceeding the WHO guidelines for arsenic of 10 µg/L in areas of Chihuahua, Mexico.

- *Potentially important study-specific confounders:* Arsenic.
  - *Direction/magnitude of effect*: The direction and magnitude of effects is unknown. There is potential for arsenic to occur in the study area, but it is not known how it relates to fluoride exposure. If they occur together in the water, it will bias away from the null; however, if they occurred in different areas, there is potential to bias toward the null.
- *Basis for rating:* Probably high risk of bias based on indirect evidence that there is potential for exposure to arsenic that was not sufficiently addressed.

- ***Attrition:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* A total of 161 of 245 children were included in the study. Exclusion criteria are presented and are unrelated to outcome or exposure. For the 161 children, there are no missing outcome data.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
- ***Exposure:***
  - *Rating:* Probably low risk of bias (+);Probably high risk of bias (-)
  - *Summary:* **Urinary Fluoride (probably low risk of bias):** First morning void urine samples were collected based on NIOSH methods. Water samples were also stated to be collected, but it does not appear that methods followed any particular standard, and there is no indication that subjects were provided with collection containers. Analysis was based on a calibration curve using fluoride ion selective electrode. QC methods were mentioned. Based on results, there were values below detection limits, but LODs or % below LOD were not reported.

    Daily fluoride exposure (probably high risk of bias): Daily fluoride exposure was based on the water fluoride level, drinking water consumption (based on parental report of how many glasses of water consumed), and body weight.
    - *Direction/magnitude of effect*: Spot urine samples that did not account for dilution could have exposure misclassification. The misclassification is likely non-differential and is not likely to bias in any specific direction. Daily exposure was based partially on parental report of water consumption. The direction and magnitude of effect is unknown.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using well-established methods that directly measured exposure. The daily fluoride exposure is probably high risk of bias because there is indirect evidence that the exposure was assessed using methods of unknown validity.
- ***Outcome:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Intellectual ability was evaluated using Raven's Colored Progressive Matrices by an independent examiner. Some details were provided, but it was not stated that the tests were assessed blind; however, there is no indication that subjects were from high fluoride areas and the assessor would not have knowledge of the urine or water fluoride

levels. Results for children were converted into a percentile according to age (details not provided) and overall scores were assigned an intellectual grade of I to V as described in the report.
    - *Basis for rating:* Probably low risk of bias based on indirect evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
    - *Rating:* Definitely low risk of bias (++)
    - *Summary:* All outcomes outlined in the abstract, introduction, and methods were reported in sufficient detail.
    - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
    - *Rating:* Probably low risk of bias (+)
    - *Summary:*
        - *Statistical analyses:* The Kolmogorov-Smirnov test was used to determine variable distribution. The Kruskal Wallis test was used to compare exposure levels between IQ grades with a Dunn's post hoc test. Multivariate logistic regression was used the estimate the association between presence of dental caries and various risk factors. Fluoride levels in drinking water and urine and fluoride exposure dose are compared across intellectual grades. Children were from 13 schools selected via stratified cluster sample design. There was no adjustment for clustering at the school level or for the sampling design. Although the analysis used individual-level exposures rather than area-level exposures, if the exposure levels within a certain school are highly correlated (which might be expected), then the results might still be biased. The large number of clusters (13 schools) makes clustering less of a concern and the impact on the effect estimates is expected to be minimal.
        - *Other potential concerns:* None identified.
    - *Basis for rating:* Probably low risk if bias based on indirect evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Probably low risk-of-bias ratings in exposure and outcome. Study strengths include individual exposure measurements and outcomes blindly assessed, but is limited by the cross-sectional study design, lack of accounting for urine dilution, and by not addressing potential exposures to arsenic in the study area. Although the study is considered to have low potential for bias overall, the focus of the study was to evaluate the relationship between fluoride exposure and lower rates of dental caries. In terms of evaluating an association between fluoride exposure and IQ scores, the study is limited by the way that the data were reported.

Sudhir *et al.* (2009)

Study Details:

- ***Study design*:** Cross-sectional

- ***Population*:** Children aged 13–15 years
- ***Study area:*** Nalgonda district (Andhra Pradesh), India
- ***Sample size:*** 1,000 children
- ***Data relevant to the review:*** Mean IQ grade (not standard scores) or IQ distribution by water fluoride strata (<0.7, 0.7-1.2, 1.3-4.0, and >4.0 ppm).
- ***Reported association with fluoride exposure:*** Yes: Significant increase in mean and distributions of IQ grades (i.e., increase in proportion of children with intellectual impairment) with increasing drinking water fluoride levels.

Risk of Bias:

- **Author contacts:**
  - Authors were contacted in September of 2017 for additional information related to risk-of-bias evaluation, but no response was received.
- ***Population selection:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Children were selected from the same general population during the same time frame and were then broken down into nearly equal exposure groups. A cross-sectional study was conducted among 13–15-year-old school children of Nalgonda district, Andhra Pradesh between August and October 2006. Data were collected from the school children who were life-long residents of Nalgonda district, Andhra Pradesh and who consumed drinking water from the same source during the first 10 years of life. A stratified random sampling technique was used. The entire geographical area of Nalgonda district was divided into four strata based on different levels of naturally occurring fluoride in the drinking water supply. Children were randomly selected from schools in the different strata. It was noted that the 1,000 selected children were equally divided among all four strata, however, each group did not have 250 children (but instead 243−267 in each group). Participation rates are not reported. Exclusion criteria included: children who had a history of brain disease and head injuries, children whose intelligence had been affected by congenital or acquired disease, children who had migrated or were not permanent residents, children with orthodontic brackets, and children with severe extrinsic stains on their teeth. Age and gender data are presented in Table 1 of the study, but this information is not presented by the different fluoride groups.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that subjects were similar and were recruited using the same methods during the same time frame.
- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Data were collected using a self-administered questionnaire and clinical examination. The self-administered questionnaire requested information on demographic data (appears to cover age and sex), permanent residential address, staple food consumed, liquids routinely consumed, and aids used for oral hygiene maintenance (fluoridated or nonfluoridated). SES was measured using the Kakkar socio-economic status scale (KSESS) with eight closed-ended questions related to parental education, family income, father's occupation, and other factors. All children were asked to fill out the form, and the answers obtained were scored using Kakkar socio-economic status

scoring keys. Based on this scoring, children were divided into three groups—lower class, middle class, or upper class. Age, sex, and SES were not found to be significantly associated with IQ. Other confounders including smoking were not addressed. Co-exposures such as arsenic and lead were not addressed; however, there is no indication that lead is a co-exposure in this population and arsenic is not likely a major concern in this area based on water quality maps.

- *Potentially important study-specific confounders:* Key confounders age, gender, and measures of SES were similar between exposure groups; however, arsenic was not taken into account. Arsenic often occurs in the drinking water along with fluoride in some Indian populations; however, based on water quality maps, this does not appear to be an issue in the Nalgonda district of Andhra Pradesh. Iodine deficiencies are not mentioned.
    - *Direction/magnitude of effect***:** Conceptually, the presence of arsenic would potentially bias away from the null if present with fluoride. Deficiencies in iodine would bias away from the null if present in areas of high fluoride, but toward the null if present in areas of non-high fluoride. Neither of these were considered issues in this study for reasons noted above.
- *Basis for rating:* Probably low risk of bias based on indirect evidence that the key confounders are considered, co-exposure to arsenic is likely not an issue in this area, and methods used for collecting the information were valid and reliable.

- ***Attrition:***
    - *Rating:* Definitely low risk of bias (++)
    - *Summary:* Results were available for the 1,000 children selected to participate.
    - *Basis for rating:* Definitely low risk of bias based on direct evidence of no attrition.
- ***Exposure:***
    - *Rating:* Probably low risk of bias (+)
    - *Summary:* Children were placed into one of four strata based on the level of fluoride in drinking water. Collection of water samples was done in the districts. The placement into strata was based on fluoride levels obtained from documented records of District Rural Water Works Department. Once the children were assigned to strata, it was confirmed that the fluoride level of their drinking water was within the strata assigned. This was done using the methodology followed in National Oral Health Survey and Fluoride Mapping 2002–2003. During the initial visits to the schools, the children were interviewed regarding their history of residence and source of drinking water from birth to 10 years. The first child meeting criteria was given a bottle for water collection and the next child was only given a bottle for collection if the water source was different than that of a previous child. Children were asked to collect the sample of water from the source that was used in the initial 10 years of their life and was collected the next day. It was not reported if all bottles were returned. The water samples collected were subjected to water fluoride analysis using an ion-specific electrode, Orion 720A fluoride meter at District Water Works, Nalgonda to confirm the fluoride levels in the water before commencement of clinical examination. LOD and QA/QC details were not reported.
        - *Direction/magnitude of effect***:** There is some potential for exposure misclassification based on recall of the children on the source of water used in

their first 10 years of life. The misclassification is likely non-differential and not likely to bias in any specific direction. Children who had changed water since birth were excluded, but it was not specifically noted that the fluoride in the water source was stable over the years.

- *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using well-established methods that directly measured exposure.

- ***Outcome:***
  - *Rating:* Probably high risk of bias (NR)
  - *Summary:* The Raven's standard progressive matrices (1992 edition) was used to assess IQ. This Raven's test is a standard test and although there is no information provided to indicate that the methods were reliable and valid in the study population, this test was created to be culturally fair (+ for methods). Blinding or other methods to reduce potential bias were not reported (NR for blinding). No response was received to an e-mail request for clarification in September 2017. Overall rating for methods and blinding = NR.
  - *Basis for rating:* Probably high risk of bias based on indirect evidence that the outcome was not assessed blind and could bias the results.
- ***Selective Reporting:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* All outcomes outlined in the abstract, introduction, and methods are reported in sufficient detail.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:*
    - *Statistical analyses:* Chi-square test and Spearman rank correlation were used to assess the association between four different fluoride levels and IQ grades. Area-level exposures were used. Clustering of children within the four areas was not accounted for in the analysis; however, because multiple villages were included in each fluoride exposure level, clustering is less of a concern and the impact on the effect estimates is expected to be minimal.
    - *Other potential concerns:* None identified.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Probably low risk-of-bias ratings in confounding and exposure. Study strengths include verification of exposure measurements and the addressing of potential key confounders, but it was limited by the cross-sectional study design and lack of information on blinding during outcome assessment.

Till *et al.* (2020)

Study Details:

- ***Study design*:** Prospective cohort
- ***Population*:** MIREC participants (pregnant mothers and their children aged 3–4 years)
- ***Study area:*** 10 cities, Canada
- ***Sample size:*** 398 mother−child pairs (247 from non-fluoridated areas, 151 from fluoridated areas; 200 breastfed as infants, 198 formula-fed as infants)
- ***Data relevant to the review:*** Adjusted linear regression models evaluating associations between IQ with water fluoride concentration (with or without adjusting for maternal urine) in formula-fed or breast-fed infants or by fluoride intake from formula.
- ***Reported association with fluoride exposure:*** Yes: Significantly lower performance IQ with water fluoride by breastfeeding status (adjusted βs = −9.26 formula-fed, −6.19 breastfed) and fluoride intake from formula (adjusted β = −8.76); significantly lower full-scale IQ with water fluoride in formula-fed (adjusted β = −4.40); no significant changes in full-scale IQ for water fluoride in breastfed children or fluoride intake from formula-fed children; no significant changes in verbal IQ scores with fluoride exposure.

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Pregnant women were recruited between 2008 and 2011 by the MIREC program from 10 cities across Canada. Inclusion and exclusion criteria were provided. Additional details were stated to be available in Arbuckle *et al.* (2013). A total of 610 children were recruited to participate in the developmental follow-up with 601 children completing all testing. The demographic characteristics of women included in the current analyses (n = 398) were not substantially different from the original MIREC cohort (N = 1945) or the subset without complete water fluoride and covariate data (n = 203). A table of characteristics of the study population is provided. Approximately half of the children lived in nonfluoridated cities and half lived in fluoridated cities.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that the exposed groups were similar and were recruited with the same methods during the same time frame.
- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Covariates were selected a priori that have been associated with fluoride, breast feeding, and children's intellectual ability. Final covariates included child's sex and age at testing, maternal education, maternal race, second-hand smoke in the home, and HOME score. City was considered but was excluded from the models. Confounders that were not assessed include: parental mental health, iodine deficiency/excess, parental IQ, and co-exposure to arsenic and lead. Co-exposure to arsenic is less likely an issue in this Canadian population because the population mainly received water from municipal water supplies that monitor for lead and arsenic, and the lack of information

is not considered to appreciably bias the results. In addition, a previous study on this population (Green *et al.* 2019) conducted sensitivity analyses on co-exposures to lead and arsenic. Results from these sensitivity analyses support that co-exposures to lead and arsenic are not likely a major concern in this study population.

- *Potentially important study-specific confounders:* All key confounders were considered in this study.
  - *Direction/magnitude of effect*: Not applicable.
- *Basis for rating:* Probably low risk of bias based on direct evidence that key confounders were addressed and indirect evidence that the methods used to collect the information were valid and reliable and co-exposures were not an issue.

- ***Attrition:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Of 610 children, 601 (98.5%) in the MIREC developmental study who were ages 3–4 years completed the neurodevelopment testing. Of the 601 children who completed the neurodevelopmental testing, 591 (99%) completed the infant feeding questionnaire and 398 (67.3%) reported drinking tap water. It was noted that the demographic characteristics were not substantially different from the original MIREC cohort or the 203 subjects without complete water fluoride or covariate data.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
- ***Exposure:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Information on breastfeeding was obtained via questionnaire at 30–48 months. Fluoride concentration in the drinking water was assessed by daily or monthly reports provided by water treatment plants. Water reports were first linked with mothers' postal codes and the daily or weekly amounts were averaged over the first 6 months of each child's life. Additional details can be found in Till *et al.* (2018). Maternal urinary exposure was used to assess fetal fluoride exposure. Procedures can be found in Green *et al.* (2019).
    - *Direction/magnitude of effect*: There is not any specific direction or magnitude of bias expected. Urinary fluoride levels are reflective of recent exposure. The possibility of the exposure misclassification would be similar in all subjects and would be non-differential. For the fluoride intake from formula, exposure was based on the fluoride levels in the water at the residence and the proportion of time that the infant was not exclusively breastfed. This exposure misclassification would also be non-differential.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using well-established methods that directly measured exposure.
- ***Outcome:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Intelligence was tested using the Wechsler Preschool and Primary Scale of Intelligence III. This is appropriate for both the study population and age group. This is considered a gold standard test. It was not reported whether the evaluators were blind to the child's fluoride exposure status during the assessment. Although it is unlikely that

the assessors had knowledge of the specific drinking water levels or maternal urine levels, there is potential that the outcome assessors had knowledge of the city the child lived in and if the city was fluoridated or non-fluoridated. Correspondence with the study authors on the outcome assessment for Green *et al.* (2019) indicated that it was unlikely that the testers had knowledge of the city's fluoridation. The same is assumed here. Specific measurements included were identified.

- *Basis for rating:* Probably low risk of bias based on indirect evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.

- ***Selective Reporting:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* All outcomes outlined in the abstract, introduction, and methods were reported in sufficient details.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:*
    - *Statistical analyses:* Regression diagnostics were used to test assumptions for linearity, normality, and homogeneity. There were two potential influential observations (based on Cook's distance), and sensitivity analyses re-estimated the models without these two variables. Effect modification by breastfeeding status was evaluated. Interestingly, all regression coefficients were divided by 2 to represent change in IQ per 0.5-mg/L change in fluoride. One concern is posed by the lack of accounting for city in the regression models, ideally as a random effect. The authors explored including city as a covariate in the models; however, city was not included either because it was strongly multi-collinear with water fluoride concentration (VIF > 20) (model 1, with water fluoride concentration) or because fluoride intake from formula is a function of water fluoride concentration (assessed at the city level) and was therefore deemed redundant (model 2). However, the models use city-level water fluoride concentrations—and, in sensitivity analyses, adjust for maternal urinary fluoride—which warrants exploration of city as a random effect rather than a fixed effect (as would be the case by having it included as a covariate). Even including individual-level maternal urinary fluoride might not fully account for lack of a city effect, given that the subjects were from six different cities, with half of them fully on fluoridated water. Hence, even individual-level exposures are likely to be correlated at the city level. Based on a previous analysis (Green *et al.* 2019), it is unlikely that exclusion of city from models (as a fixed or random effect) would significantly impact the effect estimates.
    - *Other potential concerns:* None identified.
  - *Basis for rating:* Probably low risk if bias based on indirect evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.

- ***Basis for classification as low risk-of-bias study overall:*** Probably low risk-of-bias ratings in confounding, exposure, and outcome. Study strengths include individual exposure measurements, prospective cohort design, and the addressing of potential key confounders.

Trivedi *et al.* (2012)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** Children aged 12−13 years
- ***Study area:*** Kachchh, Gujarat, India
- ***Sample size:*** 84 children
- ***Data relevant to the review:*** Mean IQ scores and distribution by low and high fluoride villages.
- ***Reported association with fluoride exposure:*** Yes: Significantly lower mean IQ score in the high fluoride villages (92.53 ± 3.13) compared to the low fluoride villages (97.17 ± 2.54) in boys and girls combined (and by gender).

Risk of Bias:

- **Author contacts:**
  - Authors were contacted in September of 2017 to obtain additional information for risk-of-bias evaluation.
- ***Population selection:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* There is insufficient information provided on the sampling methods to determine if the populations were similar. Although it was noted that samples were obtained for groundwater quality from March to May of 2011, there is no indication that the children were selected at the same time or during a similar time frame. Correspondence with the author indicates that children were selected within a week of the water collection based on random selection of a school in the village. Study participants were selected from six different villages of the Mundra region of Gujarat, India. Subjects were grouped into high and low villages based on the level of fluoride in the drinking water of those villages. The number of subjects per village were not reported, but it was noted that there were 50 children in the low fluoride group and 34 children in the high fluoride group. It is not clear if the differences in numbers were based on different participation rates or if there were fewer children in the high fluoride villages. Recruitment methods including any exclusion criteria and participation rates were not provided. SES was stated to be low and equal based on questionnaire information, but the results were not provided. It should also be noted that only regular students (having attendance more than 80%) of standard 6^{th} and 7^{th} grades were selected, but it was not noted if attendance varied by village. Correspondence with the study author indicated that there was an average of 20 students per class with an average of 40 students per village. It appears that keeping the requirement for 80% attendance was a limiting factor that caused different numbers of children by area; however, this was applied similarly to both groups.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that subjects were similar and recruited using the same methods during the same time frame.

- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Children were stated to be students of the 6th and 7th standard grades. Age was not addressed, but the children would all be of similar age based on the grades included. Results were reported for males and females separately as well as combined. SES and iodine consumption were stated to be analyzed via a questionnaire and were standardized on the basis of the 2011 census of India. Although it was noted in the abstract that the SES was equal (no data provided), the study report did not mention the iodine results. Although the study authors did not address arsenic or lead, they did provide physicochemical analyses for the water samples from the six different villages. While the authors did not specifically analyze lead or arsenic in the water samples, these physicochemical analyses suggest that differential lead or arsenic exposure were unlikely. Moreover, based on water quality maps, arsenic is not expected to be a major concern in this study area. Based on the information from the water quality maps and the physiochemical analysis of the water provided, there is indirect evidence that neither arsenic nor lead were a concern in this study population.
  - *Potentially important study-specific confounders:* Key confounders age, gender, and measures of SES were similar between exposure groups; however, arsenic was not taken into account. Arsenic often occurs in the drinking water along with fluoride in some Indian populations; however, based on water quality maps, arsenic does not appear to be an issue in the study area.
    - *Direction/magnitude of effect***:** Conceptually, the presence of arsenic would potentially bias away from the null if present with fluoride or toward the null if present in the reference group; however, for reasons noted above, arsenic is not considered a concern in this study population.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the methods used to collect the information were valid and reliable, that potential co-exposures were not an issue, and that key confounders were addressed.
- ***Attrition:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Results were provided for 84 children, but the methods do not indicate how many children were initially selected to participate nor were any exclusion criteria provided. It was noted in the results that 84 children had their groundwater and urine tested, but it was not noted if analyses were restricted to these children or if exposures were assessed in all the children who had IQ measurements. Correspondence with the study author indicated that the main reason for exclusion was a <80% attendance rate, with fluoride and IQ measured on all 84 children who met the criteria.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence of no attrition.
- ***Exposure:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Children in villages were grouped based on fluoride levels that were assessed in groundwater (low F villages versus high F villages). The average concentration of these levels was considered to be the levels in the drinking water with confirmation using urinary fluoride levels. The groundwater samples were selected to cover major parts of the taluka and represent overall groundwater quality. Ten samples were

obtained from each village. Fluoride was measured in the groundwater using ion exchange chromatography. Although urine levels were also significantly higher in the high fluoride village, no information was provided on how or when the urinary samples were obtained or how they were measured. However, correspondence with the study author indicated that the groundwater and urine fluoride levels were available for all 84 children indicating that the urine measures were available for the children that had IQ measures. The urine samples were stated to be collected at the same time that the second water sample was collected.

        - *Direction/magnitude of effect*: Fluoride levels were measured in both the drinking water and urine. Although there is some variability in the measurements, there is no overlap between the two groups and the urine and drinking water levels in the children support each other. Any potential exposure misclassification would be non-differential and direction and magnitude are unknown.
    - *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using well-established methods that directly measured exposure.
- ***Outcome:***
    - *Rating:* Probably low risk of bias (+)
    - *Summary:* Outcome methods were only noted to be reported in Trivedi *et al.* (2007), which was scored as follows: IQ was measured in the children of both areas using a questionnaire prepared by Professor JH Shah, copyrighted by Akash Manomapan Kendra, Ahmedabad, India, and standardized on the Gujarati population with 97% reliability rate in relation to the Stanford-Binet Intelligence Scale (+ for methods). Blinding or other methods to reduce bias are not reported, but correspondence with the study author indicated that the teachers were blind to the status of fluoride. The teachers administered the tests in the presence of a research fellow. It is not completely clear who scored the tests, but it is assumed the teachers. (+ for blinding). Overall rating for methods and blinding = +.
    - *Basis for rating:* Probably low risk of bias based on indirect evidence that the outcomes were blindly assessed using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
    - *Rating:* Definitely low risk of bias (++)
    - *Summary:* All outcomes outlined in the abstract, introduction, and methods are reported in sufficient detail.
    - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
    - *Rating:* Probably high risk of bias (-)
    - *Summary:*
        - *Statistical analyses:* Mean IQ scores in low and high fluoride villages were compared using a t-test. Consideration of heterogeneity of variances was not reported. Results are reported as means and standard errors of the means, with p-values for significant differences. Area-level exposures were used. There was no accounting for clustering of children within the villages, and comparative

analyses did not account for potential confounders. Urinary fluoride was not considered in the comparative analyses. The lack of individual exposure levels and the lack of accounting for clustering are likely to bias the standard error of the difference in mean IQ levels between the high and low fluoride villages and make the differences appear stronger than they actually are.

- *Basis for rating:* Probably high risk of bias based on indirect evidence that the statistical analyses did not account for clustering, and this lack of accounting could bias the association. There were no other potential threats to risk of bias identified.

- ***Basis for classification as low risk-of-bias study overall:*** Probably low risk-of-bias ratings in confounding, exposure, and outcome. Study strengths include individual exposure measurements and the addressing of potential key confounders, but the study was limited by the cross-sectional study design. Another limitation of the study was lack of accounting for clustering, which may bias the standard error of the differences making the effect appear stronger than it actually is; however, this does not change the nearly 5-point difference in IQ score between the two villages.

Wang *et al.* (2012)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** Children aged 8–13 years (possibly the same study population as Xiang *et al.* (2003a))
- ***Study area:*** Wamiao and Xinhuai villages located in Sihong County, Jiangsu Providence, China
- ***Sample size:*** 526 school children
- ***Data relevant to the review:*** Mean IQ and % low IQ (< 80) by total fluoride intake.
- ***Reported association with fluoride exposure:*** Yes: Significantly lower mean IQ in the endemic versus non-endemic regions, as reported in Xiang *et al.* (2003a); when high exposure group was broken into 4 exposure groups based on fluoride intake, a dose-dependent decrease in IQ and increase in % with low IQ observed; significant correlation between total fluoride intake and IQ ($r = -0.332$); for IQ<80, adjusted OR of total fluoride intake was 1.106 (95% CI: 1.052, 1.163).

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* The study appears to be the same study population as Xiang *et al.* (2003a) and Xiang *et al.* (2011); however, the study does not cite these studies as providing additional information and numbers of children differ; therefore, it may be a separate analysis on the same villages. The years of testing were not provided so it cannot be determined if study subjects are the same. Two villages, Wamiao and Xinhuai, located 64 km apart in Sihong County, Jiangsu Province were selected for the study. Wamiao is a village in a region with severe endemic fluorosis and Xinhuai is a village in a non-endemic fluorosis region. Neither village has fluoride pollution from coal or industrial sources. Villages were stated to be similar in terms of annual per capita income,

transportation, education, medical conditions, the natural environment, and lifestyle. All primary students ages 8–13 years currently in school in either village were surveyed with exclusions noted. Of 243 children from Wamiao, 236 (97.12%) were included, and of 305 children from Xinhuai, 290 (95.08%) were included. No table of subject characteristics was provided.

- *Basis for rating:* Probably low risk of bias based on indirect evidence that the exposure groups were similar and were recruited using the same methods within the same time frame, with direct evidence that there was no difference in participation/response rates.

- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Logistic regression of low IQ rate and total fluoride intake adjusted for age and sex. Both villages had hand-pumped well water for drinking water, but the authors do not mention if arsenic was also present in the drinking water. However, a publication by Xiang *et al. (*2013) on this study area indicates that Xinhuai (the low fluoride area) had significantly higher arsenic levels compared to Wamiao (the endemic fluorosis area), which would bias toward the null. Areas were stated to be similar in annual per capita income, transportation, education, medical conditions, the natural environment, and lifestyle; however, no details were provided. This study did not address other co-exposures, but other studies on populations in these villages (Xiang *et al.* 2011, Xiang *et al.* 2003a) indicate that iodine and lead are not concerns.
  - *Potentially important study-specific confounders:* Arsenic often occurs in the drinking water along with fluoride in some Chinese populations; however, based on information provided in Xiang *et al.* (2013), arsenic concentrations were higher in the low fluoride area compared to the high fluoride area. Because there were significant effects on IQ observed in the high fluoride areas, the impact of co-exposure to arsenic is less of a concern. The presence of arsenic in the control village may cause an underestimation of the effect of fluoride, and despite this potential impact, there was still a significant association between fluoride exposure and IQ.
    - *Direction/magnitude of effect***:** Presence of arsenic in this study population would potentially bias toward the null.
  - *Basis for rating:* Probably low of risk bias because there is indirect evidence that the key confounders are take into account, methods used for collecting the information were valid and reliable, and co-exposures to arsenic and lead and iodine deficiency are not attributing to the effect observed in this area. The potential bias toward the null combined with the reporting of an effect increases confidence that there is an effect.

- ***Attrition:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Data are reported for all 526 children noted to be included in the study. There is a slight discrepancy in the reported total number of children from the high-fluoride village and the number of participants from the high-fluoride village between this paper (236 participated of 243 total children) and the 2003 and 2011 publications on the same study population (222 of 238). This discrepancy is not explained but is not expected to appreciably bias the results.

- *Basis for rating:* Probably low risk of bias based on indirect evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.

- ***Exposure:***
  - *Rating:* Probably low risk of bias (+); Probably high risk of bias (-)
  - *Summary:* **Water fluoride (+ probably low risk of bias):** Exposure was based on drinking water levels and fluoride intake. Residents in the Wamiao village were divided into five groups based on fluoride levels in the drinking water. Clean, dry polyethylene bottles were used to collect 50 mL of drinking water from each student's household and fluoride content was measured.

    Total fluoride intake (- probably high risk of bias): Six families from each of the five Wamiao groups were randomly selected as dietary survey households. Intakes of various foods by each person at each meal and intakes of unboiled water, boiled water, and tea were surveyed for four consecutive days. Methods for food collection were described. Five representative households from each village were selected based on geographic location, population distribution, housing structure, and other conditions. Indoor air samples were collected once daily for five consecutive days; outdoor air was sampled at two points once daily for five days. Methods for determining fluoride content in samples were noted to follow specific guidelines. Calculation of total fluoride intake was stated to follow Appendix A of the People's Republic of China Health Industry Standard with some details provided. Although it is assumed the method is valid, it was not detailed how each fluoride determination was made for each subject, and it appears that total fluoride intake was determined based on data from select subjects and not all subjects.
    - *Direction/magnitude of effect***:** There is potential for exposure misclassification based on calculating fluoride intake based on measurements from a few select subjects rather than all subjects. The direction and magnitude of effect cannot be assessed based on the information provided.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using well-established methods that directly measured exposure. The intake is probably high risk of bias because there is indirect evidence that the exposure was assessed using methods of unknown validity.

- ***Outcome:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* IQ of each child was measured with the Combined Raven's Test for Rural China (CRT-RC) (++ for methods). The test was stated to be administered to the children independently in a school classroom under the supervision of three exam proctors. Testing methods, testing language, and testing conditions were all in strict accordance with the CRT-RC guidebook. Major testing personnel received necessary training by the Psychology Department of East China Normal University. The children undergoing IQ testing and the test scorers were kept double-blinded throughout the testing process. (++ for blinding). Overall rating= ++.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.

- ***Selective Reporting:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* All outcomes outlined in the abstract, introduction, and methods are reported in sufficient detail.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:*
    - *Statistical analyses:* Logistic regression analysis was used to determine the odds of having low IQ with increasing fluoride intake. Analyses and methods are not well described. There is no mention of what tests were used for the mean IQ comparison by village; however, statistical software (SPSS) was used, suggesting appropriate tests were applied. Simple linear regression analyses were conducted to evaluate associations between total fluoride intake and children's IQ or low IQ rate. There is no evidence that regression diagnostics were used to test model assumptions for linearity, normality, and homogeneity. Clustering at the village level was not accounted for in the analyses. The overall impact of these factors on effect estimates is expected to be minimal given the use of individual-level data and adjustment for potential confounders.
    - *Other potential concerns:* None identified.
  - *Basis for rating:* Probably low risk if bias based on indirect evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Definitely or probably low risk-of-bias ratings in confounding, exposure, and outcome. Study strengths include individual exposure measurements with blinding at outcome assessment but is limited by the cross-sectional study design and not using individual measurements to calculate fluoride intake. All key confounders were accounted for in the study design or analysis, but there is potential for the presence of arsenic to bias toward the null.

Wang *et al.* (2020b)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** School children aged 7–13 years
- ***Study area:*** Tianjin City, China (possibly a subset of the children from Yu *et al.* (2018))
- ***Sample size:*** 571 school children
- ***Data relevant to the review:*** IQ scores by urine and water fluoride levels.
- ***Reported association with fluoride exposure:*** Yes: Significant associations between IQ score and water fluoride (adjusted β = −1.587 per 1-mg/L increase) and urinary fluoride (adjusted β = −1.214 per 1-mg/L increase) in boys and girls combined based on both quartiles and continuous measures. No significant modification effect of gender.

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Subjects were from a cross-sectional study conducted in 2015, but no citation was provided on this cohort (presumably the Yu *et al.* (2018) cohort). It was noted that the subjects in that cohort were from districts with historically high or normal fluoride levels. Subjects for this study were selected by using a stratified and multistage random sampling approach. Brief description was provided. The study area consisted of three historically high fluoride areas and four nonendemic areas. A flow diagram was provided for inclusion and exclusion, but this detail was given for all children and not by area. Therefore, it cannot be determined if the participation differed by area. However, there was a 93% recruitment rate, and the 13 excluded due to missing data are not likely excluded due to exposure. Detailed characteristics of the study population are provided. Exclusion criteria included: "children who had congenital or acquired diseases affecting intelligence, or a history of cerebral trauma and neurological disorders, or those with a positive screening test history (like hepatitis B virus infection, Treponema palladium infection and Down's syndrome) and adverse exposures (smoking and drinking) during maternal pregnancy, prior diagnosis of thyroid disease, and children who had had missing values of significant factors (2.2%) were also excluded."
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that the exposed groups were recruited using similar methods during the same time frame and that any differences between the exposed groups were accounted for in the statistical analyses.
- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Study authors noted that the study areas are not exposed to other neurotoxins such as lead, arsenic, or mercury nor were they iodine-deficient. Final models included child's age, child's gender, child's BMI, maternal and paternal education, household income, and low birth weight. Other potential confounders that were considered is unclear as they only noted that the confounders were selected based on current literature. Reasons for exclusion included history of disease affecting intelligence, history of trauma or neurological disorders, positive screening test history, or exposures such as smoking or drinking during pregnancy. Information was obtained by questionnaire or measurements. Variables such as parental BMI, behavioral and mental health disorders, IQ, and quantity and quality of the caregiving environment were not addressed.
  - *Potentially important study-specific confounders:* All key confounders were considered in this study.
    - *Direction/magnitude of effect***:** Not applicable.
  - *Basis for rating:* Probably low risk of bias because there is direct evidence that the key confounders are taken into account, indirect evidence that the methods for collecting the information were valid and reliable, and co-exposure to arsenic is not an issue in this area.

- ***Attrition:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* A detailed chart of the recruitment process is presented. The study had a 93% recruitment rate and only 2.2% of subjects with missing data for certain covariates were excluded.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
- ***Exposure:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Children provided spot urine samples, presumably at the time of examination. Water samples were randomly collected from public water supplies in each village. Fluoride concentrations were analyzed using fluoride ion-selective electrode according to the national standardized method in China. There is no indication if the urine samples accounted for dilution.
    - *Direction/magnitude of effect***:** Not accounting for dilution could cause there to be some exposure misclassification. The direction and magnitude would depend on where the differences occurred.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using acceptable methods that provide individual levels of exposure.
- ***Outcome:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Assessments of IQ scores were conducted by graduate students at the School of Public Health, Tongji Medical College at the Huazhong University of Science and Technology. Each team member was assigned a single task, meaning that only one person would have conducted the IQ tests. A Combined Raven's Test for Rural China was used. Therefore, the test was appropriate for the study population (++ for method). It was note that the examiner was trained and blind to the exposure (++ for blinding). Overall = ++
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* All outcomes in the abstract, introduction, and methods are reported in sufficient details.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:*
    - *Statistical analyses:* Logistic and multivariate regression models accounting for potential confounders were used. Results are presented as betas or ORs and 95% CIs. Regression diagnostics were conducted for all models, including

examination of multicollinearity, heteroscedasticity, and influential observations. Mediation and interaction analyses were appropriate. There is no evidence that the stratified and multistage random sampling approach for subject selection was accounted for in the analyses by using sampling weights or accounting for clustering using random effect models; however, selected villages are similar in population and general demographic characteristics. Given the use of individual-level data and adjustment for potential confounders, the impact on the regression coefficients is likely to be minimal.
        - *Other potential concerns:* None identified.
    - *Basis for rating:* Probably low risk of bias based on indirect evidence that the statistical analyses were appropriate and no other potential threats to risk of bias were identified.
- ***Basis for classification as low risk-of-bias study overall:*** Definitely or probably low risk-of-bias ratings in confounding, exposure, and outcome. Study strengths include individual exposure measurements but is limited by the cross-sectional study design and lack of accounting for urine dilution. All key confounders were considered in the study design or analysis.

Xiang *et al.* (2003a)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** Children aged 8–13 years
- ***Study area:*** Wamiao and Xinhuai villages located in Sihong County, Jiangsu Providence, China
- ***Sample size:*** 512 school children
- ***Data relevant to the review:*** Comparison of IQ (mean and distribution) between Wamiao County (a severe endemic fluorosis area) and Xinhuai County (non-endemic fluorosis area); additional breakdown of the Wamiao area into 5 water fluoride exposure groups.
- ***Reported association with fluoride exposure:*** Yes: Significantly lower IQ scores observed with water fluoride levels of 1.53 mg/L or higher. Percent of subjects with IQ scores below 80 was significantly increased at water fluoride levels of 2.46 mg/L or higher. Significant inverse correlation between IQ and urinary fluoride (Pearson correlation coefficient −0.164). Mean IQ scores for children in the non-endemic region (100.41 ± 13.21) were significantly higher than the endemic region (92.02 ± 13.00).

Risk of Bias:

- **Author contacts:**
    - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
    - *Rating:* Probably low risk of bias (+)
    - *Summary:* Two villages, Wamiao and Xinhuai, located 64 km apart in Sihong County, Jiangsu Province were selected for this study, which was conducted between September and December 2002. Wamiao is located in a severe fluorosis endemic area, and Xinhuai is located in a non-endemic fluorosis area. Neither village has fluoride pollution from burning coal or other industrial sources. All eligible children in each village were included; children who had been absent from either village for 2 years or longer or who had a history of brain disease or head injury were excluded. In Wamiao, 93% of the

children (222 out of 238) were included for the study; in Xinhuai, 95% were included (290 out of 305). The children in Wamiao were divided into five subgroups according to the level of fluoride in their drinking water: <1.0 mg/L (group A), 1.0–1.9 mg/L (group B), 2.0–2.9 mg/L (group C), 3.0–3.9 mg/L (group D), and >3.9 mg/L (group E). Children in Xinhuai (0.18–0.76 mg F/L in the drinking water) served as a control group (group F). Demographic characteristics are not presented, and statistical analyses are not adjusted, but mean IQ scores are stratified by child's age, child's gender, family income, and parental education.

- *Basis for rating:* Probably low risk of bias based on indirect evidence that the exposure groups were similar and were recruited using the same methods within the same time frame, with direct evidence that there was no difference in participation/response rates.

- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Although information was stated to be collected on personal characteristics, medical history, education levels of the children and parents, family SES, and lifestyle, only child's gender, child's age, family income, and parental education were addressed. Other potential co-exposures, such as arsenic, were not addressed. A separate publication in 2003 [(Xiang *et al.* 2003b), letter to the editor], indicated that blood lead levels were not significantly different between the two areas. Although arsenic was not addressed specifically in this publication, Xiang *et al.* (2013) measured both fluoride and arsenic in the Wamiao and Xinhuai areas. Xinhuai (the low fluoride area) had significantly higher arsenic levels compared to Wamiao (the endemic fluorosis area). This is likely to bias toward the null; however, the study observed a significantly lower IQ score in the endemic fluorosis area. Iodine was tested in a subset of the children and found not to be significantly different between the two groups.
  - *Potentially important study-specific confounders:* Arsenic often occurs in the drinking water along with fluoride in some Chinese populations; however, based on information provided in Xiang *et al.* (2013), arsenic concentrations were higher in the low fluoride area compared to the high fluoride area. Because there were significant effects on IQ observed in the high fluoride areas, the impact of co-exposure to arsenic is less of a concern. The presence of arsenic in the control village may cause an underestimation of the effect of fluoride, and despite this potential impact, there was still a significant association between fluoride exposure and IQ.
    - *Direction/magnitude of effect***:** Presence of arsenic in this study population would potentially bias towards the null.
  - *Basis for rating:* Probably low risk of bias because there is indirect evidence that the key confounders are taken into account, methods used for collecting the information were valid and reliable, and co-exposures to arsenic and lead and iodine deficiency are not attributing to the effect observed in this area. The potential bias toward the null combined with the reporting of an effect increases confidence that there is an effect.

- ***Attrition:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Data are complete. IQ results were reported for all 512 children included in the study (222 in the endemic area and 290 in the nonendemic area).

- *Basis for rating:* Definitely low risk of bias based on direct evidence that there was no attrition.

- ***Exposure:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Exposure was based on drinking water and urinary levels of fluoride. The two study areas were selected to reflect a severe endemic area and a nonendemic area. Drinking water was collected from wells and early-morning spot urine samples were collected from a randomly-selected subsample of children. Both water and urine samples were measured using fluoride ion-selective electrode, but no quality control was discussed. Both absolute and creatinine-adjusted urine results were reported.
    - *Direction/magnitude of effect***:** There is potential for exposure misclassification because only current levels were assessed. Migration of subjects in or out of the area was not assessed, but the study authors noted that, if the children had been absent from the village for 2 or more years, they were excluded. Misclassification would likely be non-differential, which could bias the results in either direction.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using well-established methods that directly measured exposure.
- ***Outcome:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* The IQ of each child was measured with the Combined Raven's Test for Rural China (CRT-RC) (++ for methods). The test was stated to be administered to the children independently in a school classroom, in a double-blind manner, under the supervision of an examiner and two assistants, and in accordance with the directions of the CRT-RC manual regarding test administration conditions, instructions to be given, and test environment. (++ for blinding). Overall rating= ++
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* All outcomes outlined in the abstract, introduction, and methods are reported in sufficient detail.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:*
    - *Statistical analyses:* There is no mention of the tests conducted, but data were stated to be analyzed using SAS suggesting appropriate tests were applied. Results provided in the tables indicate that t-tests comparing IQ values between the villages (overall and by gender) were conducted, but it was not reported that heterogeneity of variance was assessed. In addition, correlations between IQ and age, family income, and parents' education level were tested with Pearson's correlation. There is no evidence that a test for trend was conducted

to evaluate the stated "significant inverse concentration-response relationship between the fluoride level in drinking water and the IQ of children."

- A potential concern raised by the NASEM (2020) peer review was the lack of accounting for relationships in exposure between persons from the same village. Given only two villages were included and the analyses consisted of village-level comparisons (no use of individual-level covariate data), it is likely that the standard error of the difference in mean IQ between fluoride in water exposure groups will be biased, making differences appear stronger than they actually are. Without controlling for village effects and given the large differences in fluoride concentrations and IQ levels between villages, the apparent dose-response relationship could be due to a village effect in addition to a fluoride effect. However, the dose-response relationship is apparent within the "exposed" village, diminishing the concern for a village-only effect and likely minimizing the impact on the effect estimates.
- *Other potential concerns:* None identified.

- *Basis for rating:* Probably low risk of bias based on indirect evidence that statistical analyses were appropriate and that there were no other threats to risk of bias.

- ***Basis for classification as low risk-of-bias study overall:*** Definitely or probably low risk-of-bias ratings in confounding, exposure, and outcome. Study strengths include individual exposure measurements and outcomes blindly assessed but is limited by the cross-sectional study design and lack of accounting for urine dilution. All key confounders were considered in the study design or analysis, but there is potential for the presence of arsenic to bias toward the null.

Xiang *et al.* (2011)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** Children aged 8–13 years (same study population as Xiang *et al.* 2003a)
- ***Study area:*** Wamiao and Xinhuai villages located in Sihong County, Jiangsu Providence, China
- ***Sample size:*** 512 school children
- ***Data relevant to the review:*** Mean IQ scores and odds ratio for having an IQ < 80 presented by serum fluoride quartiles.
- ***Reported association with fluoride exposure:*** Yes: Significant linear trend across quartiles of serum fluoride and children's IQ score < 80 (adjusted ORs for Q1 and Q2; Q1 and Q3; and Q1 and Q4, respectively: 1; 2.22 [95% CI: 1.42, 3.47]; and 2.48 [95% CI: 1.85, 3.32]); significant effects observed at ≥ 0.05 mg/L serum fluoride.

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* The study population is the same as that was used in the Xiang *et al.* (2003a) study, but a few more measurements were available and different analyses were conducted. The comparison population is considered the same as previously based on

the study populations being recruited from similar populations, using similar methods, during the same time frame. Demographic characteristics were not provided.

- *Basis for rating:* Probably low risk of bias based on indirect evidence that the exposure groups were similar and were recruited using the same methods within the same time frame, with direct evidence that there was no difference in participation/response rates.

- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* As was noted in the 2003 publication, information was collected on personal characteristics, medical history, education levels in the children and parents, family SES, and lifestyle. In the logistic regression model, age and gender were adjusted in the analysis. In the previous report, no significant associations were observed between groups for family income and parents' education. Urinary iodine and blood lead levels were also stated to be measured and were noted not to be significantly different between the groups. Although the iodine levels were reported in the previous publication, the lead levels were not reported nor were the methods. Lead information is reported in a letter to the editor (Xiang *et al.* 2003b) and was not significantly different between the areas. Although arsenic was not addressed specifically in this publication, Xiang *et al.* (2013) measured both fluoride and arsenic in the Wamiao and Xinhuai areas. Xinhuai (the low fluoride area) had significantly higher arsenic levels compared to Wamiao (the endemic fluorosis area). This is likely to bias toward the null; however, the study observed a significantly lower IQ score in the endemic fluorosis area and with increasing serum fluoride.
  - *Potentially important study-specific confounders:* Arsenic often occurs in the drinking water along with fluoride in some Chinese populations; however, based on information provided in Xiang *et al.* (2013), arsenic concentrations were higher in the low fluoride area compared to the high fluoride area. Because there were significant effects on IQ observed in the high fluoride areas, the impact of co-exposure to arsenic is less of a concern. The presence of arsenic in the control village may cause an underestimation of the effect of fluoride, and despite this potential impact, there was still a significant association between fluoride exposure and IQ.
    - *Direction/magnitude of effect***:** Presence of arsenic in this study population would potentially bias toward the null.
  - *Basis for rating:* Probably low of risk bias because there is indirect evidence that the key confounders are taken into account, methods used for collecting the information were valid and reliable, and co-exposures to arsenic and lead and iodine deficiency are not attributing to the effects observed in this area. The potential bias toward the null combined with the reporting of an effect increases confidence that there is an effect.
- ***Attrition:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Data are reported for all 512 children noted to be included in the study.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that there was no attrition.
- ***Exposure:***
  - *Rating:* Probably low risk of bias (+)

- *Summary:* Fluoride levels were measured in serum with a fluoride ion-selective electrode. A fasting venous blood sample was used. No details are provided on validation (including correlation with drinking water levels) or QA. Children who did not reside in their village for at least 2 years were excluded. Results were provided in quartiles, but they combined the lower two quartiles. After combining the two lower quartiles into one, the exposure levels ranged from <0.05 mg/L (Q1 + Q2) to >0.08 mg/L (Q4).
  - *Direction/magnitude of effect***:** Serum fluoride may not be the best estimate for exposure. There is potential for exposure misclassification because only current levels were assessed. Migration of subjects in or out of the area was not assessed, but the study authors noted that, if the children had been absent from the village for 2 or more years, they were excluded. Misclassification would likely be non-differential, which could bias results in either direction.
- *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using well-established methods that directly measured exposure.

- ***Outcome:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* IQ was assessed as part of the 2003 evaluation. IQ was measured with the Combined Raven's Test for Rural China which is appropriate for this population (++ for methods). Although this study does not provide details, the original study article from 2003 provides specific details. The study authors indicate in the 2003 publication that the tests were conducted in a double-blind manner and these are the same results and population (++ for methods). Overall rating=++
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* All outcomes outlined in the abstract, introduction, and methods are reported in sufficient detail.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:*
    - *Statistical analyses:* Statistical analyses conducted were appropriate for the study. Chi square tests were used to compare categorical variables, and multiple logistic regression was used to evaluate the association between serum fluoride levels and risk of low IQ. A potential concern raised by the NASEM (2020) peer review was the lack of accounting for relationships in exposure between persons from the same village. Although only two villages were included, in the analyses which consisted of village-level comparisons it is likely that the standard error of the difference in mean IQ between villages will be biased. This is less of a concern for the mean IQ comparisons across quartiles of serum fluoride levels, and for the logistic regression analyses of risk of low IQ and

individual-level serum fluoride levels. Without controlling for village effects and given the large differences in fluoride concentrations and IQ between villages, the apparent dose-response relationship could be due to a village effect in addition to a fluoride effect. However, the dose-response relationship is still present within the "exposed" village, diminishing the concern for a village-only effect and likely minimizing the impact on the effect estimates.

        - *Other potential concerns:* None identified.
    - *Basis for rating:* Probably low risk if bias based on indirect evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Definitely or probably low risk-of-bias ratings in confounding, exposure, and outcome. Study strengths include individual exposure measurements with blinding at outcome assessment but is limited by the cross-sectional study design and use of serum concentrations. All key confounders were considered in the study design or analysis, but there is potential for the presence of arsenic to bias toward the null.

Yu *et al.* (2018)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** Children aged 7–13 years
- ***Study area:*** Tianjin City, China
- ***Sample size:*** 2,886 school children
- ***Data relevant to the review:*** IQ for normal (≤ 1 mg/L) versus high (> 1 mg/L) water fluoride; betas for IQ score by water and urine fluoride groupings; ORs by IQ category using water and urine fluoride levels.
- ***Reported association with fluoride exposure:*** Yes: Significant difference in mean IQ scores in high water fluoride areas (>1.0 mg/L; 106.4 ± 12.3 IQ) compared to the normal water fluoride areas (≤1.0 mg/L; 107.4 ± 13.0) water fluoride areas. Distribution of IQ scores was also significantly different (p = 0.003). Every 0.5-mg/L increase in water fluoride (between 3.40 and 3.90 mg/L) was associated with a 4.29 lower IQ score (95% CI: −8.09, −0.48).

Risk of Bias:

- **Author contacts:**
    - Authors were contacted in September 2018 to obtain additional information for the risk-of-bias evaluation.
- ***Population selection:***
    - *Rating:* Definitely low risk of bias (++)
    - *Summary:* School children (2,886), aged 7–13 years, were recruited from the rural areas of Tianjin City, China. After exclusion, 1,636 children were assigned to the "normal-fluoride" exposure group and 1,250 were assigned to the "high-fluoride" exposure group based on a cut-off water fluoride level of 1.0 mg/L. A multi-stage random sampling technique, stratified by area, was performed to select representative samples among local children who were permanent residents since birth. Detailed characteristics of the study population are provided. Exclusion criteria included: 1) children who had

congenital or acquired diseases affecting intelligence, 2) children with a history of cerebral trauma and neurological disorders, 3) children with a positive screening test history (like hepatitis B virus infection, Treponema palladium infection and Down's syndrome), and 4) children with adverse exposures (smoking and drinking) during maternal pregnancy. A table of characteristics was provided by fluoride level with differences adjusted in the analysis.

- *Basis for rating:* Definitely low risk of bias based on direct evidence that the exposed groups were recruited using similar methods during the same time frame and that any differences between the exposed groups were considered in the statistical analyses.

- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Demographic data were collected by trained investigators during a face-to-face interview with the recruited children and their parents. Questionnaires were not stated to be validated. The developmental status of the children was further assessed by calculation of BMI, and all measurements were conducted by nurses based on recommended standard methods. Variables that presented differential distribution between the normal-fluoride and high-fluoride exposure groups were adjusted in the linear regression analysis of IQ data and included age, sex, paternal and maternal education levels, and low birth weight. Children exposed to smoking in utero were excluded from the study. Sensitivity analyses were conducted by modifying covariates adjusted in multivariable models among demographics (age and sex); development (BMI); socioeconomics (maternal education, paternal education, and household income); history of maternal disease during pregnancy (gestational diabetes, malnutrition, and anemia); and delivery conditions (hypoxia, dystocia, premature birth, post-term birth, and low birth weight). None of the study sites selected were in areas endemic for iodine deficiency disorders nor were other potential neurotoxins like lead, arsenic, and mercury present. Variables such as parental BMI and behavioral and mental health disorders were not addressed.
  - *Potentially important study-specific confounders:* All key confounders were considered in this study.
    - *Direction/magnitude of effect***:** Not applicable.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that methods of obtaining the information were valid and reliable and direct evidence that all key confounders and co-exposures were addressed.
- ***Attrition:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* There were 1,636 children assigned to the "normal-fluoride" exposure group based on water fluoride, and 1,250 children were assigned to the "high-fluoride" exposure group. Exclusion from the original group of 2,886 children was adequately described. A total of 2,380 children provided urine samples. There is no indication that the data presented excludes any additional children or urine samples, but results do not indicate a sample size for all results.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.

- ***Exposure:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* According to the annual surveillance data from the CDC, the drinking water sources and water fluoride concentrations in each village had remained at stable levels over the past decade. During the investigation, water samples were collected randomly from the public water supplies in each village. Spot (early-morning) urine samples from every child and water samples from each village were collected in pre-cleaned, labeled polythene tubes and transported to the lab within 24 hours while frozen. Samples were stored at −80°C until analysis. Concentrations of fluoride ions (mg/L) were analyzed using the national standardized ion-selective electrode method in China; the detection limit was 0.01 mg/L. Samples were diluted with an equal volume of total ionic strength adjusted buffer (TISAB) of pH 5–5.5 for optimal analysis. Double-distilled deionized water was used throughout the experiment. There is no reporting of any QC methods.
    - *Direction/magnitude of effect***:** Spot urine samples may lead to non-differential exposure misclassification. The large population size likely dilutes any potential effects of occasional misclassification. Because the drinking water sources of fluoride had been noted to be stable for the past decade and the children were 13 years or younger, there would only be exposure misclassification if there was a lot of migration between areas.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using well-established methods that directly measured exposure.
- ***Outcome:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* IQ scores were measured using the second edition of Combined Raven's Test-The Rural in China (CRT-RC2) for children aged 7–13 years (++ for methods). The test was completed by each participant within 40 minutes according to the instruction manual. For each test, 40 children were randomly allocated to one classroom to take the test independently under the supervision of four trained professionals. There is no mention of whether the evaluators were blinded to the fluoride group of each child (normal vs. high fluoride) or whether there were steps taken to ensure consistency in scoring across the evaluators. It is also not clear if the 40 children randomly assigned to the classroom were specific to the village or if a local center was used. Correspondence with the study authors indicated that the four professionals worked together throughout the examination without knowledge of the child's fluoride exposure (++ for blinding).
  - *Basis for rating:* Definitely low risk of bias based on the direct evidence that the outcome was blindly assessed using instruments that were valid and reliable.
- ***Selective Reporting:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* All outcomes outlined in the abstract, introduction, and methods are reported in sufficient detail.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
  - *Rating:* Probably low risk of bias (+)

- *Summary:*
  - *Statistical analyses:* Statistical analyses used were appropriate for the study. Univariate and multivariable piecewise linear regression models were used to estimate the associations between water fluoride or urinary fluoride levels and IQ scores. Multiple logistic regression analysis was used to evaluate the association between water or urinary fluoride levels and IQ degree using the normal intelligence group as the control. Sensitivity analyses were conducted. There is no evidence that residual diagnostics were used to examine model assumptions or that the complex sampling design (stratified multistage random sampling) was accounted for in the analysis using sampling weights and adjustment for clustering. The impact of these factors on the effect estimates is expected to be minimal given the use of individual-level data and adjustment for and numerous potential confounders.
  - *Other potential concerns:* None identified.
- *Basis for rating:* Probably low risk if bias based on indirect evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.

- ***Basis for classification as low risk-of-bias study overall:*** Definitely or probably low risk-of-bias ratings in confounding, exposure, and outcome. Study strengths include individual exposure measurements with blinding at outcome assessment but is limited by the cross-sectional study design and lack of accounting for urine dilution. All key confounders including potential co-exposures were considered in the study design or analysis.

Zhang *et al.* (2015b)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** Children aged 10–12 years
- ***Study area:*** Tianjin City, China
- ***Sample size:*** 180 children
- ***Data relevant to the review:*** IQ by control and high fluoride groups; IQ correlations with water, serum, or urinary fluoride levels; betas for IQ with urinary fluoride levels (by genotypes)
- ***Reported association with fluoride exposure:*** Yes: S Significant correlation between IQ score and children's serum fluoride (r = –0.47) and urinary fluoride (r = –0.45); significant difference in mean IQ score for high-fluoride area (defined as >1 mg/L in drinking water; 102.33 ± 13.46) compared with control area (<1 mg/L; 109.42 ± 13.30).

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary*: Subjects were similar and recruited during the same time frame using the same methods. Authors recruited schoolchildren from a high fluoride area (1.40 mg/L) and a control area (0.63 mg/L) in Tianjin City, China. In accordance with the principles of

matching social and natural factors such as educational standard, economic situation, geological environments as much as possible, two areas with different fluoride concentrations in the groundwater were selected by a stratified cluster random sampling of this region. A total of 180 5th grade children aged 10 to 12 years from two primary schools located 18 km apart in the Jinnan District were recruited—Gegu Second Primary School (from an endemic fluorosis area) and Shuanggang Experimental Primary School (from a non-endemic fluorosis area). The areas are not affected by other drinking water contaminants, such as arsenic or iodine. All subjects were unrelated ethnic Han Chinese and residents in Tianjin with similar physical and mental health status. The authors excluded subjects with known neurological conditions including pervasive developmental disorders and epilepsy. Descriptive statistics of the study population are presented by exposure group in Table 1 of the study. A number of potential differences are taken into account in the statistical analyses.

- *Basis for rating*: Definitely low risk of bias based on direct evidence that the exposure groups were similar and recruited using similar methods during the same time frame.

- **Confounding:**
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Covariates included in the statistical models were child's age, child's gender, educational levels of parents, drinking water fluoride (mg/L), and levels of thyroid hormones (T3, T4, and TSH). Authors report that the study areas are not affected by other contaminants such as arsenic or iodine and residents were of similar physical and mental health status. Other important confounders (maternal demographics, smoking, reproductive health) were not considered. Covariate data were obtained from a study questionnaire.
  - *Potentially important study-specific confounders:* All key confounders were considered in this study.
    - *Direction/magnitude of effect*: Not applicable.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the methods used to collect the information were valid and reliable and direct evidence that key confounders including potential co-exposures were addressed.
- ***Attrition:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Results are complete for the 180 children selected for the study.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that there was no attrition.
- ***Exposure:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Drinking water samples (10 mL) were collected from the tube wells of each child's household. Three fasting venous blood samples were also collected. Urine samples were collected in the early morning before breakfast. Fluoride contents in drinking water (W-F), serum (S-F), and urine (U-F) were measured using an ion analyzer EA940 with a fluoride ion-selective electrode (Shanghai constant magnetic electronic technology Co, Ltd, China) according to the China standard GB 7484-87. All reference solutions for the fluoride determinations were double-deionized water. Parallel samples were set for determination and averages were taken. The quantitation limits of this

method for W-F, S-F, and U-F were 0.2, 0.012, and 0.5 mg/L, respectively. Recovery rates for this method were in the range of 94.3%–106.4%. The intra- and inter-assay coefficients of variation for fluoride were 2.7% and 6.7%, respectively. Dilution of the urinary fluoride was not addressed.
        - *Direction/magnitude of effect*: Not applicable.
    - *Basis for rating:* Definitely low risk of bias based on direct evidence that the exposure was consistently assessed using well-established methods that directly measured exposure.
- ***Outcome:***
    - *Rating:* Definitely low risk of bias (++)
    - *Summary:* A Combined Raven's Test for Rural China (CRT-RC) was taken to evaluate the IQ of each child (++ for methods). The study report stated that all tests were administered at school by a trained examiner who was masked to participants' drinking water fluoride levels (++ for blinding). Overall rating for methods and blinding=++.
    - *Basis for rating:* Definitely low risk of bias based on direct evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
    - *Rating:* Definitely low risk of bias (++)
    - *Summary:* All results outlined in the abstract, introduction, and methods sections were reported in sufficient detail.
    - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
    - *Rating:* Probably low risk of bias (+)
    - *Summary:*
        - *Statistical analyses:* Associations between serum and urinary fluoride levels and IQ score were estimated using general linear models and multivariate linear regression by COMT polymorphism. Normality (Kolmogorov-Smirnov test) was evaluated for all continuous variables. There is no evidence that residual diagnostics were used to examine model assumptions or that the complex sampling design (stratified multistage random sampling) was accounted for in the analysis using sampling weights and adjustment for clustering. The impact of these factors on the regression effect estimates is expected to be minimal given the use of individual-level data and adjustment for numerous potential confounders.
        - *Other potential concerns:* None identified.
    - *Basis for rating:* Probably low risk if bias based on direct evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Definitely or probably low risk-of-bias ratings in confounding, exposure, and outcome. Study strengths include individual exposure measurements, outcomes blindly assessed, and assessment of potential key confounders including potential co-exposures.

Other Neurodevelopmental Studies

Barberio *et al.* (2017b)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** Canadian Health Measures Survey (cycles 2 and 3) participants (children aged 3−12 years)
- ***Study area:*** general population of Canada
- ***Sample size:*** 2,221 children (1,120 from Cycle 2, 1,101 from Cycle 3)
- ***Data relevant to the review:*** Associations between learning disability or ADHD (Cycle 2 only) assessed by parent or child self-report and urinary fluoride.
- ***Reported association with fluoride exposure:*** Yes: Significant increase in adjusted OR for learning disability with unadjusted urinary fluoride (1.02; 95% CI: 1.00, 1.03) when Cycle 2 and 3 were combined. No significant associations with creatinine-adjusted or specific gravity-adjusted urinary fluoride. No significant association between urinary fluoride and ADHD.

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* The comparison groups were selected from Cycles 2 and 3 of the Canadian Health Measures Survey. This is a nationally representative sample of residents living in 10 provinces, with clear exclusion criteria provided. Exclusion only represented about 4% of the target population (all Canadian residents 3−79 years old living in 10 provinces). A table of characteristics of the study population is provided.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that the subjects were recruited from the same population using the same methods during the same time frame and exposure groups were similar.
- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* The study adjusted for sex, age (3–12 years old), household education, and household income adequacy. Variables to discern fluoride source, including drinking water and dental products, were also considered. Cycle 2 data also included adjustments for: 1) children for whom tap water (vs. bottled or other) was the primary source of drinking water at home or away from home and 2) children who had lived in his or her current home for 3 or more years. Confounders such as parental behavioral and mental health disorders, smoking, and nutrition were not discussed. The study used data from the Canadian Health Measures Survey which consists of a nationally representative sample of Canadians. Most Canadians (~89%) receive water from municipal water supplies, which monitor for levels of lead and arsenic. Therefore, co-exposure to lead and arsenic are less likely an issue in this population and the lack of information is not considered to appreciably bias the results.
  - *Potentially important study-specific confounders:* All key confounders were considered in this study.

        - *Direction/magnitude of effect*: Not applicable.
    - *Basis for rating:* Probably low risk of bias based on direct evidence that key confounders were addressed and indirect evidence that the methods used to collect the information were valid and reliable and that co-exposures were not an issue.
- ***Attrition:***
    - *Rating:* Probably low risk of bias (+)
    - *Summary:* Covariate data were missing for less than 5% of all analyses, apart from household income; household income was reported for only 71–77% of participants and was imputed for the remainder.
    - *Basis for rating:* Probably low risk of bias based on indirect evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
- ***Exposure:***
    - *Rating:* Probably low risk of bias (+)
    - *Summary:* Estimates of urinary fluoride (µmol/L) from spot urine were available for a subsample of respondents. Analysis was performed under standardized operating procedures at the Human Toxicology Laboratory of the Institut National de Santé Publique du Québec (accredited under ISO 17025). Fluoride content of urine samples was analyzed using an Orion pH meter with a fluoride ion-selective electrode with limits of detection of 20 µg/L (Cycle 2) and 10 µg/L (Cycle 3). Urinary dilution was addressed by using creatinine-adjusted levels as well as specific gravity-adjusted levels. In Cycle 3 only, estimates of the fluoride concentration of tap water samples collected from randomly selected households were available. The subsample of households selected for tap water sample collection corresponded to the person-level urine fluoride subsample. Analysis of the fluoride concentration of tap water was performed using a basic anion exchange chromatography procedure, with a limit of detection of 0.006 mg/L. QC methods were not addressed.
        - *Direction/magnitude of effect*: There is not any specific direction or magnitude of bias expected. Urinary fluoride levels are reflective of a recent exposure. Having a single concurrent measurement may not be reflective of the exposure associated with the outcome, but if subjects lived in the same area throughout life the exposure may be an adequate representation. Although there is possible exposure misclassification it would be non-differential.
    - *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using well-established methods that directly measured exposure.
- ***Outcome:***
    - *Rating:* Probably high risk of bias (-)
    - *Summary:* The primary outcome variable, diagnosis of a learning disability by a health professional, was based on a single item from a household survey asked to all respondents: "Do you have a learning disability?". Answer options were: "yes", "no", "don't know", or the participant refused to answer. For Cycle 2, those who indicated having a learning disability were also asked what kind, with the answer options of: "ADD", "ADHD", "dyslexia", or "other". This question was omitted in Cycle 3 and the reason for omission is not described. Parents or guardians answered all questions for children aged 3–11 years, while children 12 years and older answered questions

themselves. The self-reporting of a learning disability did not appear to have been confirmed by medical records or a health professional. (- for methods based on self-report of diagnosis by a health care professional also in Cycle 3 no specific disabilities were described). Blinding was not a concern as spot urine samples were sent to a separate lab and self-reports would not have knowledge of their urine or tap water exposure level (+ for blinding). Overall rating = -.

- *Basis for rating:* Probably high risk of bias based on indirect evidence that the outcome was measured using an insensitive method in the study population.

- ***Selective Reporting:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* All outcomes outlined in the abstract, introduction, and methods sections were reported in sufficient detail.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:*
    - *Statistical analyses:* Logistic regression analyses, adjusted and unadjusted for covariates, examined the associations between fluoride exposure and diagnosis of learning disability. Analyses were performed for Cycle 2 only (urinary fluoride and type of learning disability diagnosis), Cycle 3 only (urinary fluoride, water fluoride, and learning disability diagnosis), and Cycles 2 and 3 combined. Analyses used survey weights and bootstrapped weights to ensure proper computation of variance estimates. Results are reported as unadjusted and adjusted ORs with 95% CIs.
    - *Other potential concerns:* None identified.
  - *Basis for rating:* Definitely low risk if bias based on direct evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Probably low risk-of-bias ratings in confounding and exposure. Study strengths include individual exposure measurements and the addressing of potential key confounders but was limited by the cross-sectional study design and insensitive outcome measures.

Bashash *et al.* (2017)

Study Details:

- ***Study design*:** Prospective cohort
- ***Population*:** Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) participants (pregnant mothers and their children aged 4 or 6–12 years).
- ***Study area:*** Mexico City, Mexico
- ***Sample size:*** 299 mother–child pairs, of whom 287 had data for the general cognitive index (GCI).
- ***Data relevant to the review:*** Adjusted and unadjusted associations between GCI and maternal or child's urinary fluoride concentrations.

- ***Reported association with fluoride exposure:*** Yes: Significant association between maternal urinary fluoride and GCI score (adjusted β = −3.15; 95% CI: −5.42, −0.87). No significant associations with children's urinary fluoride.

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Study participants were selected from two different cohorts from three hospitals in Mexico City that serve low-to-moderate income populations. One cohort was from an observational study of prenatal lead exposure and neurodevelopment outcomes and the other was from a randomized trial of the effect of calcium on maternal blood lead levels. The authors state that participants had no history of psychiatric disorders, high-risk pregnancies, gestational diabetes, illegal drug use, or continuous prescription drugs, but they do not include any information on smoking habits. Study populations appear to be similar, but there may be some differences because subjects were selected from two different cohorts that were recruited from slightly different time periods.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the exposure groups were similar despite the subjects coming from different original study populations where different methods were used for recruitment.
- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Data were collected via questionnaire on maternal age, education, marital status at first prenatal visit, birth order, birth weight, gestational age at delivery, maternal smoking, maternal IQ, and HOME scores. All models were adjusted for gestational age at birth, child's sex, birth weight, birth order, child's age at testing, maternal marital status, smoking history, age at delivery, maternal IQ, education, and cohort, with additional testing for children's urinary fluoride, mercury, lead, and calcium. Sensitivity analyses additionally adjusted for HOME score. Confounders not considered included BMI, iodine deficiency, arsenic, and maternal mental health and nutrition. Arsenic is assumed not to be a potential co-exposure in this population as the study authors did not discuss it as an issue but did discuss other co-exposures. Arsenic is included in the water quality control program in Mexico City and is not considered a concern in this population.
  - *Potentially important study-specific confounders:* All key confounders were addressed.
    - *Direction/magnitude of effect***:** Not applicable.
  - *Basis for rating:* Probably low risk of bias based on direct evidence that key confounders including other potential co-exposures were addressed and indirect evidence that the methods used to collect the information were valid and reliable and that arsenic is not likely to be an issue in this study population.
- ***Attrition:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Although there was a large amount of attrition, the study authors clearly describe all reasons for attrition and also provide characteristics to compare those

participants included to those excluded. There were some slight differences between those included and those excluded, but there is nothing to indicate that the attrition would potentially bias the results.
    - *Basis for rating:* Probably low risk of bias based on indirect evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
- ***Exposure:***
    - *Rating:* Definitely low risk of bias (++)
    - *Summary:* Urinary fluoride concentrations were determined in spot urine samples (2nd morning void) collected from mothers (during at least one trimester) and children ages 6–12 years. Fluoride content was measured using ion-selective electrode-based assays. QC methods were described including between laboratory correlations. All samples were measured in duplicate. Extreme outliers were excluded. Urinary dilution was addressed by using creatinine-adjusted levels.
        - *Direction/magnitude of effect***:** Not applicable.
    - *Basis for rating:* Definitely low risk of bias based on direct evidence that exposure was consistently assessed using well-established methods that directly measured exposure.
- ***Outcome:***
    - *Rating:* Definitely low risk of bias (++)
    - *Summary:* Outcome was assessed using the McCarthy Scales of Children's Abilities (MSCA) in 4-year-old children (translated into Spanish) and the Wechsler Abbreviated Scale of Intelligence (WASI) in 6–12-year-olds. The WASI is a well-established test and the validity of both tests is well documented by the authors. Inter-examiner reliability was evaluated and reported with a correlation of 0.99 (++ for methods). The study report stated that psychologists were blind to the children's fluoride exposure (++ for blinding). Overall rating for methods and blinding = ++.
    - *Basis for rating:* Definitely low risk of bias based on direct evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
    - *Rating:* Definitely low risk of bias (++)
    - *Summary:* All outcomes outlined in the abstract, introduction, and methods are reported in sufficient detail.
    - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
    - *Rating:* Definitely low risk of bias (++)
    - *Summary:*
        - *Statistical analyses:* Statistical analyses used were appropriate for the study. Statistical tests of bivariate associations [using Chi-square tests for categorical variables and analysis of variance (ANOVA)] were used to compare the means of the outcomes or exposure within groups based on the distribution of each covariate. Generalized additive models (GAMs) were used to estimate the adjusted association between fluoride exposure and measures of children's intelligence. Residual diagnostics were used to examine model assumptions and

identify any potentially influential observations. Results are reported as adjusted effects and 95% CIs. In sensitivity analyses, regression models accounted for clustering at the cohort level by using cohort as a fixed effect in the models. Although using cohort as a random effect would be more appropriate, using individual-level exposure data and accounting for numerous potential confounders in the models likely captured the cohort effect. Additional models with cohort as a random effect were also subsequently made available via personal communication with the study authors and showed similar results to the main model.

        - *Other potential concerns:* None identified.
    - *Basis for rating:* Definitely low risk if bias based on direct evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Definitely or probably low risk-of-bias ratings in confounding, exposure, and outcome. Study strengths include individual exposure measurements, outcome blindly assessed, and the prospective cohort study design.

Bashash *et al.* (2018)

Study Details:

- ***Study design*:** Prospective cohort
- ***Population*:** ELEMENT participants (pregnant mothers and their children aged 6–12 years)
- ***Study area:*** Mexico City, Mexico
- ***Sample size:*** 210 mother–child pairs
- ***Data relevant to the review:*** Associations between ADHD and other attention/impulsivity scores and maternal urinary fluoride concentrations.
- ***Reported association with fluoride exposure:*** Yes: Significant associations between maternal urinary fluoride and Conners' Rating Scales-Revised (CRS-R) scores, including Cognitive Problems and Inattention Index (adjusted β = 2.54; 95% CI: 0.44, 4.63), DSM-IV Inattention Index (adjusted β = 2.84; 95% CI: 0.84, 4.84), DSM-IV ADHD Total Index (adjusted β = 2.38; 95% CI: 0.42, 4.34), and ADHD Index (adjusted β = 2.47; 95% CI: 0.43, 4.50).

Risk of Bias:

- **Author contacts:**
    - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
    - *Rating:* Probably low risk of bias (+)
    - *Summary:* Participants were a subset of mother-child dyads enrolled in various longitudinal birth cohort studies of the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) project. Subjects were included from two of the four cohorts for which maternal urinary samples were available. Participants in cohort 2A were recruited between 1997 and 1999, and participants in cohort 3 were recruited from 2001 to 2003. Inclusion and exclusion criteria were applied consistently across the two cohorts. A table of subject characteristics was provided in the study and any differences were considered in the analysis. Study populations appear to be similar, but there may be some

differences because subjects were selected from two different cohorts: one from an observational study on prenatal lead exposure and the other from a randomized trial on the effects of calcium on blood lead levels. In addition, they were recruited from slightly different time periods.

- *Basis for rating:* Probably low risk of bias based on indirect evidence that the exposed groups were similar, and any differences were taken into account in the analysis.

- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Questionnaires were used to collect information on maternal age, maternal education, history of smoking, and marital status during the first pregnancy visit. Child information at birth included birth weight, sex, birth order, and gestational age as calculated by the nurse. Mothers also responded to an SES questionnaire during the visit when the psychometric tests were administered. The Home Observation for Measurement of the Environment (HOME) score was evaluated in a subset of participants. Covariates were selected a priori. Models adjusted for maternal age at delivery, years of education, marital status, smoking history, gestational age at birth, age at outcome assessment, child's sex, birth order, SES, cohort, and calcium intervention. Arsenic is included in the water quality control program in Mexico City and is not considered a concern in this population.
  - *Potentially important study-specific confounders:* None identified, although this study did not specifically address arsenic or other co-exposures. Bashash *et al.* (2017) addressed potential co-exposure to lead and mercury but did not address arsenic. Arsenic was potentially addressed as part of the water quality program in Mexico City.
    - *Direction/magnitude of effect***:** Not applicable.
  - *Basis for rating:* Probably low risk of bias based on direct evidence that key confounders were addressed and indirect evidence that the methods used to collect the information were valid and reliable and that arsenic and other potential co-exposures are not likely to be an issue in this study population.
- ***Attrition:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Although there was a large amount of attrition from the original cohorts, it was unlikely related to outcome or exposure and there were very little missing data from those included in the study. Of the 231 mothers with a minimum of one maternal urine fluoride measurement and matching outcome identified for the project, only 17 were excluded based on incomplete demographic and outcome information.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
- ***Exposure:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Mothers provided at least one spot urine sample during pregnancy. As described in Bashash *et al.* (2017), urinary concentrations were determined on second morning void. Fluoride content was measured using ion-selective electrode-based assay. Bashash *et al.* (2017) describes QC methods. All samples were measured in duplicate

and extreme outliers were excluded. Urinary dilution was addressed by using creatinine-adjusted levels.
        - *Direction/magnitude of effect*: N/A
    - *Basis for rating:* Definitely low risk of bias based on direct evidence that exposure was consistently assessed using well-established methods that directly measured exposure.
- ***Outcome:***
    - *Rating:* Definitely low risk of bias (++)
    - *Summary:* Behaviors associated with ADHD were assessed using the Spanish version of the Conners' Rating Scales-Revised, which has been validated for the evaluation of ADHD. Mothers completed the CRS-R at the same follow-up visit that the child completed the CPT-II tests. All tests were applied under the supervision of an experienced psychologist (++ for methods); however, a limitation of the study noted by the authors was only using parent reports and not teacher reports as they can vary from one another. Blinding was not reported, but it is unlikely that the mothers were aware of their urinary fluoride levels. Although mothers may have had knowledge that they were receiving fluoride through fluoridated salt or naturally occurring fluoride in their water, they would not have knowledge that this was relevant to the study purpose as the ADHD tests were conducted for the original cohort (as was acknowledged by the study authors in the discussion). (++ for blinding). Overall rating = ++.
    - *Basis for rating:* Definitely low risk of bias based on direct evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
    - *Rating:* Definitely low risk of bias (++)
    - *Summary:* All outcomes outlined in the abstract, introduction, and methods were reported in sufficient detail.
    - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
    - *Rating:* Definitely low risk of bias (++)
    - *Summary:*
        - *Statistical analyses:* Bivariate analyses included Chi-square tests for categorical variables and ANOVA for continuous outcomes. Appropriate univariate statistics and transformations were performed before bivariate analyses. Residuals from fully adjusted linear regressions were checked and suggested skewness. Gamma regression with an identity link was used to examine the adjusted association between prenatal fluoride and each neurobehavioral outcome (instead of using log transformation). Generalized additive models were used to visually examine potential non-linearity. Sensitivity analyses examined impact of other potential confounders. Diagnostics were used to assess violations of the model assumptions and to identify remaining influential observations. The Benjamini–Hochberg false discovery rate (FDR) procedure was used to correct for multiple testing.
        - *Other potential concerns:* None identified.

  - *Basis for rating:* Definitely low risk if bias based on direct evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Definitely or probably low risk-of-bias ratings in confounding, exposure, and outcome. Study strengths include individual exposure measurements, outcome blindly assessed, and the prospective cohort study design.

Choi *et al.* (2015)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** First grade children (ages 6–8 years)
- ***Study area:*** Mianning County in southern Sichuan, China
- ***Sample size:*** 51 first grade children
- ***Data relevant to the review:*** Associations between learning, memory, visual motor ability, motor ability, and manual dexterity with continuous urine or drinking water fluoride levels. Study also had information based on dental fluorosis score.
- ***Reported association with fluoride exposure:*** No: None of the outcomes were significantly associated with fluoride exposure.

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Subjects were selected during the same time frame using the same methods. Fifty-one first-grade children residing in Mianning County in southern Sichuan, China were included in this pilot study. It is not specified if the 51 children represented all the first-grade children from this area or if some refused to participate. Children who did not speak Chinese, were not students at the Primary School of Sunshui Village in Mianning County, or those with chronic or acute disease that might affect neurobehavioral function tests were excluded. Demographic characteristics are presented in Table 1 of the study, which indicates that subjects were similar. Potential confounders are adjusted for in the statistical analyses.
  - *Basis for Rating:* Definitely low risk of bias based on direct evidence that the exposure groups were similar and were recruited within the same time frame using the same methods with no evidence of differences in participation/response rates.
- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* The parents or guardians completed a questionnaire on demographic and personal characteristics of the children (sex, age at testing, parity, illnesses before age 3, and past medical history) and caretakers (age, parity, education and occupational histories, residential history, and household income). A 20-µL capillary blood sample was collected at the school by a Mianning County Center for Disease Control (CDC) health practitioner and tested for possible iron deficiency which could be used as a

covariate of neurodevelopmental performance. Confounders that were not assessed include: maternal BMI, parental mental health, maternal smoking status, maternal reproductive factors, parental IQ, and HOME score. However, the study authors noted that confounding bias appeared to be limited due to the minimal diversity in the social characteristics of the subjects. The study authors indicated that CDC records documented that levels of other contaminants including arsenic and lead were very low in the area. Iodine differences were not specifically addressed, but there is no indication from the information provided that this might be a concern.

- *Potentially important study-specific confounders:* All key confounders were considered in this study.
  - *Direction/magnitude of effect*: Not applicable.
- *Basis for rating:* Probably low risk of bias because there is direct evidence that the key confounders are taken into account and indirect evidence that co-exposure to arsenic is likely not an issue in this area and that methods used for collecting the information were valid and reliable.

- ***Attrition:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* The majority of results were reported for the 51 children stated to be included in the pilot study. In Table 5 of the study, the N for each dental fluorosis category only totals 43, but the text indicates 8 children did not have a Dean Index because permanent teeth had not erupted.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
- ***Exposure:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* The study used three different measurements of fluoride exposure: well water fluoride concentrations from the residence during pregnancy and onwards, fluoride concentrations from children's first morning urine samples, and degree of children's dental fluorosis. Fluoride concentrations in community well water were measured and recorded by Mianning County CDC; specific methods were not reported, but they likely used standard methods as they were conducted by the CDC and were likely the same as those used to measure the fluoride in urine. Migration of subjects was noted to be limited. Well water fluoride concentrations of the mother's residence during pregnancy and onward were used to characterize a child's lifetime exposure. To provide a measure of the accumulated body burden, each child was given a 330-mL (11.2-oz) bottle of Robust© distilled water (free from fluoride and other contaminants) to drink the night before the clinical examinations, after emptying the bladder and before bedtime. The first urine sample the following morning was collected at home, and the fluoride concentration was determined on a 5-mL sample using an ion-specific electrode at the Mianning CDC. There is no indication that urinary fluoride levels accounted for dilution nor was it clear that the method of administering water to the children and collection methods sufficiently controlled for differences in dilution. One of the investigators, a dentist, performed a blinded dental examination on each child's permanent teeth to rate the degree of dental fluorosis using the Dean Index. The Dean

Index is a commonly used index in epidemiological studies and remains the gold standard in the dentistry armamentarium. The Index has the following classifications: normal, questionable, very mild, mild, moderate, and severe. Quality control (QC) procedures are not reported but were likely appropriate.

- *Direction/magnitude of effect*: Current levels were used to assess lifetime exposure. This is likely to be a non-differential exposure misclassification and direction of bias is unknown. Because subject migration appears to be limited, it is likely that the current fluoride levels are adequate reflections of past exposure. Dental fluorosis would be an indicator that exposure occurred in the past and there was a fair correlation between degree of dental fluorosis and current urine and water fluoride levels, with both increasing with increasing levels of dental fluorosis.

- *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using well-established methods that directly measure exposure.

- ***Outcome:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* The study authors adopted culture-independent tests considered feasible for children aged 6 to 8 years. The Wide Range Assessment of Memory and Learning (WRAML) was used for the assessment of memory and learning. Three subtests were also used. The Finger Windows subtest assesses sequential visual memory. The Design Memory subtest assesses the ability to reproduce designs from memory following a brief exposure. The Visual Learning subtest assesses the ability to learn the locations of pictured objects over repeated exposures. The Wechsler Intelligence Scale for Children-Revised (WISC-IV) included digit span for auditory span and working memory and block design for visual organization and reasoning. The grooved pegboard test assesses manual dexterity. The tests used have been validated on a western population. Although there is no information provided to indicate that they were validated on the study population, the study authors indicated that the tests were culture-independent (+ for methods). Blinding of the outcome assessors or steps to minimize potential bias was not reported. However, it is unlikely that the assessors had knowledge of the individual exposure as children all came from the same area, and water and urine levels were tested at the CDC. (+ for blinding). Overall = +.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that all outcomes were assessed blindly using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* All outcomes outlined in the abstract, introduction, and methods are reported in sufficient details.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:*

        - *Statistical analyses:* Statistical analyses were appropriate. Multiple regression models evaluate the associations between exposure indicators and test scores after adjusting for potential confounders. Specific regression models are not described or refenced, just stated to be "standard regression analysis with confounder adjustment." The distributions of fluoride concentrations in urine and water were skewed and were log10-transformed to approximate a Gaussian distribution (test not specified). Results are reported as adjusted effects and 95% CIs. There is no evidence that residual diagnostics were used to examine model assumptions; however, the impact on the effect estimates is expected to be minimal.
        - *Other potential concerns:* It should be noted that this study was a pilot study and, therefore, had a relatively small sample size (i.e., 51 children).
    - *Basis for rating:* Probably low risk if bias based on indirect evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Probably low risk-of-bias ratings in the confounding, exposure, and outcome risk-of-bias domains. Study strengths include individual fluoride measurements with blinding at outcome assessment likely. All key confounders and many other confounders were taken into account in the study design or analysis.

Li *et al.* (2004) [translated in Li *et al.* 2008a]

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** Full term, normal neonates 24−72 hours old from healthy mothers
- ***Study area:*** Zhaozhou County, Heilongiang Province, China
- ***Sample size:*** 91 neonates (46 males and 45 females)
- ***Data relevant to the review:*** Comparison of neurobehavioral capacity between children in the high-fluoride area compared to the control area.
- ***Reported association with fluoride exposure:*** Yes: Significant differences in neurobehavioral assessment total scores between high-fluoride (36.48 ± 1.09) and control (38.28 ± 1.10) groups; significant differences in total neurobehavioral capacity scores as measured by non-biological visual orientation reaction and biological visual and auditory orientation reaction between the two groups (11.34 ± 0.56 in controls compared to 10.05 ± 0.94 in high-fluoride group).

Risk of Bias:

- **Author contacts:**
    - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
    - *Rating:* Probably low risk of bias (+)
    - *Summary:* There is indirect evidence that the exposure groups were similar. They were recruited during the same time frame using the same methods. From 2002 to 2003, 273 neonates were born in a hospital in Zhaozhou County, China. Ninety-one of 273 full-term neonates (46 males, 45 females) were randomly selected. Mothers ranged in age

from 20 to 31 years, met multiple health criteria, and had not changed residence during pregnancy. Authors report that the two study groups are located in the same area with similar climate, living habits, economic and nutritional conditions, and cultural backgrounds, but do not provide these data in the manuscript. There is no statistically significant difference in the mode of delivery, birth weight, infant length, or sex. Subjects were separated into exposure groups after random selection.

- *Basis for Rating:* Probably low risk of bias based on indirect evidence that the exposure groups were similar and were recruited within the same time frame using the same methods with no evidence of differences in participation/response rates.

- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* No confounders were specifically controlled in the analysis. The study authors note similarities in characteristics in the two populations (i.e., living habits, economic and nutritional conditions, and cultural backgrounds), but do not provide these data nor do they indicate what specific characteristics were considered. There were no significant differences in infant gender, birth method, gestational age, or infant weight and length. All tests were conducted when children were 1–3 days old. No potential co-exposures were discussed. Although arsenic is considered a potential issue in China, water quality maps indicate that there is a 25–50% probability that the drinking water in that area exceeds the WHO guideline for arsenic of 10 µg/L.
  - *Potentially important study-specific confounders:* Key confounders, including child's age, child's gender, and measures of socioeconomic status (SES), were similar between exposure groups; however, arsenic was not taken into account. Arsenic often occurs in the drinking water along with fluoride in some Chinese populations; however, based on water quality maps, arsenic does not appear to be an issue in Zhaozhou County of the Heilongjiang Province. Iodine deficiencies are not mentioned.
    - *Direction/magnitude of effect***:** Conceptually, the presence of arsenic would potentially bias away from the null if it were present with fluoride. Deficiencies in iodine would potentially bias away from the null if it were present in areas of higher fluoride, but toward the null if it were present in areas of lower fluoride. Neither of these are considered a concern in this study for reasons detailed above.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the key confounders are taken into account, co-exposure to arsenic is likely not an issue in this area, and methods used for collecting the information are valid and reliable.
- ***Attrition:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Although authors did not discuss why they only randomly selected 91 of the 273 neonates available, results were available for all 91 subjects.
  - *Basis for rating:* Definitely low risk of bias based on results being available for all subjects.
- ***Exposure:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Subjects were split into control and high-fluoride groups based on fluoride levels in their places of residence. Although the levels were provided (1.7–6.0 mg/L for

the high-fluoride group compared to 0.5–1.0 mg/L for the control group), it was not reported how or when these levels were measured. Urine was collected when women were hospitalized, but before labor began. Urine samples were sent to a specific lab for measurement using fluoride ion-selective electrode. It was noted that this procedure strictly followed the internal controls of the laboratory indicating quality control. Level of detection (LOD) was not provided. Urinary fluoride levels were significantly higher in the high-fluoride mothers (3.58 ±1.47 mg/L) compared to the control-group mothers (1.74±0.96 mg/L). There was indirect evidence that exposure was consistently assessed using well-established methods that directly measure exposure. Although results were mainly based on exposure area, they were supported by urine data making exposure misclassification less of a concern.

        - *Direction/magnitude of effect*: There is high variability in both water fluoride and urine fluoride in the subjects from the high-exposure area. Although there is no overlap in the water fluoride levels in the exposure areas, there is some overlap in the urine concentrations in the mothers from the two areas. This may reflect the single measurement and pose no specific bias, or it could indicate that some mothers in the high-fluoride area have lower fluoride exposure, which could bias the results toward the null.
    - *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using well-established methods that directly measure exposure.
- ***Outcome:***
    - *Rating:* Probably low risk of bias (+)
    - *Summary:* A standard neonatal behavioral neurological assessment method was carried out by professionals in the pediatric department working in neonatal section trained specifically for these programs and passing the training exams. (+ for methods). The examinations were carried out 1 to 3 days after delivery. Because urine samples were collected on the day of delivery and sent to a separate laboratory, it is likely that the outcome assessors were blind. Although the subjects were separated by fluoride exposure area, it is not likely that the professionals were aware of the exposure as the tests were conducted in the hospital (+ for blinding).
    - *Basis for rating:* Probably low risk of bias based on indirect evidence that the outcome was assessed blindly using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
    - *Rating:* Probably low risk of bias (+)
    - *Summary:* The study authors reported numerous endpoints in sufficient detail; however, because they did not provide a list of endpoints tested there is no direct evidence that all were reported.
    - *Basis for rating:* Probably low risk of bias based on indirect evidence that all the study's measured outcomes were reported.
- ***Other potential threats:***
    - *Rating:* Probably low risk of bias (+)
    - *Summary:*
        - *Statistical analyses:* Statistical analyses are described only as a t-test. Consideration of heterogeneity of variance was not reported. Results are

reported as mean and standard deviations of neurological scores. Maternal urinary fluoride levels were only used to compare exposures between exposed and control groups. Infants in the control group were from four villages, and those in the exposed group were from five villages within the same district. Infants were randomly selected before they were assigned to exposed or control groups. In the comparisons, there was no accounting for clustering at the village level. It is likely that the standard error of the difference in mean neurobehavioral assessment scores between the high fluoride group and control group will be biased, making differences appear stronger than they actually are. However, the use of multiple villages per exposure group is likely to mitigate some of the impact of this lack of accounting for clustering, and the overall impact on effect estimates is expected to be minimal.

        - *Other potential concerns:* It should be noted that, although the study states that subjects were randomly selected, it is unclear why only 91 subjects were included and if they were randomly selected to obtain equal groups in the high-fluoride and control groups.
    - *Basis for rating:* Probably low risk of bias based on indirect evidence that statistical analyses were appropriate and that there were no other potential threats to risk of bias.
- ***Basis for classification as low risk-of-bias study overall:*** Probably low risk-of-bias ratings in the confounding, exposure, and outcome risk-of-bias domains. Study strengths include individual fluoride measurements to support the differences in the two areas. Tests were noted to be conducted at the hospital providing indirect evidence that blinding was not a concern during the outcome evaluation. Although there was some potential for bias due to the lack of accounting for arsenic or iodine deficiencies, co-exposure to arsenic is likely not a major concern according to groundwater quality maps.

Riddell *et al.* (2019)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** Canadian Health Measures Survey (cycles 2 and 3) participants (children aged 6−17 years)
- ***Study area:*** general population, Canada
- ***Sample size:*** 3,745 children
- ***Data relevant to the review:*** Adjusted odds ratios for ADHD and attention symptoms per 1 unit increase in urinary fluoride, by water fluoride in the tap water, or community fluoridation status.
- ***Reported association with fluoride exposure:*** Yes: Significantly increased odds of ADHD diagnosis (adjusted OR = 6.10; 95% CI: 1.60, 22.8) or hyperactivity/inattentive symptoms (adjusted β = 0.31; 95% CI: 0.04, 0.58) per 1-mg/L increase in tap water fluoride. Also, a significant association between ADHD diagnosis (adjusted OR = 1.21; 95% CI: 1.03, 1.42) or hyperactivity/inattentive symptoms (adjusted β = 0.11; 95% CI: 0.02, 0.58) and community water fluoridation status. No significant associations with urinary fluoride levels.

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Subjects were part of Cycles 2 and 3 of the Canadian Health Measures Survey. This is a nationally representative sample of residents living in 10 provinces. Specific inclusion criteria were provided. This study was restricted to children 6–17 years of age with different fluoride measurements that consisted of three participant samples. One of the samples was only available in Cycle 3.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that the exposed groups were similar and were recruited with the same methods during the same time frame.
- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Covariates included in all models included child's age at test, child's sex, ethnicity, BMI, parents' education, total household income, exposure to cigarette smoke inside the home, and log-transformed concurrent blood lead levels. Confounders such as parental behavioral and mental health disorders, quantity and quality of caregiving environment, and co-exposure to arsenic were not discussed. The study used data from the Canadian Health Measures Survey which consists of a nationally representative sample of Canadians. Most Canadians (~89%) receive water from municipal water supplies, which monitor for levels of arsenic. Therefore, co-exposure to arsenic is not likely an issue in this population. Rationale for selection of covariates was based on relationship to ADHD diagnosis and to fluoride metabolism based on literature review and consultation with an ADHD expert. There is no information of the source if data for covariates, but this is likely the questionnaires from the Canadian Health Measures Survey, which are considered standardized and validated.
  - *Potentially important study-specific confounders:* All key confounders were considered in this study.
    - *Direction/magnitude of effect***:** Not applicable.
  - *Basis for rating:* Probably low risk of bias because there is indirect evidence that the key confounders are taken into account, co-exposure to arsenic is likely not an issue in this area, and methods used for collecting the information were valid and reliable.
- ***Attrition:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* There is no information indicating that there were any data excluded due to missing covariates. All exclusions of children were described and reasonable (i.e., drinking bottled water when considered city fluoridation as a measure of fluoride exposure). Outliers were stated to be excluded, but methods for determining this were provided and it was noted that the outliers were 0.27% of the values.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.

- ***Exposure:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* **Urinary Fluoride:** Spot urine samples were collected under normal non-fasting conditions and analyzed using an Orion pH meter with a fluoride ion-selective electrode after being diluted with an ionic adjustment buffer. Analysis was performed at the Human Toxicology Laboratory of the Institut National de Sante Publique du Quebec. The precision and accuracy of the fluoride analyses, including quality control and quality assurance, were described by Health Canada (2015). The limits of detection were 20 µg/L for Cycle 2 and 10 µg/L for Cycle 3 with no values below detection. Fluoride levels were adjusted for specific gravity.
    Water Fluoride in Tap water: Tap water was collected at the subjects' homes in Cycle 3 only. Samples were analyzed for fluoride concentrations using anion exchange chromatography procedure with a LOD of 0.006 mg/L. Values below the LOD were imputed with LOD/square root 2. Of the 980 samples, 150 (16%) were below detection.
    Chlorinate Water Fluoride status: This was determined by viewing reports on each city's website or contacting the water treatment plant (provided in supplemental material). Children were excluded if they drank bottled water, had a well, had a home filtration system, lived in the current residence for 2 years or less, or lived in an area with mixed city fluoridation.
    - *Direction/magnitude of effect***:** There is not any specific direction or magnitude of bias expected. Urinary fluoride levels are reflective of a recent exposure, but the study authors adjusted to account for dilution. The possibility of exposure misclassification would be similar in all subjects and would be non-differential. There is less potential for exposure misclassification in regard to tap water or chlorinated water fluoride status as children who drank bottled water were excluded and children who had a home filtration system were excluded from the chlorinated water status.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using well-established methods that directly measured exposure.
- ***Outcome:***
  - *Rating:* Probably high risk of bias (-)
  - *Summary:*
    Strengths and Difficulties Questionnaire (SDQ): The questionnaire was administered to youths under 18 years. Children aged 6–11 years had SDQ ratings provided by parents and guardians, but youths aged 12−17 years completed the questionnaire themselves. Tests consist of 25 items with a 3-point scale. Items were divided into five subscales: emotional problems, conduct problems, hyperactivity-inattention, peer problems, and prosocial behavior. The current study only used the hyperactivity-inattention subscale. Validation of this method was not reported (- for methods).
    ADHD: Ninety percent of youths with ADHD are diagnosed after age 6 years. For children aged 6–11 years, ADHD diagnosis was provided by parents, but youths aged 12−17 years completed the questionnaire themselves. Cycle 2 asked "Do you have a learning disability?" and if yes asked to specify the type (4 options available and described). In Cycle 3, parents were asked directly whether they had ADHD, and children 12 years and older were asked if they had a physician diagnosis of ADHD and, if so, what subtype. (- for methods because different methods were used and only the children 12 years and older in cycle 3 were asked specifically about doctor diagnosis). Both were

measured in both cycles. Blinding is not likely an issue as subjects would not have knowledge of the urine or tap water fluoride levels. However, they would likely have knowledge of the city.
    - *Basis for rating:* Probably high risk of bias based on indirect evidence that the outcome was assessed using insensitive methods that varied based subject age.
- ***Selective Reporting:***
    - *Rating:* Definitely low risk of bias (++)
    - *Summary:* All outcomes outlined in the abstract, introduction, and methods sections were reported in sufficient details.
    - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
    - *Rating:* Probably low risk of bias (+)
    - *Summary:*
        - *Statistical analyses:* Robust logistic regression was used to examine the association between fluoride exposure and ADHD diagnosis, adjusting for covariates. Box-Tidewell tests were used to check the linearity of the relationship with the continuous predictors. Linear regression was used for the SDQ scores using Huber-White standard errors. Multicollinearity was evaluated using variance inflation factor (VIF) statistics. Outliers with high studentized residuals, high leverage, or large Cook's distance values were removed from all analyses with urinary fluoride. All regressions were tested for interactions between age and fluoride, and sex and fluoride. Sensitivity analyses were conducted to test the different survey cycles. There is no mention of adjustment for the complex survey design using survey weights or bootstrapped weights to ensure appropriate calculation of the estimated variances; however, the overall impact on effect estimates is expected to be minimal.
        - *Other potential concerns:* None identified.
    - *Basis for rating:* Probably low risk if bias based on indirect evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Probably low risk-of-bias ratings in confounding and exposure. Study strengths include individual exposure measurements and the addressing of potential key confounders but was limited by the cross-sectional study design and insensitive outcome measures.

Rocha-Amador *et al.* (2009)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** Children aged 6–11 years
- ***Study area:*** Durango, Mexico
- ***Sample size:*** 80 children

- ***Data relevant to the review:*** Associations between visuospatial organization and visual memory (using the Rey-Osterrieth Complex Figure Test, children's version) and urinary fluoride levels in the children.
- ***Reported association with fluoride exposure:*** Yes: Significant correlation between urinary fluoride and visuospatial organization (r = −0.29) and visual memory (r = −0.27) scores. No significant correlations with arsenic.

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Subjects were from the same population and were recruited during the same time frame using the same methods. Although this study compared three sites with antecedents of environmental pollution to mixtures of either F–As, Pb–As, or DDT–PCBs, authors evaluated each contaminant separately. The only area of interest is the area with F and As contamination. The area in Durango state (5 de Febrero) where drinking water is polluted naturally with F and As at levels exceeding 6 and 19 times, respectively, the World Health Organization (WHO) limits (WHO 2008). Children attending public schools were screened through personal interviews for study eligibility. Inclusion criteria were children between 6 and 11 years old, living in the study area since birth, and whose parents signed the agreement to participate. Children with a neurological disease diagnosed by a physician and reported by the mother were excluded from the study. The final sample for the F–As was 80. Participation rates were not reported. Selected demographic characteristics are presented in Table 1 of the study.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the populations were similar and recruited during the same time frame using the same methods.
- ***Confounding:***
  - *Rating:* Probably high risk of bias (-)
  - *Summary:* Confounding factors in children tested in the analysis included blood lead (PbB), age, gender, and height-for-age z-scores; only age had significant associations and was included in the final analysis. Arsenic was also assessed and analyzed separately from fluoride. Arsenic in urine was analyzed by atomic absorption spectrophotometer coupled to a hydride system (Perkin-Elmer model AAnalyst 100). Although the model did not adjust for arsenic, arsenic in the F–As group was not associated with either endpoint; therefore, arsenic as a co-exposure is not considered a major concern in this study. PbB was analyzed with a Perkin-Elmer 3110 atomic absorption spectrophotometer using a graphite furnace. Authors note that the mean blood lead level in the F–As study area was 5.2 µg/dL and 8% of the children had values above the reference value of 10 µg/dL. PbB was stated not to affect results and was not included in the final analysis. Other confounding data were obtained during the study interview. Father's education was provided and, in the F–As group, was stated to range from 0–16 years, but this was not considered. Maternal education, smoking, and SES were also not

considered. The authors provide an SES score of 5.9 ± 1.4 for the 5 de Febrero region (the fluoride region). It is not clear if this would vary by fluoride or arsenic levels.

- *Potentially important study-specific confounders:* SES.
  - *Direction/magnitude of effect*: There are insufficient data to determine the magnitude or direction of effect. If there is an association between fluoride exposure and SES, the direction of effect would depend on the association.
- *Basis for rating:* Probably high risk of bias based on indirect evidence that the SES was not accounted for in the study design or analysis and may have varied by fluoride levels.

- ***Attrition:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Data are complete. All 80 participants stated to be the final sample for the site of interest (F–As) were included in all analyses.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that there was no attrition.
- ***Exposure:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Fluoride in urine (FU) was analyzed according to method 8308 (''fluoride in urine'') from the National Institute of Occupational Safety and Health (NIOSH 1984) with a sensitive specific ion electrode. As a quality control check, reference standard ''fluoride in freeze dried urine'' (NIST SRM 2671a) was analyzed. The accuracy was 97.0 +/- 6.0%. Levels of FU and AsU were adjusted for urinary creatinine, which was analyzed by a colorimetric method (Bayer Diagnostic Kit, Sera-Pak1 Plus). However, details on the collection methods were not reported.
    - *Direction/magnitude of effect*: Spot urine samples in a small sample size (i.e., 80 children) may have some exposure misclassification. Adjusting for dilution reduces the potential for misclassification based on differences in dilution. Exposure misclassification would be non-differential.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using well-established methods that directly measured exposure.
- ***Outcome:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* IQ is assessed through the Rey-Osterrieth Complex Figure Test (ROCF). This is a less well-established method, although the authors provide citations suggesting it has been validated and standardized for the Mexican population (+ for methods). According to the study report, the neuropsychologist who administered the test was blinded to all exposure types and levels. (++ for blinding). Overall rating for methods and blinding = +.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* All outcomes outlined in the abstract, introduction, and methods were reported in sufficient details.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.

- ***Other potential threats:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:*
    - *Statistical analyses:* Statistical analyses used log-transformed exposure variables (although rationale was not provided). Crude and partial correlations were calculated to evaluate associations between serum fluoride levels and TOCF scores. There is no other description of the regression model, and regression diagnostics to evaluate model assumptions are not presented; however, the overall impact on effect estimates is expected to be minimal.
    - *Other potential concerns:* None identified.
  - *Basis for rating:* Probably low risk if bias based on indirect evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.
- ***Basis for classification as low risk-of-bias study overall:*** Probably low risk-of-bias ratings in exposure and outcome. Study strengths include individual exposure measurements and outcomes blindly assessed, but it is limited by the cross-sectional study design, lack of addressing SES in the study population, co-exposure with arsenic, and use of spot samples in a small population.

Valdez Jimenez *et al.* (2017)

Study Details:

- ***Study design*:** Prospective cohort
- ***Population*:** Infants aged 3–15 months
- ***Study area:*** Durango City and Lagos de Moreno, Jalisco, Mexico
- ***Sample size:*** 65 infants
- ***Data relevant to the review:*** The Bayley Scales of Infant Development II was used to assess Mental Development Index Scale and the Psychomotor Development Index scale in children 3 to 15 month and evaluated for associations with first and second trimester maternal urine fluoride.
- ***Reported association with fluoride exposure:*** Yes: Significant association between maternal urinary fluoride and MDI score during first trimester (adjusted β = −19.05; SE = 8.9) and second trimester (adjusted β = −19.34; SE = 7.46). No association between maternal fluoride during any trimester and Psychomotor Developmental Index (PDI).

Risk of Bias:

- **Author contacts:**
  - Authors were not contacted for additional information because it was not necessary.
- ***Population selection:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Subjects were recruited from two endemic areas in Mexico. The study authors do not provide information on the similarities or differences between the two areas nor do they indicate if there were different participation rates. However, recruitment methods were the same. Women receiving prenatal care in health centers located in Durango City and Lagos de Moreno, Jalisco, Mexico were recruited in 2013–2014. Participation rates are not likely to be an issue as characteristics were similar

between those who participated and those who did not. Although they did not provide characteristics by area, the characteristics provided do not indicate any differences that may be biased by the selection. Considering the age range for the non-participants, the mean age for non-participants appears to be incorrect (or the age range is incorrect); however, there does not appear to be a difference that would potentially indicate selection bias.

- *Basis for rating:* Probably low risk of bias based on indirect evidence that the exposure groups were similar and were recruited with the same methods in the same time frame, with no evidence of differences or issues with participation/response rates.

- ***Confounding:***
  - *Rating:* Probably high risk of bias (-)
  - *Summary:* Questionnaires were used to obtain information about sociodemographic factors, prenatal history, mother's health status before pregnancy (e.g., use of drugs, vaccines, diseases) and the type of water for drinking and cooking. The marginalization index (MI) was obtained from the National Population Council (CONAPO). Two additional surveys were conducted during the 2nd and 3rd trimester of pregnancy to get information about the mother's health, pregnancy evolution, and sources of water consumption. A survey was also conducted to get information about childbirth (type of birth, week of birth, weight and length of the baby at birth, Apgar and health conditions of the baby during the first month of life). This information was corroborated with the birth certificate. Linear regression models included gestational age, children's age, marginality index, and type of drinking water. Bivariate analysis was conducted on the other factors including child's gender prior to conducting multivariable regression models. Some important confounders were not considered, including parental mental health, IQ, smoking, and potential co-exposures. Water quality maps indicate a potential for arsenic to be present in the study area.
  - *Potentially important study-specific confounders:* Arsenic is a potential co-exposure in this area of Mexico.
    - *Direction/magnitude of effect***:** If arsenic were present as a co-exposure it would bias the results away from the null.
  - *Basis for rating:* Probably high risk of bias based on indirect evidence that there is a potential for co-exposure with arsenic that was not addressed.
- ***Attrition:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Out of the 90 women selected for inclusion in the study, 65 approved the participation of their infants. The authors provide a table of characteristics between women who consented to their children's cognitive evaluation and those that only participated in biological monitoring. There were no significant differences between the groups. There were fewer women who provided urine during the second and third trimesters. All specified children are included in the relevant analyses.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
- ***Exposure:***
  - *Rating:* Definitely low risk of bias (++)

- *Summary:* Fluoride exposure is assessed through morning urine samples and water fluoride levels collected from the children's homes. Sampling methodology is appropriately documented, and water levels were quantified through specific ion-sensitive electrode assays. QC was described and accuracy was >90%. Urinary fluoride was corrected by specific gravity.
  - *Direction/magnitude of effect***:** Not applicable.
- *Basis for rating:* Definitely low risk of bias based on direct evidence that exposure was consistently assessed using well-established methods that directly measured exposure.

- ***Outcome:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Neurodevelopment was assessed with the Bayley Scales of Infant Development II (BSDI-II) that was noted to be reliable and valid for evaluating children from 3 months to 5 years of age. The average age of children assessed was 8 months, with a range of 3–15 months) (++ for methods). The study report stated that a trained psychologist who was blinded about the mother's fluoride exposure evaluated the infants at home (++ for blinding). Overall rating for methods and blinding = ++.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that the outcome was blindly assessed using instruments that were valid and reliable in the study population.
- ***Selective Reporting:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* All outcomes outlined in the abstract, introduction, and methods were reported. Table 4 of the study only displays data for trimesters 1 and 2. Although 3rd trimester data were collected, they were not reported, likely because data were only available for 29 subjects. No discussion of this was provided.
  - *Basis for rating:* Probably low risk of bias because, although it appears some data were not reported, it is likely because there were insufficient data and not because the authors were selectively reporting the results.
- ***Other potential threats:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:*
    - *Statistical analyses:* Statistical analyses used log10-transformed exposure variables. Normality, homoscedasticity, and linearity assumptions were tested and satisfied for MDI and PDI scores. Bivariate analyses included correlations, t-tests, and ANOVA. Multiple linear regression models by the 1st and 2nd trimester of pregnancy were used to evaluate the association between maternal fluoride exposure and MDI and PDI scores. The best-fit model was selected using a "stepwise method" and the best-fit line was evaluated using "the curve fitting method." It is not further specified or cited what these methods entailed. Best-fit or goodness-of-fit statistics are not reported. It is unclear how a best-fit model could be selected when the authors state that all models adjusted for the same set of covariates regardless of significance, and these covariates also appear in the final model—presumably the best-fit model. It is unlikely that a stepwise method would retain all those covariates unless they were forced in the model. Residual analysis was conducted to assess model validity; however,

there is no description of the results of the residual analysis. Nonetheless, the impact on effect estimates is expected to be minimal.

- *Other potential concerns:* No other potential concerns were identified. In the peer-review report, NASEM (2020) cited the following as potential concerns: "the large difference in numbers of males and females in the offspring (20 males, 45 females), and apparently incorrect probabilities were reported for age differences between participants and nonparticipants, high rates of cesarean deliveries and premature births among participants (degree of overlap not reported), and incorrect comparisons of observed prematurity rates with national expected rates." However, these concerns were taken in consideration in other domains (***Selection, Confounding)***.

Basis for rating: Probably low risk if bias based on indirect evidence that the statistical analyses were appropriate and there were no other potential threats to risk of bias identified.

- ***Basis for classification as low risk-of-bias study overall:*** Definitely low risk-of-bias ratings in exposure and outcome. Study strengths include individual exposure measurements and outcome blindly assessed, but it is limited by the cross-sectional study design and lack of accounting for potential co-exposures to arsenic.

Wang *et al.* (2020a)

Study Details:

- ***Study design*:** Cross-sectional
- ***Population*:** School children aged 7–13 years
- ***Study area:*** Tongxu County, China
- ***Sample size:*** 325 school children
- ***Data relevant to the review:*** Associations between ADHD and other measures of learning disability with urine fluoride concentrations.
- ***Reported association with fluoride exposure:*** Yes: Significant association between psychosomatic problems and urinary fluoride (per 1-mg/L increase; adjusted β = 4.01 [95% CI: 2.74, 5.28]) and increased risk of a T-score > 70 with urinary fluoride (per 1-mg/L increase; adjusted OR = 1.97 [95% CI: 1.19, 3.27]). No significant associations with ADHD or other measures of learning disability.

Risk of Bias:

- **Author contacts:**
  - Authors were contacted in July of 2020 to obtain additional information for risk-of-bias evaluation. No response was received.
- ***Population selection:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Subjects were recruited in 2017 from Tongxu County, China. Children were selected from four randomly selected primary schools in the area. Selection was based on specified inclusion rules. It was noted that the living habits and diets of the participants from the four schools were well matched, but details were not provided. The area did not have industrial pollution within 1 km of the living environment of the children, and it was noted that the children were not exposed to other

neurodevelopmental toxicants (lead, cadmium, arsenic, or mercury). A table of subject characteristics was provided in the study, but not by school or exposure. This is a pilot study, and it is not explicitly stated if all eligible subjects participated in the study. There is no information on participation rates or if they varied by school.

- *Basis for rating:* Probably low risk of bias based on indirect evidence that the exposed groups were recruited using similar methods during the same time frame and that any differences between the exposed groups were considered in the statistical analyses.

- ***Confounding:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* It was noted that subjects were well matched in terms of living habits and diets, but there were no specifics provided. It was noted that there was no industrial exposure or exposure to other neurotoxins such as lead, cadmium, arsenic, or mercury. Covariates were collected using a standardized and structured questionnaire completed by the children and their guardians under the direction of investigators, but reliability or validity of the questionnaire was not reported. Information collected included age, gender, weight, height, parental education level, and parental migration (or work as migrant workers). IQ scores evaluated by the Combined Raven's Test-the Rural in China were used to represent basic cognitive function. Models were adjusted for age, BMI, gender, mother and father migration, and urinary creatinine. Adjustments were not made for parental education, race/ethnicity, maternal demographics (e.g., maternal age, BMI), parental behavioral and mental health disorders (e.g., ADHD, depression), smoking (e.g., maternal smoking status, secondhand tobacco smoke exposure), reproductive factors (e.g., parity), iodine deficiency/excess, maternal (and paternal) IQ, quantity and quality of caregiving environment (e.g., HOME score), or SES other than parental migration. There is no evidence to suggest that SES would differ substantially among the four rural schools in the same area of China that were randomly selected.
  - *Potentially important study-specific confounders:* SES.
    - *Direction/magnitude of effect***:** Direction and magnitude is unknown. It was noted that the subjects were matched in terms of living habits and diet and this could be an indication that SES was not different among the groups, but details were not provided.
  - *Basis for rating:* Probably low risk of bias because there is indirect evidence that the key confounders are considered, that the methods for collecting the information were valid and reliable, and that co-exposure to arsenic is not an issue in this area.
- ***Attrition:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* Data are complete. It was noted that there were 325 subjects included and results were available on all subjects.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that there was no attrition.
- ***Exposure:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:* Spot urine samples were collected from each child in the early morning into cleaned polyethylene tubes. Fluoride concentrations were measured using fluoride ion-selective electrode (with reference to Ma *et al.* (2017); however, that reference cites

Zhou *et al.* (2012)). Therefore, no QC methods or LODs were available. Fluoride concentrations were creatinine-adjusted.

- *Direction/magnitude of effect*: Spot urine samples only account for recent exposure. Although this could cause there to be some exposure misclassification, the number of subjects should help dilute any issues with the non-differential misclassification.

- *Basis for rating:* Probably low risk of bias based on indirect evidence that exposure was consistently assessed using acceptable methods that provide individual levels of exposure.

- ***Outcome:***
  - *Rating:* Probably high risk of bias (NR)
  - *Summary:* Children's behavior was assessed by the Chinese version of the Conners' Parent Rating Scale-Revised (CPRS-48). The homogeneity reliability of Cronbach α in the Chinese version of CPRS-48 was 0.932; the correlation of Spearman-brown split-half was 0.900; and the retest reliability of total score was 0.594. Raw scores for each subscale are converted into sex- and age-adjusted T-scores within a mean ± standard deviation (SD) of 50 ± 10. The guardians independently completed the CPRS-48 according to the instruction manual under the direction of trained investigators (++ for methods). Blinding is not reported. Although it is unlikely that the outcome assessors were aware of the fluoride levels in the urine, it is unclear if subjects were selected based on areas with endemic fluoride or if parents were aware of fluoride concentrations in the areas. (NR for blinding). Overall rating for methods and blinding = NR.
  - *Basis for rating:* Probably high risk of bias based on no information provided to indicate that the outcome was blindly assessed.
- ***Selective Reporting:***
  - *Rating:* Definitely low risk of bias (++)
  - *Summary:* All outcomes in the abstract, introduction, and methods are reported in sufficient details.
  - *Basis for rating:* Definitely low risk of bias based on direct evidence that all measured outcomes were reported.
- ***Other potential threats:***
  - *Rating:* Probably low risk of bias (+)
  - *Summary:*
    - *Statistical analyses:* Multiple linear regression models were used to assess the association between urinary fluoride exposure and each behavioral outcome. Logistic regression was used to assess the risk of behavioral problems (T-scores > 70) due to fluoride exposure. Sensitivity analyses were performed, with models adjusting for combinations of age, BMI, gender, mother migrated, father migrated, and urinary creatinine levels. Regression diagnostics to evaluate model assumptions are not described; however, the overall impact on effect estimates is expected to be minimal.
    - *Other potential concerns:* None identified.
  - *Basis for rating:* Probably low risk of bias based on indirect evidence that the statistical analyses were appropriate and no other potential threats to risk of bias were identified.

- ***Basis for classification as low risk-of-bias study overall:*** Probably low risk-of-bias ratings in confounding and exposure. Study strengths include individual exposure measurements, but it is limited by the cross-sectional study design and lack of details on blinding of the outcome assessment. All key confounders were considered in the study design or analysis.

Appendix 5. Mechanistic Data from Animal Studies

A number of animal studies were available that presented mechanistic data in several effect categories (see **Figure A5-1**). Limiting the data to studies with at least one exposure at or below 20 ppm fluoride drinking water equivalents (gavage and dietary exposures were back-calculated into equivalent drinking water concentrations for comparison) still provided a sufficient number of studies for evaluation of several mechanistic endpoints while allowing for a more focused look at exposure levels most relevant to human exposures. The following sections summarize the mechanistic data by the effect category. Although there is some evidence of consistency in mechanistic effects, overall, these data are insufficient to increase confidence in the assessment of findings from human epidemiology studies.

Figure A5-1. Number of Animal Mechanistic Studies for Fluoride by Mechanistic Category and Exposure Level*

	Dose Level	
Mechanism	**All**	**≤20 ppm**
Biochemical (brain/neurons)	66	25
Neurotransmitters	53	23
Oxidative stress	78	25
Histopathology	67	30
Apoptosis/cell death	19	7
Inflammation	7	5
Thyroid	50	17
Other	3	2

*Interactive figure and additional study details in Tableau® (https://public.tableau.com/app/profile/ntp.visuals/viz/Animal_Mechanisms_2021/FigureA5-1). The number of studies that evaluated mechanistic effects associated with at least one exposure at or below 20 ppm fluoride is tabulated in the "≤20 ppm" column. The total number of studies per mechanistic category are summarized in the "All" column.

Neurotransmitters

Neurotransmitter and biochemical changes in the brain and neurons were considered to be the mechanistic areas with the greatest potential to demonstrate effects of fluoride on the brain of animals in the lower dose range and provide evidence of changes in the brain that may relate to lower IQ in children (see **Figure A5-2**). Twenty of 23 neurotransmitter studies assessed changes in brain cholinesterase activity associated with fluoride exposure at or below 20 ppm fluoride. Acetylcholine is a major neurotransmitter involved in learning, memory, and intelligence (Chen 2012, Gais and Schonauer 2017). AChE is responsible for the breakdown of acetylcholine in the synapses of nerve cells. Changes in cholinesterase, acetylcholine, or AChE could be related to effects on memory. Evidence of an effect varied among the low risk-of-bias studies that assessed changes in cholinesterase or acetylcholine (n = 11 drinking water studies) (Gao *et al.* 2009, Baba *et al.* 2014, Adedara *et al.* 2017a, Khan *et al.* 2017, Gao *et al.* 2008a, Akinrinade *et al.* 2015a, Sun *et al.* 2000 [translated in Sun *et al.* 2008], Chouhan *et al.* 2010, Mesram *et al.* 2016, Liu *et al.* 2010, Nkpaa and Onyeso 2018), with the majority of studies reporting evidence of an effect that is considered inconsistent with the phenotypic outcome. Decreases in cholinesterase will cause increases in acetylcholine, which can have a positive effect on learning and

memory; however, long-term decreases in cholinesterase can lead to secondary neuronal damage occurring in the cholinergic region of the brain (Chen 2012).

Five of the 11 studies with low risk of bias (Gao *et al.* 2009, Baba *et al.* 2014, Adedara *et al.* 2017a, Khan *et al.* 2017, Nkpaa and Onyeso 2018) found statistically significant decreases in cholinesterase or AChE in brain homogenates (with some brains dissected into specific regions prior to homogenizing) with fluoride concentrations in drinking water at or below 20 ppm, and 4 of the 5 studies found statistically significant decreases in cholinesterase or AChE below 10 ppm. The 5 studies were conducted in rats (Wistar or Sprague-Dawley) with exposure ranging from 28 days to 6 months. An additional 2 out of 11 studies (Gao *et al.* 2008a, Akinrinade *et al.* 2015a) reported decreases in brain homogenate AChE at concentrations at or below 20 ppm fluoride in drinking water, but statistical significance was not reached. These studies were also conducted in rats with exposure for 30 days or 3 months. Gao *et al.* (2008a) reported a dose-dependent decrease in brain homogenate AChE in the low (5 ppm fluoride) and high (50 ppm fluoride) treatment groups compared with the control group, but the decrease was only statistically significant in the high dose group. Similarly, Akinrinade *et al.* (2015a) observed a dose-dependent decrease in percent intensity of AChE immunohistochemistry in the prefrontal cortex associated with 2.1 and 10 ppm sodium fluoride in the drinking water, but neither result was statistically significant. Gao *et al.* (2009) found lower brain homogenate AChE levels in the 5-ppm animals compared with the 50-ppm animals; therefore, the results were not always dose dependent.

Relative to the above-mentioned studies, 2 of the 11 low risk-of-bias studies observed opposite effects on brain cholinesterase levels. Sun *et al.* (2000) [translated in Sun *et al.* 2008] observed a significant increase in brain cholinesterase in Kunming mice associated with fluoride drinking water concentrations from 10 to 100 mg/L, but did not observe a dose response. Chouhan *et al.* (2010) did observe a dose-related increase in AChE levels in brain homogenate of Wistar rats with sodium fluoride concentrations of 1 to 100 ppm for 12 weeks and noted statistically significant results at 1, 50, and 100 ppm but not at 10 ppm.

Mesram *et al.* (2016) did not assess changes in AChE but observed a significant decrease in acetylcholine levels in cerebral cortex homogenate through 30 days of age in rats treated in utero with 20 ppm sodium fluoride, which may suggest an increase in AChE levels. Likewise, Liu *et al.* (2010) did not assess changes in AChE, but measured nicotinic acetylcholine receptors (nAChRs) in brain homogenate of rats following drinking water fluoride exposure, which the authors stated could modulate physiological and pharmacological functions that are involved in learning and memory-related behaviors. Significant decreases in the protein expressions of nAChR subunits at 2.26 ppm fluoride were observed; however, the corresponding receptor subunit mRNAs did not exhibit any changes (Liu *et al.* 2010).

The studies that assessed other neurotransmitters of the brain and neurons were too heterogeneous or limited in number to make any determination on mechanism, even before limiting the review of the data to low risk-of-bias studies. There were only five studies that evaluated dopamine and/or metabolites (Tsunoda *et al.* 2005, Chouhan *et al.* 2010, Reddy *et al.* 2014, Banala *et al.* 2018, Sudhakar and Reddy 2018). Four of the studies observed decreases in dopamine levels in the brain with exposures less than 20 ppm fluoride (Reddy *et al.* 2014, Chouhan *et al.* 2010, Banala *et al.* 2018, Sudhakar and Reddy 2018); however, the fifth study (Tsunoda *et al.* 2005) observed increased dopamine and metabolites at fluoride exposures below 20 ppm (with statistical significance achieved only for the metabolite homovanillic acid in one brain region). No differences from the control group were observed at levels above 20 ppm fluoride. Other neurotransmitters were evaluated at or below 20 ppm fluoride exposure, but generally only in a couple of studies.

Biochemistry (Brain/Neurons)

Similar to above, the endpoints measured in brain biochemistry studies were too heterogeneous or limited in number to make any determination on potential relevance of mechanism, even before limiting the review of the data to low risk-of-bias studies (see **Figure A5-2**). Endpoints related to biochemical changes in the brain or neurons included carbohydrate or lipid changes, RNA or DNA changes, changes in gene expression, or changes in protein expression. For the most part, only a single study was available for any given endpoint. The largest body of evidence on biochemistry was on protein level in various brain regions. Eleven low risk-of-bias studies were identified that evaluated protein levels; however, few studies evaluated the same proteins or areas of the brain. In the few cases where the same protein was evaluated, results were not always consistent. These data are insufficient to increase confidence or support a change to hazard conclusions.

Histopathology

Histological data can be useful in determining whether effects are occurring in the brain at lower fluoride concentrations; however, author descriptions of these effects may be limited thereby making it difficult to directly link histological changes in the brain to learning and memory effects. Histopathology of the brain was evaluated in 31 studies with concentrations at or below 20 ppm fluoride, of which 15 studies were considered low risk-of-bias studies (Adedara *et al.* 2017b, Akinrinade *et al.* 2015a, Bhatnagar *et al.* 2002, Bhatnagar *et al.* 2011, Chouhan *et al.* 2010, Guner *et al.* 2016, Jiang *et al.* 2014, Lou *et al.* 2013, McPherson *et al.* 2018, Mesram *et al.* 2016, Niu *et al.* 2018, Pulungan *et al.* 2016, Nageshwar *et al.* 2018, Zhao *et al.* 2019, Jia *et al.* 2019). In all but one low risk-of-bias study [Pulungan *et al.* (2016); gavage], animals were exposed to fluoride via drinking water. All low risk-of-bias studies were conducted in rodents, and all but three studies were conducted in rats (Wistar [seven studies]; Sprague-Dawley [four studies]; Long-Evans hooded [one study]). Overall, the low risk-of-bias studies that evaluated histopathology in the brain had low potential for bias for key questions regarding randomization and exposure characterization; however, eight studies were rated as probably high risk of bias for the key risk-of-bias question regarding outcome assessment based on lack of reporting of blinding of outcome assessors and/or inadequate description of outcome measures or lesions. Moreover, low image quality in some of the studies hampered the ability to verify the quality of the data. Further technical review of the 15 low risk-of-bias studies was conducted by a board-certified pathologist. Based on confidence in the results for each study, the technical reviewer further categorized the low risk-of-bias studies as studies with higher or low confidence in the outcome assessment, which is reflected in the following summary of the brain histopathology results. Main limitations of the histopathology data identified by the pathologist included lack of information on methods of euthanasia and fixation. Perfusion fixation is generally considered the best practice for lesions of the central nervous system in addition to complete fixation of the brain prior to its removal from the skull (Garman *et al.* 2016). Four of the low risk-of-bias studies reported that they used this method (Bhatnagar *et al.* 2002, Bhatnagar *et al.* 2011, McPherson *et al.* 2018, Pulungan *et al.* 2016). Two of the low risk-of-bias studies handled the brains before fixation was complete, which can produce artifacts that can resemble dead neurons (Zhao *et al.* 2019, Nageshwar *et al.* 2018). Fixation and brain removal details were inadequately described in the remaining low risk-of-bias studies.

Although there was heterogeneity in the endpoints reported (e.g., cell size, shape, and counts; nuclei fragmentation; increased vacuolar spaces) and some variation in the consistency of the evidence based on the area of the brain evaluated, the majority of the low risk-of-bias studies (11 of 14 drinking water studies) found some histological change in the brain of rats or mice treated with fluoride at concentrations at or below 20 ppm, of which 8 studies reported histological changes in the brain at or below 10 ppm. Histological changes in the hippocampus (one of the areas of the brain most evaluated for histological changes) associated with fluoride exposures at or below 20 ppm were reported in three

of four low risk-of-bias studies with higher confidence in the outcome assessment (Bhatnagar *et al.* 2002, Bhatnagar *et al.* 2011, Guner *et al.* 2016) and in three of four low risk-of-bias studies with lower confidence in the outcome assessment (Jiang *et al.* 2014, Niu *et al.* 2018, Nageshwar *et al.* 2018). McPherson *et al.* (2018) was the only drinking water study (with higher confidence in the histopathology outcome assessment) that did not observe any histological changes in hippocampus at 10 or 20 ppm fluoride in male Long-Evans hooded rats exposed in utero through adulthood (>PND80). Although there are too few studies to definitively explain the inconsistency in results, McPherson *et al.* (2018) also did not observe any associations between fluoride exposure and impairments to learning and memory, which is inconsistent with the majority of developmental exposure studies that observed learning and impairments associated with fluoride exposure for other strains of rats. Similarly, histological changes in the cortex were reported in three of the four low risk-of-bias drinking water studies with higher confidence in the outcome assessment (Chouhan *et al.* 2010, Bhatnagar *et al.* 2011, Akinrinade *et al.* 2015a) and in three of four low risk-of-bias studies with lower confidence in the outcome assessment (Lou *et al.* 2013, Mesram *et al.* 2016, Nageshwar *et al.* 2018).

Histological changes were also consistently reported in other areas of the brain in studies with higher confidence in the outcome assessment, including the amygdala, caudate putamen, cerebellum, and hypothalamus, although each of these areas of the brain were only evaluated in one low risk-of-bias study (Bhatnagar *et al.* 2011, Guner *et al.* 2016). Pulungan *et al.* (2016), one of two low risk-of-bias studies with higher confidence in the outcome assessment that did not report histological changes in the brain, observed a decreasing trend in the number of pyramidal cells in the prefrontal cortex with increasing dose, but this was not changed at concentrations below 20 ppm (study administered sodium fluoride via gavage; the 5-mg/kg-day dose was considered to be equivalent to 15.3 ppm fluoride in drinking water) nor were any of the results statistically significant.

Oxidative Stress

Oxidative stress is considered a general mechanistic endpoint that cannot be specifically linked to neurodevelopmental or cognitive effects in humans; however, like histopathology, it may help in identifying changes in the brain occurring at lower concentrations of fluoride. Oxidative stress in the brain was evaluated in 25 studies that examined concentrations at or below 20 ppm fluoride, of which 15 studies had low potential for bias (Adedara *et al.* 2017a, Adedara *et al.* 2017b, Akinrinade *et al.* 2015b, Chouhan *et al.* 2010, Gao *et al.* 2008b, Guner *et al.* 2016, Mesram *et al.* 2016, Nkpaa and Onyeso 2018, Shan *et al.* 2004, Zhang *et al.* 2015a, Chouhan and Flora 2008, Gao *et al.* 2009, Khan *et al.* 2017, Bartos *et al.* 2018, Nageshwar *et al.* 2018). All of the low risk-of-bias studies were conducted in rats (mainly Wistar or Sprague-Dawley) and administered fluoride via drinking water with exposure durations ranging from 28 days to 7 months. Although there was heterogeneity in the endpoints reported (i.e., varying measures of protein oxidation, antioxidant activity, lipid peroxidation, and reactive oxygen species [ROS]) and some variation in the consistency of the evidence based on the endpoint, the majority of the studies (13 of 15 studies) (Adedara *et al.* 2017a, Adedara *et al.* 2017b, Akinrinade *et al.* 2015b, Gao *et al.* 2008b, Gao *et al.* 2009, Guner *et al.* 2016, Mesram *et al.* 2016, Nkpaa and Onyeso 2018, Shan *et al.* 2004, Zhang *et al.* 2015a, Khan *et al.* 2017, Nageshwar *et al.* 2018, Bartos *et al.* 2018) found evidence of oxidative stress in the brains of rats treated with fluoride at concentrations at or below 20 ppm, of which 10 studies reported oxidative stress in the brain below 10 ppm fluoride. The most consistent evidence of oxidative stress in the brain was reported through changes in antioxidant activity. Eleven of the 12 low risk-of-bias studies that evaluated antioxidant activity reported an effect at concentrations at or below 20 ppm (Adedara *et al.* 2017a, Adedara *et al.* 2017b, Akinrinade *et al.* 2015b, Gao *et al.* 2008b, Gao *et al.* 2009, Guner *et al.* 2016, Mesram *et al.* 2016, Nkpaa and Onyeso 2018, Khan *et al.* 2017, Bartos *et al.* 2018, Nageshwar *et al.* 2018). Decreases in antioxidant activity using measures of superoxide dismutase (SOD) activity were reported in seven of

eight low risk-of-bias studies (Adedara *et al.* 2017a, Adedara *et al.* 2017b, Akinrinade *et al.* 2015b, Mesram *et al.* 2016, Nkpaa and Onyeso 2018, Khan *et al.* 2017, Nageshwar *et al.* 2018) and, among these seven studies, all that also measured changes in catalase (CAT) activity (n = 6 studies) also reported decreased activity (Adedara *et al.* 2017a, Adedara *et al.* 2017b, Mesram *et al.* 2016, Nkpaa and Onyeso 2018, Khan *et al.* 2017, Nageshwar *et al.* 2018). A decrease in total antioxidant capacity (T-AOC) as a measure of antioxidant activity was also consistently reported in two low risk-of-bias studies (Gao *et al.* 2008b, Gao *et al.* 2009), and a decrease in glutathione peroxidase (GPx) activity was reported in two of three low risk-of-bias studies (Adedara *et al.* 2017b, Nkpaa and Onyeso 2018).

Relative to the above-mentioned studies, 2 of the 15 low risk-of-bias studies (Chouhan and Flora 2008, Chouhan *et al.* 2010) did not observe statistically significant effects on oxidative stress in the brain with concentrations at or below 20 ppm fluoride; however, the measure of oxidative stress evaluated in Chouhan and Flora (2008) and Chouhan *et al.* (2010) (glutathione [GSH] to oxidized glutathione [GSSG] ratio as an indication of antioxidant activity and ROS levels) were not evaluated in any other low risk-of-bias study. Chouhan and Flora (2008) observed a dose-dependent increase in ROS levels associated with 10, 50, and 100 mg/L sodium fluoride in the drinking water; however, results were not statistically significant at any dose. In Chouhan *et al.* (2010), the levels of ROS were significantly higher at 50 ppm sodium fluoride in drinking water, but statistical significance was not met at doses below 20 ppm fluoride (1 and 10 ppm sodium fluoride) or at 100 ppm sodium fluoride; yet, hydrogen peroxide levels as a measure of ROS were found to be significantly increased at 15 ppm sodium fluoride in drinking water in studies conducted by another group of authors (Adedara *et al.* 2017a, Adedara *et al.* 2017b).

Apoptosis/Cell Death

Seven low risk-of-bias studies were identified that evaluated apoptosis with concentrations at or below 20 ppm fluoride. Results from these studies were inconsistent and were insufficient for evaluating fluoride-induced apoptosis. These data are insufficient to increase confidence or support a change to hazard conclusions.

Inflammation

Five low risk-of-bias studies were identified that evaluated potential effects of fluoride on inflammation with concentrations at or below 20 ppm. The inflammation markers were too heterogeneous or limited in number to make any determination on potential relevance of mechanism, even before limiting the review of the data to low risk-of-bias studies. These data are insufficient to increase confidence or support a change to hazard conclusions.

Thyroid

Seventeen studies were identified that evaluated potential effects of fluoride on the thyroid with concentrations at or below 20 ppm (see **Figure A5-1**). These animal thyroid data are not further described because this endpoint has been directly evaluated in a number of human studies that have failed to identify consistent evidence to suggest that thyroid effects are a requisite mechanism by which fluoride causes neurodevelopmental or cognitive effects in humans.

Figure A5-2. Number of Low Risk-of-bias Animal Studies that Evaluated Biochemical, Neurotransmission, and Oxidative Stress Effects at or Below 20 ppm by Mechanism Subcategory and Direction of Effect*

Mechanism	Mechanism Subcategory	Direction of Effect ↑	↓	NS	Grand Total
Biochemistry (brain/neurons)	Carbohydrate/lipid-related		1	1	2
	Gene expression		1		1
	Protein levels associated with brain function	8	7	7	11
	Other biochemical	2			2
Neurotransmitters	Cholinesterase	2	7	3	11
	Dopamine and metabolites		1		1
	Other neurotransmitters	2	2	1	2
Oxidative stress	Antioxidant activity	1	10	4	12
	Lipid peroxidation byproduct	7		4	11
	Protein oxidation	2		1	2
	ROS	2		2	4

*Interactive figure and additional study details in Tableau® (https://public.tableau.com/app/profile/ntp.visuals/viz/Fluoride_Animal_SelectMechanisms_2021/FigureA5-2). This figure displays study counts for low risk-of-bias studies, as these counts are most relevant to the text in this section. Counts for high risk-of bias studies or all studies combined can be accessed in the interactive figure in Tableau®. Study counts are tabulated by significance—statistically significant increase (↑), statistically significant decrease (↓), or not significant (NS). For example, the "↑" column displays numbers of unique studies with at least one endpoint in the mechanistic subcategory with significantly increasing results at fluoride exposure levels of ≤20 ppm. These columns are not mutually exclusive (i.e., a study may report on multiple endpoints with varying results within a single mechanistic subcategory and therefore may be reflected in the counts for the "↑", "↓", and NS columns, but would only be counted once in the Grand Total column). Endpoints, species, strain, sex, and exposure duration are available for each study in the interactive figure in Tableau®.

National Toxicology Program
U.S. Department of Health and Human Services

DRAFT NTP MONOGRAPH ON THE

SYSTEMATIC REVIEW OF FLUORIDE EXPOSURE AND NEURODEVELOPMENTAL AND COGNITIVE HEALTH EFFECTS*

Revised September 16, 2020

*The September 6, 2019 draft monograph was peer reviewed by a committee convened by the National Academy of Sciences, Engineering, and Medicine (NASEM). This current draft incorporates changes in response to that review and is being submitted to the same NASEM committee for an additional round of peer review.

Office of Health Assessment and Translation
Division of the National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOREWORD

The National Toxicology Program (NTP), established in 1978, is an interagency program within the Public Health Service of the U.S. Department of Health and Human Services. Its activities are executed through a partnership of the National Institute for Occupational Safety and Health (part of the Centers for Disease Control and Prevention), the Food and Drug Administration (primarily at the National Center for Toxicological Research), and the National Institute of Environmental Health Sciences (part of the National Institutes of Health), where the program is administratively located. NTP offers a unique venue for the testing, research, and analysis of agents of concern to identify toxic and biological effects, provide information that strengthens the science base, and inform decisions by health regulatory and research agencies to safeguard public health. NTP also works to develop and apply new and improved methods and approaches that advance toxicology and better assess health effects from environmental exposures.

NTP conducts literature-based evaluations to determine whether exposure to environmental substances (e.g., chemicals, physical agents, and mixtures) may be associated with adverse health effects. These evaluations result in hazard conclusions or characterize the extent of the evidence and are published in the NTP Monograph series, which began in 2011. NTP Monographs serve as an environmental health resource to provide information that can used to make informed decisions about whether exposure to a substance may be of concern for human health.

NTP conducts these health effects evaluations following pre-specified protocols that apply the general methods outlined in the "Handbook for Conducting a Literature-Based Health Assessment Using the OHAT Approach for Systematic Review and Evidence Integration."[1] The protocol describes project-specific procedures tailored to each systematic review in a process that facilitates evaluation and integration of scientific evidence from published human, experimental animal, and mechanistic studies.

The key feature of the systematic review approach is the application of a transparent framework to document the evaluation methods and the basis for scientific judgements. This process includes steps to comprehensively search for studies, select relevant evidence, assess individual study quality, rate confidence in bodies of evidence across studies, and then integrate evidence to develop conclusions for the specific research question. Draft monographs undergo external peer review prior to being finalized and published.

NTP Monographs are available free of charge on the NTP website and cataloged in PubMed, a free resource developed and maintained by the National Library of Medicine (part of the National Institutes of Health). Data for these evaluations are included in the Health Assessment and Workspace Collaborative.

For questions about the monographs, please email NTP or call 984-287-3211.

[1] OHAT is the abbreviation for Office of Health Assessment and Translation, which is within the Division of the National Toxicology Program at the National Institute of Environmental Health Sciences.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

TABLES:

FIGURES:

DATA FIGURES:

RISK-OF-BIAS FIGURES: