Scientific review on

the link between the narcotic effects of solvents and (developmental) neurotoxicity

Final report

Prepared for ECHA

Contract Number : ECA.6677-E3

DMG de Groot
Cerespark 1, 5844AE Stevensbeek, NL

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Prepared for the European Chemicals Agency (ECHA)
by
DMG de Groot PhD, ERT
Cerespark 1, 5844AE Stevensbeek, NL
Tel: +31 644 163 121 Email: didima_de_groot@hotmail.com

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Disclaimer

The views and propositions expressed herein are, unless otherwise stated, those of DMG de Groot and do not necessarily represent any official view of ECHA.

Table of Contents

Glossary

ATE, acute toxicity estimate
ATSDR, Agency for Toxic Substances and Disease Registry
BGS, brain growt spurt
BGSP, brain growth spurt period
C, ceiling value
Ca, potential occupational carcinogen
CAS, Chemical Abstracts Service
CDC, Centers for Disease Control and Prevention
CID, compound identifier
CNS, central nervous system
CRC, CRC Press, a publishing group
DHHS, Department of Health and Human Services
EEG, electroencephalography
EPA, Environmental Protection Agency
ER, evaporation rate
FDA, Food and Drug Administration
FOB, functional observational battery
FP, flash point
GD, gestational day
GESTIS, Gefahrstoffinformationssystem
IDLH, immediately dangerous to life or health
IFA, Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung
IUPAC name, International Union of Pure and Applied Chemistry
LD_{50}, median lethal dose
LD_{Lo}, lowest lethal dose
LOAEL, lowest observed adverse effect level
Log Kow, log octanol-water partition coefficient
Log Pow, log octanol-water partition coefficient
MSDS, material safety data sheet
NIOSH, National Institute for Occupational Safety and Health
NOAEC, no observed adverse effect concentration
NOAEL, no observed adverse effect level
NS, nervous system
OECD TG, Organization for Economic Co-operation and Development test guideline
OSHA, Occupational Safety and Health Administration
OT, odor threshold
OTS, Office of Technology Solutions
PEL, permissible exposure limit
PNDT, prenatal developmental toxicity
QSAR, quantitative structure–activity relationship
RA, radioactive
RDT, repeated dose toxicity

REL, recommended exposure limit
ST, short term
STOT SE 3: H336, specific target organ toxicity, single exposure, category 3, may cause drowsiness or dizziness
TK, toxicokinetics
TLV, threshold limit value
TWA, time-weighted average
VP, vapor pressure

Executive summary

The present review is a scientific evaluation of the current literature on the specificity of 'adverse' effects on the nervous system, of organic solvents with known narcotic properties (dizziness, drowsiness and central nervous system (CNS) depression), and on the underlying mechanisms and modes of action. At present, neurotoxicity in adults is considered as of particular concern for developmental neurotoxicity. However, whether narcosis in adults is to be considered as part of the spectrum of neurotoxicity and thus of a concern for developmental neurotoxicity needs further clarification.

To explore this, a selection of narcotic organic solvents –mostly registered under REACH and having harmonized classification as STOT SE 3 H336 (i.e. may cause dizziness, drowsiness, CNS depression)– were *grouped* based on their chemical structure, and the groups were ranked with increasing polarity. Information on narcosis and (developmental) neurotoxicity reported in acute and repeated dose toxicity studies, and developmental neurotoxicity (as far as available) was collected for each substance in a group.

Evaluation of the available literature on the mechanisms and modes of action for narcosis and neurotoxicity induced by organic solvents led to the following conclusions:

1. There is strong evidence that *narcosis* following *acute* exposure to organic solvents is –for a large part– caused by partitioning of organic solvents into the phospholipid bilayer of neuronal cells, and there is evidence that the physico-chemical properties of a particular solvent are important determinants for its specific effects on membrane geometry.
2. Specific *neurotoxic* effects, which may be observed on *sub-chronic or chronic (often low-level)* exposure to solvents, are more likely to involve interactions of solvents or their biotransformation products with specific targets in the nervous system, in particular NMDA and GABA receptors.
3. Organic solvents with narcotic properties in repeated dose toxicity studies such as toluene partly act via NMDA receptors and may thus be mechanistically linked with learning and memory impairments in the (developing) brain. However, there is no further information on other substances with similar narcotic properties to support this assumption.
4. Most recently it has been suggested that the *inhibitory* action of $GABA_A$ receptors contributes to the narcotic/anesthetic-like activities of toluene, and of general anesthetics such as propofol. Importantly, $GABA_A$ receptors act differently during development compared to adulthood; the early exposure leads to *excitation* rather than inhibition of $GABA_A$ receptors. This excitation, together with NMDA receptor activation, may potentially trigger excess apoptosis affecting neural development.

Only specific organic solvents (n-hexane; the aromatic hydrocarbons benzene, toluene, styrene, and xylene; the halogenated solvents trichloroethylene and 1-bromopropane; the alcohols n-propanol,

and n-butanol) displayed narcotic effects after an acute exposure, neurotoxicity or narcosis after repeated dosing, and developmental neurotoxicity, suggesting a possible link between narcosis and (developmental) neurotoxicity. Most of the substances with narcotic effects after an acute exposure did *not* show neurotoxicity in adults or in developing organisms. In this respect however it should be noted that investigations for developmental neurotoxicity are often lacking, potentially due to lack of identified concerns from the studies in adults or with structurally similar substances. It is hypothesised that interaction with GABA and/or NMDA receptors might mechanistically explain (developmental) neurotoxicity. However, a clear and undisputed mechanistic link between narcosis or anesthesia and (developmental) neurotoxicity has not been established in the literature. On a general level, it cannot be concluded that narcotic effects exerted by organic solvents after high acute exposure levels or even after lower and repeated exposure are automatically linked to neurotoxicity or developmental neurotoxicity for a specific substance.

1. INTRODUCTION

1.1 Introduction

In the context of REACH, chemicals are subjected to a number of tests to evaluate their safety. In 2011, OECD accepted the OECD test guideline (TG) 443 for an Extended One-Generation Reproductive Toxicity Study (EOGRTS) as a formal test guideline (OECD, 2011a). The test method was included in REACH information requirements in March 2015 with a requirement to include investigations on developmental neurotoxicity based on a particular concern as specified in REACH Annexes and ECHA Guidance on information requirements and chemical safety assessment, endpoint specific guidance R.7a, reproductive toxicity R. 7.6, version 1.1, October 2015.

With regard to a cohort for testing of developmental neurotoxicity of the first generation offspring (F1-animals) the OECD test guideline 443 recognizes *'Cohort 2A'*, i.e. assessment of developmental neurotoxicity (DNT) as adults (auditory startle, functional observation battery, motor activity, neuropathology (mature nervous system: perfusion fixation, brain weight, full neuro-histopathology), and *'Cohort 2B'*, i.e. assessment of developmental neurotoxicity (DNT) at weaning (neuropathology of developing nervous system); optional: perfusion fixation, brain weight, microscopic examination of the brain).
The DNT cohort should be included if specific external triggers are documented or predicted. Recognized triggers are e.g. functional or morphological alterations observed in mature nervous system; chemical class or structure/activity relationships (SAR); evidence of specific mechanisms/modes of action of the substance with an association to (developmental) neurotoxicity (e.g cholinesterase inhibition or relevant changes in thyroidal hormone levels associated to adverse effects); thyroid weight and pathology; altered surface righting reflex.

The main research question for this review that focuses on the evaluation of toxicity of organic solvents with narcotic properties is
whether narcosis in adults is to be considered as part of the spectrum of neurotoxicity, and thus a concern for developmental neurotoxicity. This needs clarification.

1.2 Organic solvents

1.2.1 How are organic solvents defined in this review?

The term 'solvents' refers to liquid organic chemicals –here we address *'liquid at room temperature'*– used to dissolve, suspense or extract one or more other substances, *without causing a chemical change to either the compound or the solvent itself* (Garlantezec et al., 2009). *Organic* solvents are carbon-based solvents, i.e. unlike inorganic solvents they contain carbon in their molecular structure. Traditional organic compounds only comprise carbon and hydrogen (hydrocarbons). These traditional hydrocarbon solvents with only carbon and hydrogen are nonpolar; however, many organic solvents may contain functional groups with electronegative elements (i.e. oxygen), making the molecule polar. Typically, organic solvents are in a liquid state at ambient temperature and pressure.

Organic solvents comprise a large and structurally diverse group of chemical compounds and form most prevalent sources of chemical exposures in working populations (Teaf, 2000). They are used routinely in commercial industries and are present in most everyday products. The chemical structures of organic solvents have often been used to categorize them.

An updated table (March 2016) listing the most common solvents and their properties is posted on the website of the American Chemical Society (Division of Organic Chemistry), but more are available from different sources
https://www.organicdivision.org/orig/organic_solvents.html

1.2.2 Solvent properties: volatility, lipophilicity and small molecule size

The large groups of organic solvents share but few chemical properties. They also share little physical properties. However, when considered from a biological relevant perspective, they do have three very important properties in common. The first is their volatility; volatile chemicals produce vapors readily, at room temperature and normal atmospheric pressure. Vapors escape easily from volatile liquid chemicals. Solvents can vary widely in their volatility, with low boiling point solvents being much more volatile. Evaporation of solvents in air allows easy entrance into an individual's lungs and, from there, directly into brain and other body organs before reaching the liver where metabolism of the solvents primarily occurs by P450 enzymes.
Second, is their fat solubility (lipophilicity); the lipophilicity of solvents enables fast entrance into lipid rich structures such as the nervous system (myelin and neurons).
Third, is a small molecule size; small molecule size means that organic solvents can be absorbed through the skin and can readily enter the blood stream.

In most cases the metabolism results in reduced toxicity and increased elimination of the resulting products, but exceptions do exist. The toxicity of toluene, for example, is reduced when liver enzymes change the compound so that it does not readily cross cell membranes; the toxicity of benzene, however, is increased after being changed into a compound that can attack the blood-forming cells of the bone marrow, causing leukemia. Also genetic variability impacts on an individual's ability to metabolize certain solvents, resulting in increased or decreased toxicity. Furthermore, the liver itself may be prone to toxic damage of the solvent such as e.g. carbon tetrachloride (CCl_4) which may even be worsened by pre-exposure to alcohol (Gilbert, 2011).

Solvents are eliminated from the body by metabolism or exhalation. Concentrations in exhaled air are determined by volatility and fat-solubility, i.e. the more volatile and fat-soluble the solvent, the greater its concentration in exhaled air. In addition, activity of the individual plays a role (the more active, the more ventilation).

1.2.3 Narcotic effects of organic solvents

Despite the fact that organic solvents have been in use for over 100 years, until the 40s of the past century little information was available in the medical literature about their effects on the nervous system. Their narcotic/anesthetic effects have been known for a long time although poorly understood. Diethylether and chloroform were reported to be in use as anesthetics in 1846 and 1847, respectively, and when their safety risks were recognized they were replaced by e.g. fluorinated ethers and other narcotic hydrocarbons some of which are still in use to date.

Narcosis appears to be caused by substances with differences in structure, which suggests a common interaction pathway between the molecules of the substance and the sensitive cells of the nervous system. At the cellular level, the narcotic effects have been associated with a reduction in neuronal excitability caused by changes in membrane structure and function. However, the exact location and mechanism of this action are still largely unknown although much research has been done in this area (reviewed e.g. by Jayakar et al., 2014; Lugli et al. 2009; Olsen and Guo-Dong, 2011; Seeman, 1972; Urban, 2008; Woll et al., 2016).

To learn about underlying mechanisms of the narcotic potency of organic solvents, it appears

significant to appreciate what is known about the mechanisms underlying the narcotic/anesthetic potency of *hydrocarbon general narcotics and anesthetics*. After all, it seems very likely that narcotic organic solvents and hydrocarbon narcotics and anesthetics like aliphatic alcohols, aliphatic ketones, acetylene hydrocarbons and ethers –at least in part– have common mechanisms with regard to their narcotic potency.

There is some overlap between the terms 'narcosis' and 'anesthesia'. For the sake of a discussion on narcotic properties of organic solvents and underlying mechanisms, it is relevant to define the different terms 'narcosis' and 'anesthesia' as used here.

1.2.4 How are 'narcosis', 'anesthesia' and 'narcotic organic solvents' defined in this review?

Narcosis in fact is an imprecise term; generally, it is not an intentional situation but rather an unplanned /accidental complication, as can for example result from intake of too many painkillers, but also from exposure to narcotic organic solvents. ECHA Guidance on the Application of the CLP Criteria (Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures) describes the criteria for narcotic effects as follows:

a) central nervous system depression including narcotic effects in humans such as drowsiness, narcosis, reduced alertness, loss of reflexes, lack of coordination, and vertigo are included. These effects can also be manifested as severe headache or nausea, and can lead to reduced judgement, dizziness, irritability, fatique, impaired memory function, deficits in perception and coordination, reaction time, or sleepiness.
b) narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure.

Anesthesia is a reversible reduced level of consciousness with unresponsiveness to painful stimuli, administered and balanced under the control of an expert and well-trained staff, e.g. an anesthesiologist/ anesthetist. As such, a more comprehensive definition of anesthesia could include control of pain (analgesia), muscle relaxation, suppression of reflexes, prevention of nausea and vomiting and even reduction of long-term effects such as postoperative cognitive dysfunction (Urban and Bleckwenn, 2002).

Here we consider **'narcotic organic solvents'** causing –mostly unplanned/ undesirable– transient narcotic effects like dizziness, drowsiness, loss of righting reflex, lack of coordination, lethargy, ataxia and CNS depression. Notice that CNS depression also refers here to the physiological depression of the CNS which may lead to decreased rate of breathing, decreased heart rate, and loss of consciousness possibly leading to coma or death (NIH, 2011).

Many organic solvents found in industrial environment produce narcotic effects, i.e. depression of bioelectrical activity in the central and peripheral nervous system. Usually, narcosis occurs without serious systemic effects unless the dose is substantial. The rapidity of onset and depth of effect depend on the concentration present; the rate at which the solvent distributes to organs of the body from the blood depends on the blood-to-air partition coefficient. Narcotic organic solvents having a high blood-to-air partition coefficient, such as diethyl ether, pass into organs from

the blood at slow rate. But, halothane for example, an *anesthetic* having a low blood-to-air partition coefficient, distributes to organs more rapidly (Andrews and Snyder, 1991). The narcosis ranges from mild to complete loss of consciousness. In accidents involving exposure to very high concentrations, breathing may become extremely shallow and death may follow due to asphyxiation, i.e. the body does not receive adequate oxygen in order to function.

High-level exposure to narcotic organic solvents results in immediate signs and symptoms of a disturbance of the central nervous system (CNS). Short-term, high-level solvent exposure causes fatigue and dizziness that slow reaction time, and attenuates rational judgment (see e.g. Dick, 1988; Viaene, 2002). In general, the symptoms stop as soon as the exposure ends. In case the appearance of symptoms is delayed, a metabolite rather than the substance itself is responsible for the effects.

1.2.5 On the mechanism(s) of narcotic/neurologic effects of narcotic organic solvents

The narcotic effects caused by *acute high-level* exposure to organic solvents originally were believed to be caused by partitioning of organic solvents into the phospholipid membrane of neuronal cells –the 'classic lipid hypothesis of anesthetic action'– reviewed for example by Lugli et al. (2009), Seeman (1972) and Urban (2008). Lately, it has been recognized that this hypothesis –i.e. that organic solvent partitioning is associated with a common perturbation of membrane structure– cannot completely account for the commonly observed narcotic effects of different organic solvents. Other physicochemical properties seem to contribute as well. For example, Meulenberg et al (2016) just recently tested whether changes in membrane geometry contribute to the narcotic effects exposing cultured human SH-SY5Y neuroblastoma cells to certain organic solvents; they measured the solvent-induced changes in cell membrane capacitance with the whole-cell patch clamp technique for real-time capacitance. The theory on membrane capacitance was applied to deduce changes in membrane geometry caused by solvent partitioning. Meulenberg et al. (2016) found that the cyclic hydrocarbons m-xylene, toluene, and cyclohexane caused a rapid and reversible increase of membrane capacitance; the aliphatic, nonpolar n-hexane did not cause a detectable change of whole-cell membrane capacitance, whereas the amphiphiles n-hexanol and n-hexylamine caused an increase of membrane capacitance and a concomitant reduction in membrane resistance; the chlorinated hydrocarbons 1,1,2,2-tetrachloroethane and tetrachloroethylene caused a similar magnitude increase in membrane capacitance, despite a large difference in dielectric properties. The results of Meulenberg et al. (2016) demonstrated that solvent partitioning predominantly leads to an increase in membrane surface area and to a lesser extent to an increase in membrane thickness as was earlier believed (Mori et al., 1984). Moreover, the results indicated that the physicochemical properties of each solvent are important determinants for its specific effects on membrane geometry.

Also the mechanisms underlying the effects of *chronic* solvent exposure are not straightforward; they might even be distinct from the mechanisms associated with the (narcotic) effects of short-term, high-level solvent exposure. Although the findings of Meulenberg et al. (2016) indicated that changes in membrane capacitance of solvents cannot exclusively explain narcosis, the membrane effects were measured at high concentrations of solvents, which are generally associated with 'narcotic' or even with lethal effects. Two case studies, containing compiled available data on toluene concentrations during acute intoxication, report brain concentrations between 189 µM and

755 µM. In fatal cases, postmortem brain concentrations ranged up to 5.6 mM (Tanaka et al., 2016; Yajima et al., 2005). As outlined by Meuleman et al. (2016) in their introduction, specific *neurotoxic* effects, which may be observed on sub-chronic or chronic and often low-level exposure to solvents, more likely involves interactions of solvents or of their biotransformation products with specific targets in the nervous system. Several mechanisms have been suggested; most recent developments suggest $GABA_A$ receptor activation (Jayakar et al., 2014; Olsen and Guo-Dong, 2011; Woll et al., 2016).

1.2.6 Solvents: usage and exposure

See **Annex 1**

1.3 Organic solvents and (developmental) neurotoxicity

1.3.1 Research on Organic Solvents and (developmental) Neurotoxicity

Research on the neurotoxicity of organic solvents started in the late 1960s (Hartman, 1995) and since then, a number of organic solvents with narcotic properties has been listed as neurotoxins in adults; well known are e.g. n-hexane, tetrachloroethylene, and toluene. However, despite extensive research in man and animal studies, little attention was given to effects of organic solvents to the *developing* nervous system. This has changed with the understanding that the mature and developing nervous system differ to a large extend thereby making the developing nervous system more prone to the hazardous effects of chemicals including organic solvents. It has been acknowledged to date that –'*Children are no adults*'– in that they have an immature blood-brain barrier, incomplete detoxification capabilities and different metabolic competencies. Most important is the window of exposure relative to the targeted developmental neural processes/structures, and the dose, duration and frequency of exposure (James and Glen, 1980; Jevtovic-Todorovic et al., 2003; Uemura et al., 1985).

Notice that a general description of the developing nervous system is presented in **Annex 2**.

The chemical properties of relatively high volatility and vapor pressure that contribute to the risk of environmental exposure and human inhalation are shared by a wide range of narcotic organic solvents. An assemblage of typical symptoms characterizes exposure of **adult humans**. *Acute exposure* to organic solvents is usually followed by symptoms of fatigue, concentration problems and narcosis (i.e. dizziness, drowsiness, drunken feeling and depressed mood); generally, the symptoms disappear when exposure ends. *Chronic exposure*, however, may result in cerebellar dysfunction, encephalopathy, optic and /or cranial neuropathy, peripheral neuropathy, impairments in senses *(taste, olfaction, auditory function and vision)* and neurodegenerative diseases *(Parkinson's, Alzheimer's, multiple sclerosis, amyotrophic lateral sclerosis (ALS) or motor neuron disease)*. The functional neurotoxicity effects have been associated with cognitive deficits *(in attention, information processing, speech /verbal fluency, and memory)*.

Although effects of organic solvents on the mature nervous system have been studied extensively since the 1960th both in human (particularly in occupational settings) and in experimental (animal) settings, it took another 30 years before attention was given to effects of organic solvents on children, neonates and fetuses. In 2000, Andersen et al. published a list of substances, including solvents, pesticides, and metals rated neurotoxic in humans; twenty-four of the substances were solvents and other organic compounds. In addition, experimental neurotoxicants were reported to cause toxic adverse effects in offspring after pre- or postnatal exposure, twenty of which were solvents and other organic substances. In 2006, Grandjean and Landrigan published the results of a systematic review on published clinical and epidemiological studies into the neurotoxicity of industrial chemicals, including solvents, with a focus on developmental neurotoxicity (Grandjean and Landrigan, 2006). The authors identified five industrial chemicals that could be reliably classified as developmental neurotoxicants in humans, two of which being organic solvents (polychlorinated biphenyls and toluene), others being metals (lead, methylmercury, and arsenic). In addition, they reported 201 substances of which 39 organic solvents causing damage to the mature nervous system

–mostly in connection with exposure of individuals during occupation, suicide attempts and poisoning incidents– and they mentioned –though without further specifications– that more than 1000 substances had been reported to be neurotoxic in laboratory animal studies. In 2014, Grandjean and Landrigan published an update about the developmental neurotoxicity of industrial chemicals and concluded that in humans the number recognized in their 2006 paper had doubled during the seven years thereafter from six to twelve; hereby, tetrachloroethylene was added as another identified organic solvent (Grandjean and Landrigan, 2014).

Also, numerous animal studies reported on neurological effects in offspring following in utero exposure to narcotic organic solvents (Daniel and Evans, 1982 *(ethanol, tertiary butanol)*, Nelson et al., 1984 *(Glycol ether solvents)*, Nelson et al., 1989 *(tertiary butanol)*; Stoltenburg-Didinger et al., 1990 *(organic solvent mixtures)*). Impaired behavioral development was observed (da-Silva et al., 1990 *(toluene)*; Hass et al., 1995 *(xylene)*, 1997 *(xylene)*, 1999 *(toluene)*, 2001 *(white spirit)*; Hougaard et al., 1999 *(toluene)*; Gospe and Zhou, 1998 *(toluene)*); Ladefoged et al., 1991 *(toluene)*, 2004 *(toluene and stress)*), CNS depression (Evans & Balster, 1991 *(toluene)*); abnormal cortical and hippocampal EEG (Tomas et al., 1999 *(selected organic solvents (toluene))*; enzyme inhibition indicative of disturbed homeostatic regulatory functions of astrocytes (Vaalavirta & Tahti, 1995 *(aromatic, alicyclic and aliphatic hydrocarbons)*), changes in acetylcholine metabolism (Honma, 1983 *(toluene)*), and structural abnormalities like CNS malformations (Schardein, 2000), cochlear injury (Liu & Fechter, 1997 *(toluene)*), hydrocephalus and exencephaly (Kato, 1958 *(fuelgas)*), and cortical alterations (Gospe & Zhou, 2000 *(toluene)*).

Moreover, research got published, reporting on increased incidence of major developmental disorders in children resulting from developmental exposure to environmental toxicants, such as mercury, lead, arsenic, polychlorinated biphenyls (PCBs) and also the narcotic organic solvent toluene *(Preterm birth, Autism spectrum disorders, Pediatric bipolar disorder, Attention-deficit hyperactivity disorder)* (Grandjean and Landrigan, 2006, 2014). It should be borne in mind, however, that –despite increasing research in animals– such information is still scarce and the statement so far based on limited evidence and not specific for organic solvents (Rossignol et al., 2014).

With regard to the testing of organic solvents for developmental neurotoxicity under REACH the question arises of whether or not organic solvents showing narcotic effects at high doses (in acute studies) but not other specific neurotoxic effects, do fulfill the criteria of a particular concern for developmental neurotoxicity (DNT), or, should the observed effects rather be considered as non-specific. Thus, the question to be answered is: *'Is there a particular concern for DNT for organic solvents showing narcotic effects only supported by specific mechanism(s)/modes of action for narcotic effects, and a link to adverse neurotoxic effects'*. It seems likely that ***grouping of organic solvents*** is a logical first step when addressing this question.

To group or categorize organic solvents, often their chemical structures and physical-chemical properties have been used. There are, however, some considerations to bare in mind. Some of the considerations for grouping are given in **Annex 3**. It is relevant e.g. to ponder properties like electrophilic character (e.g. halides) as these may easily react with nucleophiles, the human body is filled with. A forehand, one would expect that substances with these structures would be more of a threat to the vulnerable developing nervous system than others. Furthermore, it should be realized that exceptions will have to be faced such as e.g. toxic metabolites as in the case of n-hexane, or increased toxicity due to subtle differences in 3D structure. Not all is known. However, as shown for

example by the studies of McKee et al., 2015 (in line with the study of Clark et al., 2013), restricted categorization may work for certain groups of solvents.

Next chapter presents the search and evaluation of a selection of narcotic organic solvents, and the results of a *grouping* attempt, based mainly on chemical structure. For the evaluation, a distinction is made between the existence of narcosis (dizziness, drowsiness and CNS depression) after acute exposure to the narcotic organic solvents, and possible narcotic effects and/or structural neuropathology and/or motor/cognitive behavioral impairments which might result in repeated dose toxicity (RDT) studies.

2 METHODS

2.1 Selection, grouping and evaluation of narcotic organic solvents

2.1.1. Selection of narcotic organic solvents (harmonized classification as STOT SE 3: H336) and developmental neurotoxicity (Annexes 4, 5, 6)

Specifically, information from studies on narcotic organic solvents in *adult* animals (acute and repeated dose toxicity studies) was collected. It was investigated primarily what the typical symptoms, especially the narcosis *(dizziness, drowsiness, CNS depression),* (structural) neuropathology and/or neurobehavioral impairments, might mean for the (developing) individual and the nervous system.

Annex 4 shows data from a number of narcotic organic solvents taken from the ECHA dissemination site. These solvents have been registered under REACH and have harmonized classification as STOT SE 3: H336; all showed narcotic effects (dizziness, drowsiness) in adulthood at some point during the investigations. Relatively few of them have also been studied for developmental neurotoxic potential, either in children or experimental animal studies. ***The substances presented in this table basically form the key selection of narcotic organic solvents used for the grouping attempt discussed below***.

This list of solvents was extended with a few well-known narcotic organic solvents to learn more about the class they belong to, i.e. benzene, styrene, xylenes, ethyl benzene, and a general anesthetic Propofol (2,6-diiso-propylphenol) –all aromatic hydrocarbons– like e.g. toluene. Furthermore, the information was substantiated with publicly available knowledge on the selected solvents. **Annex 5** presents an overview of related articles searched for this review (mostly animal studies and review articles). *Notice that each item in this table is followed by the complete reference*. **Annex 6** specifically comprises aspects of *developmental* neurotoxicity, i.e. when measurements were taken, and what symptoms and effects were found, and study information such as: type of study, study design, substance, dose and exposure scenario.
Ideally, this information might –in the end– enable to define the assembly of typical symptoms and effects, characterizing the developmental neurotoxicity following early exposure to narcotic organic solvents, and illucidate the uncertainties around the existence –all or not– of a causal link between the narcotic effects of solvents and (developmental) neurotoxicity.

2.1.2 Grouping of selected narcotic organic solvents classified as STOT SE 3: H336 (Annex 7)

Aim of the information collected in Annex 4 was to illustrate if substances classified as STOT

SE 3: H336 can also induce neurotoxicity effects after repeated dose exposure with particular interest on neurodevelopmental effects. At first sight, interpretation of this information appeared challenging, and so it seemed rational first to *group* the solvents.

Annex 7 presents the final list of the selected solvents, ranked/grouped in classes. Ranking is based on chemical structure; the classes are furthermore ranked according to polarity. The *summarized* information on narcosis and neurotoxicity from acute and repeated dose toxicity (RDT) studies is included *(for details see Annex 4)*, as are studies on (developmental) neurotoxicity, if available (three columns, right side of table). Various information like alternative names of a substance, CAS number, chemical structure, relevant isomers, or physical/chemical properties of the substances that might be of help in characterizing a substance and potential effects, has been added where applicable (three columns, left side of the table); this information is *taken from Annexes 4, 5, 6 or other publicly available sources.*
Notice that in order to distinguish between narcosis and structural/functional neurotoxicity, the cells in the table of Annex 7 are shaded. This shading is explained below under Table 1, which *summarizes* the grouping information of Annex 7.

3 RESULTS

3.1 Presentation of grouping, narcosis and neurotoxicity of selected narcotic organic solvents

3.1.1. Narcotic effects and/or (developmental) neurotoxicity of selected and grouped narcotic organic solvents (harmonized classification as STOT SE 3: H336) (Table 1)

Table 1 (below) *summarizes* the grouping information –based on chemical structure– as collected and presented in Annex 7; information on narcosis and (developmental) neurotoxicity is indicated, based on information from repeated dose toxicity studies. The cells in the 3 columns (outer right) have been shaded to distinguish between the effects observed, i.e. (transient) narcosis /nervous system depression (blue shading); neurotoxicity: structural /functional /biochemical nervous system alterations (yellow shading); absence of neurotoxic effects (no shading); 'no information available' (grey shading). Pink shading of the cells in the columns to the left indicate the aforementioned substances that were additionally evaluated (i.e. benzene, styrene, xylenes, ethyl benzene and propofol (2,6-diiso-propylphenol).
Notice that the shading of Table 1 is similar to Annex 7 but text on narcosis and (developmental) neurotoxicity is left out *(Annex 7 can be checked for details)*.

Blue-shaded cells: narcosis/CNS depression
Yellow-shaded cells: neurotoxicity
Grey cells: no information available
Empty cells: no effects observed on nervous system

Orange cells: not evaluated here
Pink cells: Evaluated here, but not included in Table 2A

Table 1. Grouping of narcotic organic solvents* and Information on narcotic effects and/or neurotoxicity

Polarity order	Class	Principle chemical structure	Selected substance **	Acute neurotox.	Repeated dose neurotox.	Developm. neurotox.
Non polar	Aliphatic hydrocarbons *Acyclic*	Straight or branched chains of carbon, **saturated** with hydrogen **Alkanes R-H**	***n-pentane***			
			isopentane			
			n-hexane			
			n-heptane			
			isoheptane			
			n-octane			
			2,2,4-trimethylpentane			
		Straight or branched chains of carbon, **unsaturated** with hydrogen **Alkanes R-H**	***ethylene***			
			2,4,4-trimethylpentene			
↓	Cyclic hydrocarbons *Not aromatic*	Ring structure saturated and unsaturated with hydrogen *cycloparaffins, naphthenes*	***cyclohexane***			
			methylcyclohexane			
↓	Aromatic hydrocarbons	Contain a 6 carbon ring structure with one hydrogen per carbon bound by energy from several resonant forms **Aromatics Ar-H**	***benzene***			
			toluene			
			styrene			
			xylenes			
			ethyl benzene			
			2,6-diiso-propylphenol (propofol)			***
↓	Halogenated hydrocarbons	A halogen atom has replaced one or more hydrogen atoms on the hydrocarbon	***trichloroethylene***			
			1-bromopropane			
			carbon tetrachloride			

(Table to be continued)

* *Table composed from various sources*

** Substances in ***italic/bold*** are presented/discussed in current review in more detail

*** Propofol-induced apoptosis of neurons and oligodendrocytes was observed during brain development, in rats, mice (in infant mice at one fourth the dose required for surgical anesthesia) and rhesus macaques (after propofol anesthesia for 5 hours). *In humans* ***propofol*** is considered safe to mature and developing nervous system. It is noted that the US FDA has set propofol to 'pregnancy category: B': 'Animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women'.

Table 1. Grouping of narcotic organic solvents*

(Table continued)

Blue-shaded cells: narcosis/CNS depression, **Yellow-shaded cells**: neurotoxicity, **Grey cells**: no information available, **Empty cells**: no effects observed on nervous system, **Orange cells**: not evaluated here, **Pink cells**: Evaluated here, but not included in Table 2A

Polarity order	Class	Principle chemical structure	Selected substance **	Acute neurotox.	Repeated dose neurotox.	Developm. neurotox.
↓	Ethers	Contain a C-O-C binding **Ethers R-O-R'**	***diethyl ether***			
			diisopropyl ether			
			2-methoxy-2-methylbutane			
↓	Esters	Formed by interaction of an organic acid with an alcohol **Esters R-COOR'**	***methyl acetate***			
			ethyl acetate			
			propyl acetate			
			isopropyl acetate			
			n-butyl acetate			
			2-ethoxy-1-methylethyl acetate			
↓	Aldehydes	Contain a double bonded carbonyl group C=O with only one hydrocarbon group on the carbon	acetaldehyde			
↓	Ketones	Contain the double bonded carbonyl group C=O with two hydrocarbon groups on the carbon **Ketones R-CO-R'**	***acetone***			
↓			***butanone***			
↓	Alcohols	Contain a single OH group **Alcohols R-OH**	methyl alcohol			
			n-propanol			
			isopropanol			
			n-butanol			
			2-methylpropan-1-ol			
			butan-2-ol			
			1-methoxy-2-propanol			
			1-ethoxy-2-propanol			
↓	Nitro-hydrocarbons	Contain an NO group	ethyl nitrate			
Polar	Glycols	Contain double OH groups	ethylene glycol			

(Table completed)

* *Table composed from various sources*

** Substances in ***italic/bold*** are presented/discussed in current review in more detail

3.2. Evaluation of grouping, narcosis and neurotoxicity of selected narcotic organic solvents

3.2.1 Narcosis and/or neurotoxicity of selected organic solvents: main grouping (Table 1)

According to **Table 1**, the following *main* grouping is obtained with respect to observed narcotic effects (blue shading) and/or neurotoxicity (yellow-shading):

Groups with substances causing (developmental) neurotoxicity in repeated dose toxicity (RDT) studies:

- aliphatic hydrocarbons (acyclic saturated)
- aromatic hydrocarbons
- halogenated hydrocarbons

Groups with substances causing (developmental) neurotoxicity and/or narcosis in RDT studies:

- alcohols

Groups with substances only causing narcotic effects in RDT studies:

- esters
- ethers

Groups with substances neither causing (developmental) neurotoxicity nor narcosis in RDT studies:

- aliphatic hydrocarbons (acyclic unsaturated)
- cyclic hydrocarbons (not aromatic)
- ketones

It is noted that for some groups examples were *not* specifically studied in this review (orange shading in Table 1 above):

- aldehydes
- nitrohydrocarbons
- glycols

3.2.2 Neurologic effects of the organic solvents presented in Table 1: individual results

All of the initially selected solvents (cf. Annex 4) have harmonized classification as STOT SE 3: H336 and may cause narcotic-like symptoms after ***acute exposure***, and many of them (mostly ethers, esters and alcohols) also in ***repeated dose toxicity (RDT) studies*** (Table 1, blue-shaded cells, third and second column from right). Moreover, some also show neurotoxicity in RDT studies (Table 1, yellow-shaded cells, second column from right): ***n-hexane*** (class: (acyclic) saturated aliphatic hydrocarbons), ***toluene*** (class: aromatic hydrocarbon), ***1-bromopropane*** (class: halogenated hydrocarbons), and the substances ***benzene***, ***styrene*** and ***xylenes*** (all three, class: aromatic hydrocarbons) the initial selection of solvents was extended with. Also ***n-heptane***, (class: (acyclic) saturated aliphatic hydrocarbons) affected the mature nervous system according to repeated dose studies, although the results were found to be somewhat conflicting as most of the effects observed

in repeated dose studies on nervous system were transient.
Detailed information is given in Annex 7.

The final outcome (see shading) of ***neurodevelopmental studies*** performed with some of the selected narcotic organic solvents is indicated in the outer right column of Table 1. Summaries, specifically of the neurodevelopmental studies, are given in Annex 6, i.e., information on type of study, model, exposure (dose, duration, time window), measurements (type and time point taken) and results. Evidence of substance-related structural/ molecular/ biochemical alterations, and/or functional deficits, measured in the developing/immature nervous system after early exposure to the selected narcotic organic solvent was found for (Table 1, yellow-shaded cells, right column):

- ***n-hexane*** (its toxic metabolite 2,5 hexanedione); *in vitro* whole chick embryo study: reduced viability and increased neuroapoptosis (Cheng et al., 2012; 2015);
- ***toluene***, *in vivo* study (rat/hamster): neurobehavioral deficits (Da-Silva et al, 1990; Hass et al., 1999; Hougaard et al., 1999; Ladefoged et al., 1991, 2004; Tomas et al., 1999);
- ***trichloroethylene***, (conflicting results): *in vivo* study (rat): no effects on neuropathology and neurobehavior (EPA, 1998); *in vivo* study (rat): altered brain redox homeostasis and neurobehavior (Blossom et al, 2016). These conflicting results might reflect differences in exposure scenario, but could also reflect differences in sensitivity of the parameters measured/test-set-up used, and/or vulnerability of the investigated individuals.
- ***1 bromopropane***, *in vivo* study (rat): Kainic acid-induced WDS (wet dog shakes) suppressed (Fueta et al., 2015);
- ***n-propanol***, *ex vivo/in vitro study:* developmental neurotoxicity measures altered (Candura et al, 1991);
- ***n-butanol***, *in vivo study* (rat): pathological changes in the CNS (Sitarek et al, 1994).

For ***isopropanol*** (non-shaded cell, right column) –*in vivo study* (rat)– investigators reported *'no evidence of developmental neurotoxicity associated with isopropanol exposure as high as 1200 mg/kg/day'* (Bates et al, 1994).

Notice that for a number of the selected solvents, information from literature on developmental neurotoxicity was not found (Table 1, grey-shaded cells, right column).

Also the additionally evaluated aromatic hydrocarbons, ***benzene***, ***styrene*** and ***xylenes*** which caused neurotoxicity in the mature nervous system, appeared to affect the developing nervous system as well. ***Ethylene benzene*** did *not* adversely affect either the mature or developing nervous system. It should be noticed that <u>*in humans*</u> ***propofol (2,6-diiso-propylphenol)*** –a general anesthetic (also aromatic hydrocarbon class)– which was also added to the list of substances in an attempt to learn more about underlying mechanisms, is considered safe to mature and developing nervous system. The US FDA has set propofol to 'pregnancy category: B': 'Animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women'. Recent studies in neonatal animals and fetuses, however, have raised concern for developmental neurotoxicity (see footnote to propofol in Table 1).

No information was available in this review on aldehydes, nitrohydrocarbons and glycols (Table 1, orange-shaded cells).

Finally, the classes in Table 1 were ranked –quite arbitrarily and roughly– according to polarity (Table 1, first column left). The results, however, do not support clear evidence of meaningful categorization of the classes of narcotic organic solvents based on polarity, although

narcotic effects in repeated dose studies might appear more in the polar classes. It should be borne in mind that large variations in polarity might exist among solvents of one and the same hydrocarbon class.
In fact, this also appeared to hold for vapor pressure –the dielectric constant– which varied even more among solvents of a specific hydrocarbon class (see Annex 7).

3.2.3 Summary / Conclusions on grouping of the narcotic organic solvents, the narcosis and neurotoxicity

Together, the information presented here (Table 1; Annex 7) on 'grouping' of the organic solvents and their possible narcotic/neurotoxic potency for the mature and developing nervous system suggests that
1) Substances in the class of *aromatic hydrocarbons* show the greatest neurotoxic potency for both mature and developing nervous systems, followed by *halogenated hydrocarbon*s and further representatives of the *aliphatic acyclic saturated hydrocarbons.*
2) *Ethers* and *esters* in general show narcotic effects in acute *and* repeated dose studies, but no clear substance related (developmental) neurotoxicity.
3) Aliphatic acyclic unsaturated hydrocarbons, cyclic hydrocarbons and ketones do not show either narcosis or neurotoxicity in repeated dose studies. Information specific for developmental neurotoxicity studies was not availbale.
4) Comparing the findings on neurotoxicity and signs of narcosis in repeated dose studies between the aliphatic, aromatic, cyclic and halogenated hydrocarbon classes on the one hand, versus the ethers, esters, ketones and alcohols on the other, the information in Table 1 suggests that the latter mainly show signs of narcotic properties in repeated dose studies, whereas the first mentioned classes show more decisive results, i.e. –yes or no– neurotoxic potency.
5) Ranking the classes according to increasing polarity *per se* appears difficult, but results suggest that grouping may be improved with inclusion of more information on the substances, like e.g. physical/chemical properties.

4. DISCUSSION

4.1 Grouping of the selected solvents based on chemical structure and polarity

4.1.1 Narcosis and neurotoxicity of grouped organic solvents based on chemical structure

The main research question for this review that focuses on the evaluation of toxicity of organic solvents with narcotic properties is '*Whether solvent induced narcosis in adults is to be considered as part of a spectrum of neurotoxicity and a concern for developmental neurotoxicity'.*
In an attempt to investigate this, a selection of narcotic organic solvents –registered under REACH and having a harmonized classification as STOT SE 3 H336 (i.e. may cause dizziness, drowsiness, CNS depression)– have been *grouped* using chemical structure and physical-chemical properties. Related narcosis and (developmental) neurotoxicity in repeated dose toxicity (RDT) studies has been indicated for each substance in a group (Table 1, Annex 7). According to this information: 1) substances in the class of *aromatic hydrocarbons* show the greatest neurotoxic potency for both the mature and the developing nervous system, followed by *halogenated hydrocarbon*s and the *aliphatic acyclic saturated hydrocarbons*; 2) *ethers* and *esters* in general show narcotic effects in acute *and* RDT studies, but no clear substance related (developmental) neurotoxicity; 3) *aliphatic acyclic unsaturated hydrocarbons*, *cyclic hydrocarbons* and *ketones* do not show either narcosis or neurotoxicity in RDT studies (notice that information specific for developmental neurotoxicity studies was not available for these classes).

It appears that 'grouping' of the selected solvents and their narcotic/neurologic properties based on chemical structure is not straight away clarifying.

4.1.2 Narcosis and neurotoxicity of grouped organic solvents: polarity ranking

Increasing polarity further ranked the classes of substances (Table 1), implying increased polarity for esters, ketones and alcohols. Comparison of the findings on neurotoxicity and signs of narcosis in RDT studies between low polarity aliphatic, aromatic, cyclic and halogenated hydrocarbons with ethers, esters, ketones and alcohols (increasing polarity), the information suggests that –over all– increased polarity mainly show signs of narcosis in RDT studies, whereas substances with low polarity show more discriminative results, i.e. presence or absence of neurotoxicity. This might suggest differences in underlying mechanisms for the narcotic and additional neurologic effects.

Differences in the mode of action of narcotic substances with distinctive polarity have long been recognised (Bradbury et al., 1989; Dearden et al., 2000; Roberts and Costello, 2003). The toxicity of industrial chemicals eliciting nonpolar narcosis can be reliably predicted by baseline toxicity models, i.e. log P (partition-coefficient). Using single chemical and combined toxic action models, several research groups have reported classes of polar compounds (for example, esters, phenols, and anilines) that elicit a narcosis-like condition (Bradbury et al, 1989). In 2003, Roberts and Castello concluded that there is a real mechanistic difference between polar and general narcosis, manifesting itself amongst others by significantly different QSARs (quantitative structure–activity

relationship).

The suggestion that there may be various mechanisms or sites of narcotic action is in line with recent studies concerning the cellular and molecular mechanisms of narcosis and neurotoxicity by organic solvents.

4.2 Evaluation of potential target molecules for narcosis and/or neurotoxicity

4.2.1 Narcotic/neurologic effects following acute exposure to organic solvents

The *narcotic* effects caused by *acute high-level* exposure to organic solvents were originally believed to be caused by partitioning of organic solvents into the phospholipid membrane of neuronal cells –the 'classic lipid hypothesis of anesthetic action'– reviewed for example by Lugli et al. (2009), Seeman (1972) and Urban (2008). Since then, it has been recognized that this hypothesis cannot completely account for the commonly observed narcotic effects of different organic solvents. Other physicochemical properties seem to contribute as well. For example, the difference between polar and general narcosis was supposed to be based on a difference in physical chemistry. General narcotic substances are acting via 3-D partition i.e. able to move in all directions in the hydrocarbon-like interior of the membrane, whereas polar narcotics are acting via 2-D partition i.e. involving binding between a functional group on the narcotic substance and the polar phosphatidylcholine head groups at the membrane surface. Based on this hypothesis, Roberts and Castello (2003) derived a mathematical model that might explain why some chemicals show additive toxicity both with general and polar narcosis. Recently, Meulenberg et al. (2016) demonstrated that the physicochemical properties of an organic solvent are important determinants for its specific effects on membrane geometry and they also concluded that specific *neurotoxic* effects, which may be observed on *sub-chronic or chronic (often low-level)* exposure to solvents, more likely involve interactions of solvents or of their biotransformation products with specific targets in the nervous system (see below).

Thus, so far, based on current literature, there is strong evidence that *narcosis* following *acute* exposure to organic solvents is –for a large part– caused by partitioning of organic solvents into the phospholipid bilayer of neuronal cells, and there is evidence that the physicochemical properties of a particular solvent are important determinants for its specific effects on membrane geometry.

4.2.2 Narcotic/neurologic effects following sub-chronic /chronic (low-level) exposure to organic solvents

The *neurotoxic* effects, observed on *sub-chronic or chronic (low-level)* exposure to organic solvents, more likely involve interactions of solvents or their biotransformation products with specific targets in the nervous system. Several mechanisms have been suggested including inhibition of excitatory ion channel receptors like NMDA-glutamate receptor (Cruz et al., 1998, 2000) and the nicotinic acetylcholine receptor (Bale et al., 2002, 2005a), and potentiation of inhibitory ion channel receptors including the gamma aminobutyric acid-A ($GABA_A$) (Beckstead et al., 2000), glycine

(Beckstead et al., 2000, 2001), and serotonin (Lopreato et al., 2003). Despite their identification, however, it remains unclear which one of these targets actually contributes directly to the narcotic /neurologic effects resulting from solvent exposure (Cruz et al., 2014). Evidence is increasing that especially Glutamate and GABA display a wide range of trophic roles in the *developing* brain, and in the transition from neonatal to adult forms of plasticity. They are widely studied in the context of organic solvent-induced narcosis and (developmental) neurotoxicity. Most recent developments suggest $GABA_A$ receptor activation as the actual target contributing directly to the narcotic/neurologic effects of organic solvents (and general anesthetics), especially when referring to exposure during neurodevelopment (Jayakar et al., 2014; Olsen and Guo-Dong, 2010; Woll et al., 2016).

Below, some receptors believed to be relevant for the possible mechanism and mode of action for organic solvent-induced narcosis and/or (developmental) neurotoxicity, are discussed in more detail. Where applicable, information on toluene (a narcotic organic solvent) and Propofol (a general anesthetic) is included as example in attempting to understand mechanisms /modes of action for the narcotic and neurologic effects of organic solvents with narcotic properties.

Notice that –for completeness– an overview of this information is given in **Annex 8** (Organic solvents and ion channel receptors), **Annex 9** (Pre-/postnatal exposure to general anesthetics), and **Annex 10** (Propofol).

4.2.3.1 Excitatory ion channel receptors: NMDA-glutamate receptor

The N-methyl-D-aspartate receptor (NMDA receptor or NMDAR) is a glutamate receptor and ion channel protein found in nerve cells.
NMDAR is a specific type of ionotropic glutamate receptor (Moriyoshi et al., 1991). It is activated when glutamate and glycine (or D-serine) bind to it; when activated it allows positively charged ions to flow through the cell membrane (Hiroyasu et al., 2005). *Ca^{2+} flux through NMDARs* is thought to be critical in *synaptic plasticity* and, therefore, NMDAR is crucial for controlling synaptic plasticity and memory function (Li and Tsien, 2009). So, organic solvents with narcotic properties may partly act via NMDA receptors and may, thus, be mechanistically linked with learning and memory impairments in the (developing**)** brain. However, there is no further information on other narcotic substances to support this assumption.

4.2.3.2 Toluene exposure and NMDA-glutamate receptor

Acute toluene exposure was shown to increase glutamate release in the developing rodent brain (Perrine et al., 2011; Win-Shwe et al., 2007). In a review article on current developments in deconstruction of neuro-behavior into relevant cellular and molecular components Bale described an example of solvent neurotoxicity demonstrating how an *in vivo* neurological defect can be linked via the N-methyl-D-aspartate (NMDA)-glutamate receptor as a common target to *in vitro* readouts. Cellular and molecular components allow for detection of specific neurotoxic effects in cell-based systems to study neurotoxic pathways and modes of actions to inform the regulatory assessment of

chemicals with potential developmental neurotoxicity. Bale confirmed that there is an interaction between toluene and the NMDA-glutamate receptor *in vivo* (Van Thriel et al., 2012).

Toluene also affected -in a dose-dependent manner- NMDA-mediated currents when studying the acute effects of toluene on NMDA receptors in rat hippocampal neurons (Bale et al., 2005). Following *acute* exposure conditions, the NMDA receptor currents were *decreased*, but following *chronic* exposure conditions, the NMDA-mediated currents were *enhanced* and there was increased expression of NMDA receptor subunits. The chronic effects were postulated to be the result of increased neuronal excitability (Bowen et al., 2006). The precision with which toluene acts in targeting ion channels was also demonstrated as toluene significantly inhibited the NMDA subtype of glutamate-activated ion channels while having little effect on the closely related AMPA subtype of ionotropic glutamate receptor and GluN2B subunits appeared considerably more sensitive to toluene inhibition than other NMDA subtypes (Cruz et al., 1998; Bale et al., 2005).

4.2.4.1 Inhibitory ion channel receptors: GABA receptor

The GABA transmission pathway has been the focus of studies on possible mechanisms involved in solvent-induced neurologic alterations, including narcosis. GABA receptors respond to the neurotransmitter gamma-aminobutyric acid (GABA), the main *inhibitory* compound in the *mature* vertebrate CNS.

GABA receptors influence cognition by coordinating with glutamatergic processes (Farahmandfar et al., 2016). There are two classes of GABA receptors: $GABA_A$ and $GABA_B$. $GABA_A$ receptors are ligand-gated ion channels, i.e. 'ionotropic receptors', whereas $GABA_B$ receptors are G protein-coupled receptors, i.e. 'metabotropic receptors'. For this review, the $GABA_A$ receptor is relevant.

In ionotropic $GABA_A$ receptors, binding of GABA molecules to their binding sites in the extracellular part of the receptor, triggers opening of a *chloride ion-selective pore*. The increased chloride conductance drives the membrane potential towards the reversal potential of the Cl^- ion, which is about –65 mV in neurons, *inhibiting* the firing of new action potentials. Notice that this mechanism is known to be responsible for the *sedative effects* of $GABA_A$ allosteric agonists such as e.g. benzodiazepines (McKernan et al., 2000; Sigel et al., 2012).

4.2.4.2 Toluene, Propofol, and GABA receptor

In various cell preparations, the narcotic organic solvent **toluene** has been shown to disrupt currents mediated by GABA receptors (Beckstead et al. 2000) and GABA receptor activity is also influenced by numerous drugs such as *general anesthetics* (Olsen et al., 2011). For example, the anesthetic effects of **Propofol** (2,6-diisopropylphenol) –a general anesthetic, added in this review to the selection of investigated organic solvents in order to learn about underlying mechanisms /modes of action– have been associated with modulation of the GABA receptor. Relatively low concentrations of propofol significantly potentiate GABA-induced current, whereby the post-synaptic membrane hyperpolarizes and so, contributes to *hypnosis* and most likely other *anesthesia phenotypes* (Sanchis-Segura et al., 2007; Zecharia et al., 2009). Reports suggest that synaptic GABAergic signaling is a critical pathway for the pharmacological effects of propofol (Eckle et al., 2015; McDougall et al., 2008; Nishikawa, 2011).

So, apart from partitioning into the phospholipid membrane of neuronal cells pending their physical/chemical properties, organic solvents may also act on GABA receptor to induce narcosis. Interestingly, during development of the nervous system GABA receptors appear to act different compared to their action in the mature nervous system (see below).

Toluene, and also trichloroethane and trichloroethylene, appeared to work at the GABA synapses of CA1 pyramidal neurons in rats to inhibit functions of these neurons (MacIver, 2009; Beckstead et al., 2000). This effect *decreased* with chronic exposure and might suggest development of a *tolerance* to these inhaled substances (Bowen et al., 2006; Bale et al., 2005). Hippocampal damage following chronic toluene exposure was demonstrated in other studies as well (Bowen et al., 2006; Zhvania et al., 2012).

The hippocampus appeared to be particularly vulnerable to the effects, suggesting a mechanism for increased hippocampal injury (Mattia et al., 1993). This appears to be most pronounced in the developing brain, including adolescence (Chen and Chan, 2011). Because the function of the hippocampus is associated with learning and memory, this would provide a rationale for memory and learning difficulties that are experienced after organic solvent exposure.

4.2.5 Unique behavior of $GABA_A$ (NMDA and AMPA) during nervous system development

Apart from *inhibitory* $GABA_A$ receptors, there are also numerous reports of *excitatory* $GABA_A$ receptors. These may be especially relevant when it comes to *developmental* neurotoxicity induced by narcotic organic solvents.

This phenomenon of *excitatory* $GABA_A$ receptors is due to increased intracellular concentration of Cl^- ions either during development of the nervous system (Ben-Ari et al., 1997; Taketo and Yoshioka, 2000) or in certain cell populations (Tomikoet al., 1983; Cherubini et al., 1991; Lamsa and Taira, 2003). Ben-Ari and co-workers (Ben-Ari et al., 1997) demonstrated that the main ionotropic receptors ($GABA_A$, NMDA and AMPA) display a *sequential* participation in neuronal excitation in the *neonatal* hippocampus: 1) GABA, the principal *inhibitory* transmitter in the *adult* CNS, acts as an *excitatory* transmitter in *early postnatal stage*; 2) Glutamatergic synaptic transmission is first purely NMDA-receptor based and lacks functional AMPA receptors. Therefore, initially glutamatergic synapses are 'silent' at resting membrane potential, NMDA channels being blocked by Mg^{2+}. When GABA and glutamatergic synapses, however, are *co-activated* during the physiological patterns of activity, $GABA_A$ receptors can facilitate the activation of NMDA receptors, playing the role conferred to AMPA receptors later on in development. Likewise, Taketo and Yoshioka (2000) demonstrated that $GABA_A$-ergic inhibitory postsynaptic currents in hippocampal CA3 pyramidal cells change developmentally and indicate that different receptor isoforms are functionally expressed between neonates and adults.

These subtle cascades of events on the one hand form an example of architecting of an impressive fine-tuned network, taking place during nervous system development; otherwise, it also demonstrates how critical the timing may be when it comes to exposure windows and the vulnerability of the developing nervous system to the toxic potency of substances like general anesthetics and/or narcotic organic solvents. For example, in the case of anesthetics, studies have found that anesthetic agents e.g *propofol* that bind to the GABA and NMDA receptors during the period of brain growth spurt (BGSP) may potentially trigger excess apoptosis and affect neuronal development (Gao et al., 1998; Ikonomidou et al., 1999; Irifune et al.,2003; Kargaran et al., 2014),

and the ***rationale*** for the increased vulnerability during this period of BGSP is that exposure to anesthetics during this period leads to *excitation*, rather than *inhibition* of the $GABA_A$ receptors (Lagercrantz, 2004; Nguyen et al., 2001).

Glutamate and GABA display a wide range of trophic roles in the developing brain, and in the transition from neonatal to adult forms of plasticity. The information given above points at critical and crucial interactions between NMDA and GABA receptors, and differences in action in the developing *vs.* the mature nervous system. So, it seems very likely that especially these receptors are involved in the processes leading to narcotic/neurologic effects of organic solvents when exposure occurs *during early development of the nervous system*. Especially exposure during the period of brain growth spurt (BGS) –the period of synaptogenesis– appears to be critical.

4.2.6 Dopamine and suspected organic solvent-induced neurodegenerative diseases

The dopamine pathway has also been evaluated for involvement in the mechanism of CNS hydrocarbon abuse (inhalant effects), in particular because of possible involvement in organic solvent-induced neurodegenerative diseases.

Dopamine receptors are implicated in many neurological processes, including motivation, pleasure, cognition, memory, learning, and fine motor control. They control neural signaling that modulates many important behaviors, such as spatial working memory as well as modulation of neuroendocrine signaling (Williams and Castner, 2006). However, dopamine receptors do not seem to be directly involved in the *narcotic* effects of organic solvents.

Dysfunction of dopaminergic neurotransmission in the CNS has been implicated in a variety of neuropsychiatric disorders (Girault and Greengard, 2004): Parkinson's disease (Fuxe et al., 2006), schizophrenia (Kienast and Heinz, 2006), neuroleptic malignant syndrome (Mihara et al., 2003), attention-deficit hyperactivity disorder (ADHD) (Faraone and Khan, 2006), and drug and alcohol dependence (Kienast and Heinz, 2006; Hummel and Unterwald, 2002). Some of these disorders are also recognized in relation to narcotic organic solvent-induced neurotoxicity.

Overall, there is a serious concern regarding a possible causal link between exposure to narcotic organic solvents and neurodegenerative diseases with a link to dopamine disturbances. However, findings are inconsistent and more research will be needed to come to a firmly based conclusion.

Studies in adult animals showed that inhalants (volatile solvents) activated mesolimbic dopamine neurons within the rat ventral tegmental area (Bowen et al., 2006; MacIver, 2009; Riegel and French, 1999; Riegel et al., 2007). Activation of these neurons, in turn, increases dopamine concentrations in the caudate nucleus and nucleus accumbens in rats (Riegel et al., 2007; Riegel et al., 2004). The authors recognized that this might be a mechanism related to *addictive* properties of inhalants (Riegel and French, 1999; Riegel et al., 2007; Howard et al., 2011).

Chronic exposure in rats to toluene caused persistent dopamine dysfunction within the basal ganglia (Lubman et al., 2008).

Together this information suggests that increased dopamine concentrations upon exposure of the adult nervous system to organic solvents with narcotic properties, may underlie addiction to these substances.

Summarizing, the information given above allows the following conclusions:

1. There is strong evidence that *narcosis* following *acute* exposure to organic solvents is –for a large part– caused by partitioning of organic solvents into the phospholipid bilayer of neuronal cells, and there is evidence that the physicochemical properties of a particular solvent are important determinants for its specific effects on membrane geometry.
2. Specific *neurotoxic* effects, which may be observed on *sub-chronic or chronic (often low-level)* exposure to solvents, are more likely to involve interactions of the solvents or their biotransformation products with specific targets in the nervous system, amongst others NMDA and GABA receptors.
3. Organic solvents with narcotic properties may partly act via NMDA receptors and may, thus, be causatively linked with learning and memory impairments in the (developing**)** brain. However, there is no further information on other narcotic substances to support this assumption.
4. Most recent developments suggest $GABA_A$ receptor activation as the actual target contributing directly to the narcotic/neurologic effects of organic solvents (and/or general anesthetics), especially when referring to anesthetic-like activities, and exposure during neurodevelopment. Interaction with GABA and NMDA receptors during the period of brain growth spurt (BGSP) may potentially trigger excess apoptosis and affect neural development. A rationale for the increased vulnerability during this period is that exposure leads to excitation, rather than inhibition, of the $GABA_A$ receptors during this period of synaptogenesis.
5. Dopamine receptors do not seem to be directly involved in the *narcotic* effects of organic solvents. Overall however, there is a serious concern regarding a possible causal link between exposure to narcotic organic solvents and neurodegenerative diseases with a link to dopamine disturbances; however, findings are inconsistent and more research will be needed to come to a firm conclusion. Increased dopamine concentrations upon exposure of the adult nervous system to organic solvents with narcotic properties, may relate to mechanisms of addiction to these substances.
6. Certain similarities are recognized for toluene and propofol with regard to possible mechanisms/modes of action for their narcotic and neurologic effects. The information from the literature suggests for both substances that (apart from partitioning of the substances into the phospholipid membrane of neuronal cells) activation of *inhibitory* $GABA_A$ receptor is involved in the narcotic/anesthetic-like action of the substances. Otherwise, during development of the nervous system, the *excitatory* action of $GABA_A$ is likely to be responsible for increased neuro-apoptosis leading to structural and functional neurodevelopmental deficits.

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ANNEX 1

A1. Solvents: usage and exposure

Exposure to organic solvents occurs in occupational industrial settings, industrial environment (air) or contaminated drinking water, and also in the home environment (e.g. cleaning products). In general, exposure to organic solvents in home environment is of short duration; chronic exposure is seen more generally in occupational settings (Logman et al., 2005). Solvent abuse (by inhalation) is another form of exposure that is documented all over the world (Dell et al., 2011; Medina-Mora and Real, 2008).

Organic solvents known for exposure to man are e.g. aromatic hydrocarbons found in glues and cleaners (e.g. toluene, benzene, xylenes); the aliphatic hydrocarbons found in fuels and cigarette lighters (e.g. butane, propane, gasoline); and the alkyl halides found in correction fluids, degreasing agents, paint strippers, and aerosol propellants (e.g. 1,1,1-trichloroethane (TCE), methylene chloride). Other inhaled substances include the aliphatic nitrites found in room air fresheners (e.g. isoamyl and isobutyl nitrite); ketones and acetone found in adhesives and nail polish remover; the volatile anesthetics (e.g. isoflurane, halothane) (Bowen and Hannigan, 2006).

ANNEX 2

A 2. Development of the nervous system

The development of the nervous system is an ongoing biological process through which nervous tissue is formed and shaped all life through, i.e. during early development, adolescence and adulthood of an individual, albeit that major changes occur during early development. Understanding of the processes involved herein will help to get more insight into the pathological and toxicological processes underlying neurodevelopmental impairments induced by chemicals such as organic solvents.

The developing brain is not well protected against environmental chemicals. First, the placenta appears not always able to prevent passage from the maternal to the fetal circulation as has e.g. been demonstrated by the presence of many chemicals detected in umbilical blood (EWG, 2005). Also, many chemicals can reach the infant via human breast milk (Needham et al., 2011). Second, it is well-known that during fetal life and early infancy, the immature blood–brain barrier provides only partial protection against the entry of chemicals into the central nervous system (Zheng et al., 2003); the many developmental processes such as e.g. the proliferation, migration and differentiation of the cells composing the nervous system, may be very vulnerable to the toxic potential of certain chemicals. Interference of a chemical into one or more of the common processes responsible for normal CNS development may result in a dis-regulating of a whole cascade of events, leading to functional deficits and disease later in life.

A2.1 Stem cells, neurons and glial cells

The cells ultimately giving rise to all specialized cells of the nervous system are the, so called, 'neural stem cells'. Stem cells are capable of self-renewal, i.e. the stem cell divides producing two cells, which, in turn can divide similarly. With time, only one of two divided cells survives and lines up in the adult brain in the ventricular zone, i.e. the zone surrounding the ventricles. Stem cells also give rise to, so called progenitor, i.e. precursor cells. These precursor cells have an unlimited cell-fate potential and can divide as well. However, as they develop, they become increasingly committed to a particular cell type and eventually produce non-dividing cells known as neuroblasts and glioblasts. These neuroblasts and glioblasts, in turn, mature to neurons and glia cells (astrocytes, and oligodendrocytes (Gage, 2000; Wang et al., 2013; Zhao et al., 2008 (review)). Neurons are the functional components of the nervous system and are responsible for information processing and transmission; astrocytes and oligodendrocytes play supporting roles that are essential for the proper functioning of the nervous system.

Another glial cell type present in the CNS is the so called microglial cell. Microglia reside in the central nervous system (CNS) and are now known to originate from the embryonic yolk sac as primitive macrophages (see Ginoux et al. 2013, for review). At the onset of circulation, these primitive macrophages exit the yolk sac and migrate into the developing brain where they will form microglia. It appears that the embryonic microglia maintain themselves until adulthood. They proliferate during late gestation and post-natal development and at a later stage in the injured adult brain in response to neuronal damage or invading pathogens. In this way, microglia inextricably link the (developing) brain to the immune system. Efficacious neurodevelopment depends on immune cells like microglia, which are involved in maintaining a normal brain development and physiology (Bilbo and Schwartz 2012; Ginhoux et al. 2013: Harry and Kraft 2012; Kettenmann et al. 2011; Svahn et al. 2012; Zhan et al. 2014).

A2.2 Neurotrophic factors

Sam Weiss and his colleagues (1996) discovered that these stem cells remain capable of producing neurons and glia not just into early adulthood, but even in an aging brain (http://stemcellfoundation.ca/en/scientist/sam-weiss/). Specific –probably chemical– signals, although still largely unknown, are needed for turning on specific genes that produce a particular cell type. One class of compounds that signals cells to develop in particular ways comprises so-called neurotrophic factors, i.e. a class of compounds acting to support growth and differentiation in developing neurons and to keep certain neurons alive in adulthood. The neurotrophins were first identified as survival factors for sympathetic and sensory neurons and have since been shown to control a number of aspects of survival, development, and function of neurons in both the central and peripheral nervous systems (Skaper, 2012 (Overview)). Neurotrophin availability is required into adulthood, where they control synaptic function and plasticity and sustain neuronal cell survival, morphology, and differentiation.

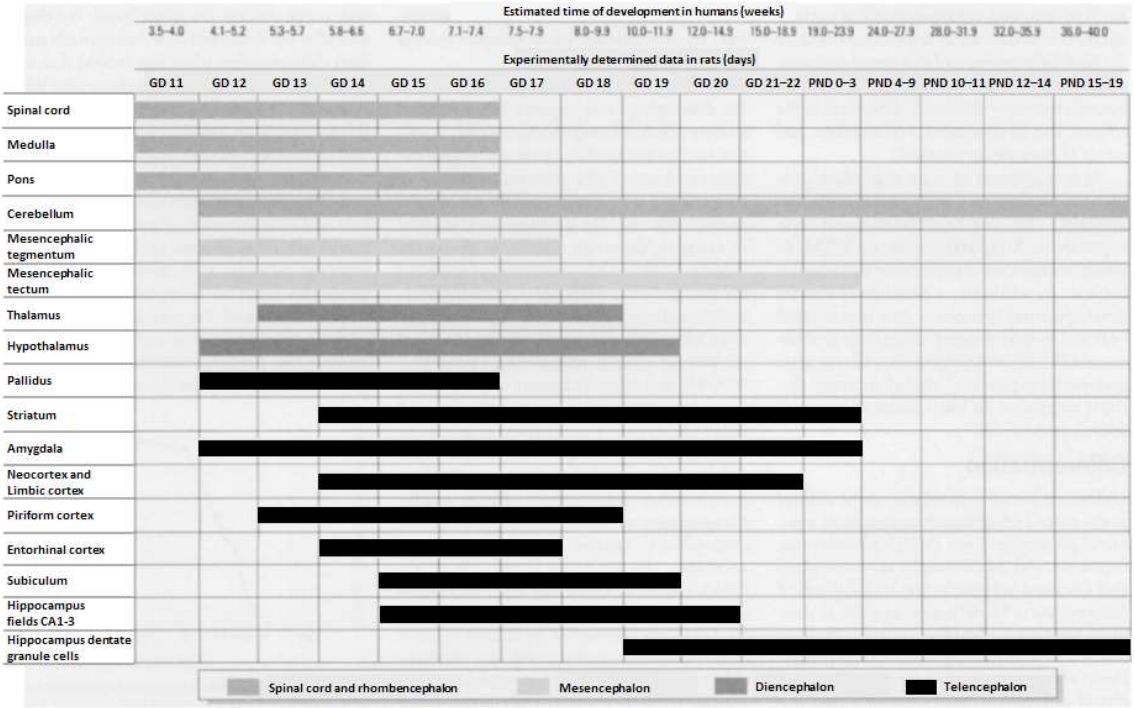
A2.3 Stages of brain development

The rapid formation of neurons and glia is just the first step in the growth of a brain. These cells must travel –*'migrate'*– to their final destination and must *'differentiate'* into the right type of neuron or glial cell, and the neurons must grow dendrites and axons and subsequently form synapses (Eagleson et al., 1997; McConnell, 1990). Interestingly, the brain also prunes back unnecessary cells and connections and so sculpts itself following the experiences and needs of an individual. Although each brain region typically distinguishes itself with region- and cell-specific time-windows for the neurodevelopmental processes, the stages generally recognizable during brain development include: cell birth (neurogenesis; gliogenesis), cell migration, -differentiation and -maturation (dendrite and axon growth), synaptogenesis (formation of synapses), cell death and synaptic pruning, and myelogenesis (formation of myelin) (Rice and Barone, 2000). These neurodevelopmental processes can be roughly divided into two classes, i.e. the activity-independent mechanisms –the hardwired

processes determined by genetic programs played out within individual neurons (differentiation, migration and axon guidance to their initial target areas) – and activity-dependent mechanisms, processes thought of as being independent of neural activity and sensory experience. Activity-dependent mechanisms come into play, once axons reach their target area: neural activity and sensory experience will mediate formation of new synapses, as well as synaptic plasticity, which will be responsible for refinement of the nascent neural circuits.

A2.4 Duration and completion of the different developmental processes

In humans, as in other vertebrates, the brain begins as part of the neural tube, the part that contains the cells from which the brain will form. As is shown in the figure below for human and rat – picture taken from Rice and Barone (2000)– the duration and completion of the different developmental processes differs per brain region and species. However, although quite different in lifespan, over all, the principle processes of neurodevelopment are recognized in all vertebrates.



Timing of pre- and early postnatal brain development in human and rats. Picture reproduced from Rice D, Barone S Jr. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect 2000; 108 Suppl 3:511-533.

Considering that the different developmental processes may take place simultaneously but

at different locations in the brain, it is clear that this increases the vulnerability of the nervous system to the toxic potential of environmental chemicals including organic solvents. So, it seems very likely that the developing nervous system is more prone to the adverse effects of environmental chemicals than the adult.

A2.5 Outgrowth of dendrites and axons

Neural maturation –i.e. the migration of cells to their final destination and the differentiation into the specific neuron type– is completed with the outgrowth of dendrites providing the surface area for synapses with other cells, and with the extension of their axons to appropriate targets initiating the formation of other synapses (Semple et al., 2013). The faster-growing axon can contact its target cell before the dendrites of that cell are completely formed. In this way, the axon may play a role in dendritic differentiation. Various molecules that attract or repel the developing axon –in turn– guide the formation of axonic connections (Dotti et al., 1987; Goldberg, 2004).

A2.6 Apoptosis / Synapse pruning

Perhaps the most surprising events in vertebrate brain development are cell death and synaptic pruning, i.e. there is first an overproduction of neurons and synapses and then a subsequent loss of them. Neurons deprived of synaptic targets, eventually die (Raff et al., 1994). This neuron death occurs because target cells produce neurotrophic factors i.e. signaling molecules that are absorbed by the axon terminals and function to regulate neuronal survival. If many neurons are competing for a limited amount of a neurotrophic factor, only some of those neurons can survive. It seems that, when neurons are deprived of a neurotrophic factor, certain genes are 'turned on' resulting in a message for the cell to die (Gorman et al., 1998). This process of cell death that is genetically programmed is called *apoptosis* and is different from the cell death caused by injury or disease.

Hence, apoptosis accounts for the death of overabundant neurons, however, not for the pruning of synapses from the cells that survive (Paolicelli et al., 2011). Synapses persist into adulthood but only when incorporated and active in a functional neural network. If not, they will eventually be eliminated from the brain. Hormones, drugs, and in particular experience can influence the formation of active neural circuits and can thus influence the processes of synapse stabilization and pruning. As such, experience can have significant effects on the organization of the nervous system.

In fact, the brain shows strong plasticity during its development and can therefore be shaped by experience. The sensitivity of the brain to experience varies with time, however. There are critical periods in the course of development when different parts of the brain are particularly sensitive to different experiences. So, the brain shows plasticity in response to external events enabling positive adaptations; however, developmental processes and cascades of events may also be interrupted leading to structural and functional impairments that may sometimes be seen only much later in life.

ANNEX 3

A3. Considerations for grouping of organic solvents for narcosis and neurotoxicity

Some basic chemistry should be considered a forehand as to take physical-chemical properties of organic solvents to categorize them to predict developmental neurotoxic potential.

A3.1 Chemical structure and physical-chemical properties

A3.1.1 Substance with strong electrophilic character

A rule of thumb in organic chemistry is that the human body is full of nucleophiles among which are DNA, RNA and proteins. This implies that, when exposure takes place to a substance with strong electrophilic character, a reaction will take place between substance and 'body nucleophiles' i.e. provided the substance can reach the body nucleophiles. This means that it must pass cell membranes and barriers like the blood-brain barrier and placenta. For example, halides (i.e. solvents with chloride and bromium atoms) in general are alkylating substances because of their electrophilic character, which is caused by the strong electronegative character of the halide. In principle, body nucleophiles are 'protected' by cell membranes against undesirable intruders and –with regard to potential developmental neurotoxic agents– the developing brain and the fetus are protected by blood-brain barrier and to a certain extend by the placenta. Traditional hydrocarbon solvents, however, despite their large variations in physical and chemical properties, all have in common that they are very volatile, have a small molecule size and show high lipophilicity allowing them to readily enter lipid rich structures like cell membrane and barriers and they can rapidly reach the lipid-rich brain (neurons and myelin).

A3.1.2 Toxic metabolite

It is also possible that a certain solvent does not have direct toxic potential, but its metabolite may have so. An example is n-hexane, which is not directly toxic but is metabolized in the body into 2,5-hexanediol (oxidation by cytochrome P450), which, in turn, is forming its tautomer 2,5-hexanedion, which is known to be responsible for the neurological effects following n-hexane exposure. Likewise, the chions forthcoming from 1-naphthol following naphthalene exposures do have the neurotoxic potential and not naphthalene.

A3.1.3 D-structure of the molecule

The toxic potential of a solvent in principle can be increased by the 3D-structure of the molecule. In this way certain molecules 'fit' exactly in important biomolecules such as DNA or certain proteins, thereby affecting their function. Well-known examples are carbon monoxide (binding to hemoglobine, excluding the oxygen molecule) and ethidiumbromide (intercalates in the DNA and RNA (strong mutagenic)). So it is possible that a specific hydrocarbon solvent is having a structure, fitting like a 'key-in-the-lock', whereas another one –with very similar structure– does not, because it does not hold similar molecules and so, may not fit as a 'key-in-the-lock'. It might be that this mechanism of 'key-in-the-lock' is not playing a significant role with regard to organic solvents and neurodevelopment, but it cannot be ruled out a forehand.

A3.1.4 The reporting by different sources on the physical-chemical properties of a substance

It is noted that the different sources reporting on the physical-chemical properties of a substance may vary. Examples are vapor pressure, solubility in water and partition coefficient (octanol-water) log Kow. In general this variation between the different sources is small, but some exceptions do exist. So, when making selections, or drawing conclusions based on physical properties of the substance it is essential to check that the values among sources do not differ too much.

A3.2 Grouping of solvents based on physical-chemical properties

A3.2.1 Example from the literature (McKee et al., 2015)

In an extensive review McKee et al. (2015) grouped solvents into 9 categories of analogous substances (McKee et al., 2015, Table 2) based on the solvent constituent's information and physical-chemical properties; hazard characterization was performed reviewing study results of representative substances. A category was considered a group of substances with similar properties, such that the results of a study of any category member would be broadly applicable to all other category members. This was in line with Clark et al. (2013) using a similar approach for petroleum products. Aim of McKee's study was to review hydrocarbon solvents –composed of constituents with carbon numbers in the range of C5 to C20– and to describe the toxicological information, to show how this information can be used to characterize and communicate toxicological hazards, which, in turn, can be used for hazard classification and basis for occupational exposure recommendations. It should be noted that alcohols, ethers etc. were not included, which in this case might have benefitted solvent grouping. The authors illustrated this with practical examples. They came to the conclusion that, although hydrocarbon solvents have complex and variable compositions, the similarities in physical-chemical properties of most of their constituents allow a generic approach to address their complexity. In general, the substances with similar physical-chemical properties have similar toxicokinetic properties and follow similar metabolic pathways. A few exceptions were

recognized by the authors –hydrocarbon substances having deviating properties– but, as outlined by the authors, these substances have extensively been characterized and the toxicological hazards well described. According to the authors, generally, unusual toxic potential of a hydrocarbon constituent is associated either with a distinct metabolic pathway –like naphthalene– or the production of an uncommon metabolite –like n-hexane. These exceptions have been extensively studied and are well described in the literature. The theory is supported by the empirical evidence that the results of toxicological studies of complex hydrocarbon solvents are similar to those of studies of individual constituents (McKee et al, 2015 (and references therein)).

The 'grouping' proposed by McKee et al. may not be ideal and may hold for restricted groups only with known 'bad guys' like n-hexane, but, considering the points mentioned in the introduction of this annex on basic chemistry, it may be this is as close as one can get when using physical chemical properties of organic solvents for grouping for potential neurotoxicity.

The authors reported an interesting case where in animal studies neurotoxicity was reported in the mature nervous system but not during development (McKee et al., 2015). This is fascinating; is it possible that a substance is neurotoxic in the mature nervous system but not during development? More research is needed to clarify whether or not such an outcome represents an exception, a specific group, or is reflecting the complexity of the developmental processes and challenges in observing the potentially different outcome and deciding on optimal time for investigations. The answer is: 'YES', i.e. 'yes, under the circumstances of the study' and so 'spoke' the results in the case mentioned in McKee's review. However –and perhaps more important– is, how to interpret the results and what conclusions to draw.

In the main text of this review (see METHODS, RESULTS, DISCUSSION) the evaluation of the grouping approach applied to the narcotic organic solvents studied here, is presented. Distinctions are made between the existence of narcosis (dizziness, drowsiness and CNS depression) after acute exposure to the narcotic organic solvents, and the narcosis, structural neuropathology and motor/cognitive behavioral impairments which might result in repeated dose toxicity studies.

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ANNEX 4

A4.1 Substances registered under REACH with harmonised classification as STOT SE 3: H336

Substances listed in the table below are registered under REACH and have harmonised classification as STOT SE 3: H336 (may cause dizzeness or drowziness). Data has been taken from the ECHA dissemination site supplemented with other publicly available information. Aim of the information included is to illustrate if substances classified as STOT SE 3: H336 can also induce neurotoxicity effects after repeated exposure with particular interest on neurodevelopmental effects.

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Note: Full references were not found for: BASF, 1980; Huff, 1990; EPA, 1998; Bushy Run Research Center, 1994, 1991; Machle et al.,1938; Bernard and David, 1996; Gamer, 1998; NTP, 1990.

A4.1.2 Table with selection of narcotic organic solvents (taken from ECHA dissemination site)

Substances Registered under REACH and having harmonised classification as STOT SE 3: H336 (may cause dizzeness or drowziness)					
EC no **Substance name**	**Acute toxicity studies in the registration dossier**	**Repeated dose toxicity studies in the registration dossier**	**Literature search for DNT information**	**Chemical properties**	**Summary on effects**
203-692-4 n-Pentane	**Frantik et al., 1994** (male rats and female mice for 4 hours and 2 hours, respectively): At 21000 ppm, 37% depression of the shortening of the tonic extension of hind limbs by 3 seconds in male rats, while at 23500 ppm, 30% depression of the lengthening of the latency of extension by 0.6 seconds in mice. **Stoughton, 1936**: acute Toxicity of n-pentane in mice via inhalation. 1st test series ("light anesthesia" test: 3.0, 3.5, and 4.2 mmol): mice lightly anaesthetized from 1.3 to 10 minutes. No mice died during the first test series. 2nd test series ("complete anaesthesia" test: 4.2, 4.5, and 4.9 mmol/L): 8 mortalities at 4.5 mmol/L, and 7 mortalities at 4.9 mmol/L. The average recovery time for the survivor was 4 to 8 minutes. The LC50 was not calculated.	**Takeuchi et al., 1981**; Schreiner, 1998; Gamer, 1998 (90 day studies via inhalation): each contains a neurotoxicity screening component. No neurotoxicity effect reported. **Takeuchi et al., 1980** (6 weeks via inhalation at 3000 ppm): no effect in prolong distal latency or disturb the conduction velocity of the motor nerve and mixed nerve in the rat's tail. No changes in the peripheral nerve, the neuromuscular junction, and muscle fibre. No changes in body weight or behaviour.	Not found	Physical state: Liquid VP: 58.59 kPa at 294.26 K Log Kow: 3.45 Water solubility: 38.5 mg/L at 20 °C	Narcosis observed from acute inhalation studies. No neurotoxicity effects were observed in the 90 day and 6 weeks inhalation studies. No neurodevelopmental effects found from the literature search.

250-610-8 Isoheptane	No acute study provided on isoheptane. **Unnamed, 1982**: The acute oral LD50value in rats was greater than 5000 mg/kg for iso-octane (CAS No. 540-84-1). Clinical observations noted one-hour post exposure in 8 of 10 animals included depression, salivation, wheezing, rough coat, and soft faeces. Two female rats appeared normal throughout the study. All animals appeared normal from day 2 through termination of the study. Unnamed, 1982: Ten male and female Sprague-Dawley rats were exposed to n-heptane (CAS No. 142-82-5) via whole body inhalation at nominal concentration of 29290 mg/m³ for 4 hours (similar to OECD 403). A slight reduction of mean male body weights was noted on day 2 post-exposure but males recovered by day 4. All animals appeared normal throughout the study and at terminal necropsy with the exception of one female observed with enlarged mandibular lymph nodes on the right side. The LC50was greater than the nominal concentration of 29300 mg/m³. Unnamed, 1982: The dermal LD50 value of iso-octane (CAS No. 540-84-1), as determined in rabbits, was greater than the limit dose of 2000 mg/kg. All rabbits appeared normal throughout the study. Very slight dermal erythema was noted in all animals on day 1 after dosing, which persisted in one male and one female on day 3. All erythema had cleared by day 7. Very slight oedema was noted in two males and one female on day 1 and cleared by day 3. Epidermal scaling was noted in one female on day 10.	There are no inhalation repeated dose toxicity data available on isoheptane in the registration dossier. **A) Ono et al., 1979** and **Takeuchi et al., 1980, 1981**: Evaluations of peripheral nerve toxicity of n-pentane, n-hexane and n-heptane male rats were exposed to 0 or 3000 ppm 12 hours/day, 7 days/week, for 16 weeks. The conduction velocity of tail nerves was measured to determine the functional status of the peripheral nerves. n-heptane: statistically significantly depressed ($p<0.01$) body weight gain after 8 weeks of exposure but gradually increased throughout the experiment (but not statistically significantly lower). No abnormal behavioral changes were observed. No statistically significant differences in motor nerve conduction velocity, distal latency or mixed nerve conduction velocity in any region of the tail. No microscopic change in nerves, muscles and neuromass junctions. n-Heptane is not a neurotoxicant in this assay system. n-hexane: induced neuropathy. NOAEC: > 3000 ppm (12470 mg/m³). Note: No information included for n-pentane in this report. **B) Unnamed, 1980**: n-heptane administered to rats via whole body inhalation at 398 and 2970 ppm for 26 weeks with a subsequent 2-week recovery period conducted similar to OECD 413. The only treatment-related observations were laboured breathing or rapid breathing and slight prostration during the first week of exposure and anogenital fur and dry rales during weekly observations. The in chamber signs were generally more numerous and severe at the higher concentration and appeared to abate by	not found	Physical state: liquid VP: 8.9 kPa log Kow: 3.7 Water Solubility: 2.5 mg/L	No studies (acute or repeated dose studies) to investigate the neurotoxic effect of n-heptane. The registrant assigned STOT SE 3 (H336) based on read-across within a category approach. n-heptane or Naphta (petroleum), light alkylate (CAS no: 64741-66-8) was not a neurotoxicant while in n-hexane induced neuropathy in 16 weeks inhalation study at 3000 ppm. No literature was found on isoheptane showing effects in the developmental nervous system.

		the second week of the study. The effects observed are consistent with acute CNS depression and generally abated by the second week of study. NOAEC: 2970 ppm corresponding to 12200 mg/m³. **C) Simonsen and Lund, 1995** ("Auditory sensitivity study"): n-heptane, male rats, inhalation (whole body), 28 days, 0, 3.3, 16.6 mg/L (re-calculated; corresponding to 0, 800, 4000 ppm). No clinical signs of neuropathy were observed; body weight gain reduced after termination of exposure and becomes statistically significant at 16.6 mg/L. A loss of auditory sensitivity equal to 10dB at 4000ppm and tested by measurement of auditory brain stem response 2 months after cessation of exposure Systemic: NOAEC 3.3 mg/L, LOAEC 16.6 mg/L; Auditory: NOAEL 3.3 mg/L, LOAEC 16.6 mg/L. D) Unnamed, 1998: ~OECD TG 413, CAS no: 64741-66-8, Sprague-Dawley rats, inhalation (whole body), test concentration (0, 668, 2220, and 6646 ppm (0, 2.4, 8.1, and 24.3 g/m3)). NOAEC for subchronic toxicity and neurotoxicity (neurobehavioural studies and neuropathological studies) = 6646 ppm (no effects except adaptive response of liver weight at 6646 ppm).			

203-625-9 Toluene	Non-human information 1) Acute Oral toxicity **A) Withey and Hall, 1975**: EU B1; rats; doses: 4000, 4560, 5200, 5930, or 6760 mg/kg of toluene. Highest dose resulted hind-limb paralysis and petechial bleeding, especially from the urinary tract, eyes and nose. The LD50 was calculated to be 5580 mg/kg. **B) Kimura et al, 1971**: Similar oral LD50 values; approximately 5600 mg/kg and 6400 mg/kg for younger and older adult rats, respectively. 2) Acute inhalation toxicity **A) BASF, 1980**: Concentrations: 6.08, 20.00, 23.98, 38.87 and 61.80 mg/L; 4 hours; 10males/females rats): LC50 > 20 mg/L (28.1 mg/L in males and females; 25.7 mg/L in males and 30 mg/L in females). According to the EU RAR (2003), animals showed watery discharge from eyes and nose, unrest, increased respiration, rocking gait, narcosis, startling movements and hyperaemia of the ears and extremities. In the highest exposure group, salivation was observed. In the group exposed to 6.08 mg/L of toluene, no adverse clinical signs were observed. All surviving rats appeared normal after 3 days following the exposure. **B) Páez-Marínez et al, 2003**: toluene, mice. The authors conclude that toluene has anxiolytic-like activity, as evidenced by its effects on burying behaviour and passive avoidance behaviour. It increased the number of electric shocks received by the animals	Non-human information 1) Oral **Huff, 1990**: Two key studies in 10 rats and mice, respectively; 312, 625, 1250, 2500, or 5000 mg toluene/kg in corn oil by gavage for 13 weeks. Deaths at 5000 mg/kg (in all rats and mice), 2500 mg/kg (8male and 1 female rat; and 4/sex mice), and 1250 mg/kg 91 mouse). Clinical signs (rats or mice): prostration, hypoactivity, ataxia, piloerection, lachrymation, and excessive salivation at 5000 and 2500 mg/kg groups. There were no treatment related effects in the haematological and serum chemical analyses or urinalyses in either species. In rats at 1250 and 2500 mg/kg there were differences in the weight of a number of organs and neuropathological changes in the brain, consisting of neuronal cell necrosis in the dentate gyrus and Ammons horn of the hippocampus. In addition to the hippocampal lesions, necrosis and/or mineralisation were present in the granular layer of the cerebellar cortex. In mice, relative brain and testis weight, and absolute kidney weight was increased in male mice at 5000 mg/kg. Myocardial degeneration was found in 3 male and 2 female mice from this group. The NOAEL for repeat dose oral toxicity is considered to be 625 mg/kg in rats and mice. 2) Inhalation **A) Huff, 1990**: 15 week study in rats, inhalation, concentration (100, 625, 1250, 2500, or 3000 ppm toluene 6.5 hours/day for 5 days/week). Death reported at 3000 ppm (8 males during week 2). Lower body weights, adverse clinical signs and differences in absolute or relative	**Da-Silvia et al, 1990**: Toluene vapor (800 mg/m3) 6 h daily from gestation days 14 to 20, and 6 to 11 in rats and hamsters resulted effect on exploratory behaviour: Male rat offspring exposed to toluene displayed shorter latencies than male controls to choose one side of a T maze in a spontaneous alternation test. Hamsters exposed to toluene performed worse in a rotating rod test.	Physical state: Liquid VP: 3088.9Pa at 21.1°C and 4130.0Pa at 26.6°C. Water solubility: 573-587 mg/l at 25°C Log Kow: 2.73	Acute exposure to toluene in human volunteers show that dizziness and sleepiness are experienced at air levels < 20 mg/L for 4h and rocking gait and narcosis were observed in rats at this same concentration). After repeated dose exposure toluene case adverse effects including impairment of auditory function and morphological evidence of cell loss in the rat cochlea, neuron loss in the central nervous system of animals and in humans neuropsychological effects, auditory dysfunction and effects on colour vision have been reported. Toluene resulted neurobehavioural effects in the rats and hamster offsprings and the effect becomes more stronger in the hamster offspring.

	during conditioned defensive burying testing, which was interpreted by the authors as indicative of a possible adverse effect on learning. **C) Unnamed, 1980**: Concentrations (Nominal: 7, 31.6, 52.2, 78.3, 104.4 mg/L; Analysed: 6.08, 20.00, 23.98, 38.87, 61.80 mg/L), ~OECD 43, toluene, rats, inhalation (whoe body), 4h. Animals showed watery discharge from eyes and nose, unrest, increased respiration, rocking gait, narcosis, startling movements and hyperaemia of the ears and extremities. In the highest exposure group, salivation was observed. In the group exposed to 6.08 mg/L of toluene, no adverse clinical signs were observed. All surviving rats appeared normal after 3 days following the exposure. 3) Acute toxicity: dermal **Smyth et al, 1969**: A single acute dermal toxicity study on toluene in the rabbit has been found. Fur was removed from the entire trunk by clipping and the dose held in contact with the skin for 24 hours (retained by impervious plastic film). The 4 animals per group were immobilised during the contact period, after which the film was removed and the rabbits caged for a 14 day observation period. The LD50 value was reported to be 14.1 mL/kg, corresponding to 12267 mg/kg (using a density of 0.87). No information on clinical signs or mortality pattern was provided. Human information The acute effects of toluene inhalation exposure have been investigated in a number of human experimental studies in volunteers. These studies indicate that toluene produces a	organ weights (particularly liver and kidney) at 3000, 2500 and 1250 ppm. Plasma cholinesterase activity decreased as exposure concentration increased. NOAEC = 625 ppm. B) No effects in neurobehavioural, or other NS effects reported in the other provided repeated dose toxicity studies in rats (NTP, 1990; Gibson and Hardisty (1983), mice (Huff, 1990). C) Unnamed,1984: Special studies addressing neurotoxicity and ototoxicity demonstrate that toluene is ototoxic in the rat and can produce neurochemical and pathological changes. However, the data are insufficient to determine NOAEC values. Human information After repeated dose exposure via inhalation in humans toluene causes a number of adverse effects including neuropsychological effects, auditory dysfunction and disturbances of colour vision. Key data published since the EU RAR (2003) addressing these effects are those of Seeber et al (2004) and Schaper et al (2003, 2004). There was no evidence that long-term exposure to toluene at 26 ppm for 21 years had any effects on cognitive function (Seeber et al, 2004). There was no evidence of ototoxicity resulting from occupational exposure to toluene below 50 ppm (mean exposure 26 ppm / 98 mg/m3) in a longitudinal study over 5 years (Schaper et al, 2003). Similarly no effect of human occupational exposures to toluene on colour vision were found in a follow up study over 4 years with three repeated examinations (Schaper et al, 2004). These studies demonstrate			

	number of subjective sensations such as headache, dizziness, feeling of intoxication, irritation and sleepiness and decreases in acute neurobehavioural performance at concentrations = 75 ppm **(EU RAR, 2003)**. **Muttray et al (2005)** exposed twenty healthy men to a constant level of 50 ppm toluene. The Pupillographic Sleepiness Test (PST) was performed before and after 4.5 hours of exposure. Acute symptoms were assessed with the Swedish Performance Evaluation System (SPES) self-assessment questionnaire, once before and 3 times during exposure. There was no effect of toluene exposure on PST or tiredness but scores for unpleasant smell and irritation to the throat were increased. A NOAEC of 50 ppm (188 mg/m3) can be determined for acute neurobehavioural effects in humans.	that 26 ppm (98 mg/m3) is a NOAEC for human adverse effects. This value will be taken forward in the risk characterisation.			

201-167-4 Trichloroethylene	The report on provided acute toxicity studies are not detail to the effect of the substance with respect to neurotoxicity.	**Maltoni et al, 1986**: Oral gavage, 52 weeks, rats, 50 or 250 mg/kg bw/day, examination includes histopathology of the central nervous system: no effects in behavioural parameters or other CNS effects. **EU RAR, 2004**: Oral gavage; 375, 750, 1500, 3000 and 6000 mg/kg/day; 13 weeks: no effects reported for behavioural parameters or other CNS effects. **EU RAR, 2004**: Inhalation, rats, (250, 800 and 2500 ppm), 7 hours/day, 5 days/week (duration of exposure not reported). NOAEL 250 mg/kg bw/day "neurotoxicity for long-term repeated exposure to trichloroethylene". **EU RAR, 2004**: Inhalation, rats, (500, 1000, 2000, 3000 ppm): No neurotoxicity effect reported. **Maltoni et al 1986**: rats, inhalation, (100, 300, 600 ppm), 104 weeks. No neurotoxicity effect reported.	**Evangelista de Duffard, 1996**: The author indicated that chlorinated hydrocarbon class that includes trichloroethylene have effects on motor, sensory, or cognitive function that are detectable using functional measures such as behavior. In additton, there is evidence that each of these chemicals are developmental toxicants if exposure occurs during critical periods of development. **EPA, 1998**: rats, 1,1,1-TCE (trichloroethylene), (75, 250, 750 mg/kg/day), oral gavage, from GD 6 to GD 10. Neurobehavioral and neuropathological parameters were measured in offsprings Result: no effect related to neurotoxicity in the offspring. **Blossom et al, 2016**: 1,1,1-TCE (trichloroethylene), Oral (drinking water) in 6 week old male offspring of dams exposed gestationally to 0, 0.01, and 0.1mg/ml resulted: a) Increased locomotor activity at 0.01 and 0.1 level. b) Alters brain redox homeostasis in the	Physical state: Liquid VP: 9.9 kPa at 25 °C Water solubility: 1.1 g/L at 20 °C Log Kow: 2.53	No detail information provided on the effect of the substance after acute exposure. No neurotoxicity effects observed after long term repeated exposure to toluene. Information from literature search showed conflicting results. One stuy concluded that trichloroethylene is develometal neurotoxicant. Similarly another prenatal study showed inflammatory and oxidative stress related effects which associated with neurotoxicity and also indicated that plasma level biomarkers can be used to predict trichloroethylene mediated neurotoxicity effects. While another PNDT study via oral gavage from GD 6 to GD 10 doses up to 750 mg/kgbw/day did not result neurobehavioural and neuropahological effects in the offspring.

			cerebellum (reduced concentration of GSH, GSSG, the glutathione redox (GSH/GSSG) ratio, and the percentage of oxidized glutathione equivalents in the cerebellum). c) Alters transsulfuration and transmethylation metabolites in plasma. d) Positive association between oxidative endpoint GSH in plasma and cerebellum e) Nitrotyrosine increased in cerebellum and plasma. f) increased plasma inflammatory biomarkers. g) increases population of memory CD4+ T cells in adult mice. h) Prenatal TCE exposure alters CD4+ T cell cytokine production Conclusion by the Author: The result suggests that the prenatal period is a critical stage of life by which the developing CNS and immune system are susceptible to long-lasting changes mediated.		

200-751-6 n-butanol	**Unnamed, 1951**: Acute oral gavage study: 3160, 3980, 5000 or 6300 mg/kg . Clinical signs includes narcosis and prostration preceded death.	**David et al, 2001**: 90 day inhalation study in rats treated with n-Butyl acetate at 500, 1500 and 3000 ppm. Clinical signs includes less movement, decreased alertness, and slower response to tapping on the exposure chamber wall (reduced reactivity) at 3000 ppm (ca. 14.1 mg/L). There were no signs of neurobehavioral effects or systemic toxicity immediately after exposure (30-60 minutes following the cessation of exposure). Histopathlology: no effects in brain, spinal cord (cervical and lumbar regions), dorsal and ventral spinal roots, dorsal root ganglia, sciatic nerve, and tibial nerve of animals. Functional Observational: No effect. Motor activity: for all male groups the mean total motor activity counts (MTMAC) and total ambulations (TA) were higher during week 4 (11% for motor activity counts) but closer to baseline values during weeks 8 and 13 and no significant differences were observed among groups). For female groups, MTMAC and TA were higher during Week 4 than at other times, but there were no statistically significant differences. Mean total motor activity counts for female rats were also closer to baseline values during Weeks 8 and 13.Schedule-Controlled Operant Behavior (SCOB): no significant concentration-time interactions in any SCOB parameters, and no significant changes in SCOB parameters following cessation of exposure relative to the last week of exposure.	**Sitarek et al, 1994**: Rats exposed to n- butanol via oral (drinking water) at 0.24, 0.8 and 4% (0.3; 1.0 and 5.0 g/kg/day) for 8 weeks before and during gestation. Result: Internal hydrocephalus was the most frequent anomaly found in the foetuses of female rats exposed to n-butanol at mid and high dose level. Pathological changes in the CNS includes (dilation of subarachnoid space, cerebral ventricles,) at all dse levels. No detectable toxic effect in the parental females. But produced congenital defects including the CNS of their offspring.	Physical state: Liquid VP: 9.31 hPa at 25 °C Water solubility: 66 g/L at 20 °C Log Pow: 1 at 25 °C	Acute oral study showed narcosis effect. 90 day inhalation study resulted reduced activity related to CNS depression at 3000 ppm (ca. 14.1 mg/L). Transient effect in motor activity was also reported. PNDT study up to 5 g/kg/day for 8 weeks resulted effects in the CNS (hydrocephalus, dilation of subarachnoid space, cerebral ventricles).

200-661-7 Isopropanol	**Unnamed, 1991**: OECD 403, tats, inhalation, 6h, test concentrations (500, 1500, 5000, 10000 ppm): At 10000 ppm group, prostration, severe ataxia, decreased arousal, slowed or labored respiration, decreased neuromuscular tone, hypothermia, and loss of reflex function was observed 1 and 6 hours after exposure. Concentration-related decreases in mean motor activity were observed for males in the 1500, 5000, and 10000 ppm groups and females in the 5000 and 10000 ppm groups. Motor activity was severely depressed for males and females in the 10000 ppm group.	**Bushy Run Research Center, 1994**: inhalation, rats, 104 weeks, test concentrations (500, 2500, 5000 ppm). At 5000 ppm; hypoactivity, lack of a startle reflex, and narcosis were identified. At 2500 ppm; hypoactivity, and a lack of a startle reflex were observed. No effects at 500 ppm. **Bushy Run Research Center, 1991**: OECD TG 413, rats and mouse, inhalation, test concentration (100, 500, 1500, 5000ppm), At 5000 ppm ataxia, narcosis, hypoactivity, and a lack of a startle reflex were observed in some rats and mice; narcosis was not observed in rats after Week 2 of the study. At 1500 ppm, narcosis, ataxia, and hypoactivity were observed in some mice, while only hypoactivity was observed in rats. No clinical signs were noted during exposures for either rats or mice of the 100 and 500 ppm groups. Clinical signs observed following exposures included a markedly increased incidence of swollen periocular tissue in rats of the 5000 ppm group (females only) and an increased incidence of perinasal encrustation in male rats of the 500, 1500, and 5000 ppm groups. Additionally, ataxia and/or hypoactivity were observed in one male rat, three female mice, and one male mouse of the 5000 ppm group immediately following exposure. Paresis was noted in one female rat of the 5000 ppm group from Week 8 to the termination of the study, although this was not believed to be a result of exposure. No exposure-related clinical signs were observed following exposures in male or female rats of the 100 ppm group; no exposure-related clinical signs were observed following exposures for male or female mice of the 100, 500, and 1500	**Bates et al, 1994**: rats, Isopropanol at 200, 700, 1200 mg/kg/day via oral gavage from GD 6 to PND 21. Neurobehavioral and neuropathological parameters were measured in offsprings The author concluded that "no evidence of developmental neurotoxicity associated with isopropanol exposure as high as 1200 mg/kg/day".	Physical state: Liquid VP: 44 hPa at 20 °C Water Solubility: "Compound is soluble, however, unable to determine degree of solubility from the word "miscible"". Log Pow: 0.05	Narcosis and increase in motor activity was observed from acute inhalation study. In Chronic inhalation study showed clinical signs of toxicity in the nervous system at 5000 ppm and at 2500 ppm (hypoactivity and lack of startle reflex). A 13 weeks inhalation study showed clinical signs of toxicity in the Nervous system including narcosis in rats (narcosis reversed after 2 weeks of the study) and mice at 5000 ppm and in some mice and rats (only hypoactivity) at 1500ppm. Increase motor activity only in female rats at 5000 ppm. No effects in functional observation battery, and no treatment related neuropathological effects. PNDT study via oral gavage from GD 6 to GD 10 at doses up to 750 mg/kgbw/day did not result neurobehavioural and neuropahological effects in the offspring.

		ppm groups. Functional observational battery: no effects. Increased motor activity for female rats in the 5000 ppm group was noted at Weeks 9 and 13. Neuropathologic examination: a minimal degree of myelin degeneration was seen in the sciatic or tibial/peroneal/sural nerves of a few rats (including controls). Since this finding was observed in only a few fibers of each affected nerve, the degeneration was believed to be spontaneous "background" myelin degeneration and not related to the exposures.			

200-746-9 n-propanol	1) Oral exposure **un named, 1953**; Smyth et al, 1954; Kennedy et al, 1991: Acute oral study (1000; 2000; 3980 mg/kg bw). LD50 =1870 mg/kg bw. Clinical effects observed: Sluggishness, prostration and narcosis. 2) Inhalation exposure **a) Unnamed, 1975**: Inhalation, OECD TG 403, test concentration (Experiment I (8h exposure): 51.91 mg/l, Experiment II (3h exposure): 62.48 mg/l), . Result: by the end of exposure, all animals were in deep narcosis. After 24h, all signs of toxicity disappeared. **b) Un named, 1991**: ~OECD TG 403, inhalation, 4h, concentration [(Experiment 1: 5185 ppm (12.9 mg/l) (12930 mg/m3), Experiment 2: 9741 ppm (24.3 mg/l) (24291.9 mg/m3), Experiment 3: 13548 ppm (33.8 mg/l) (33785.7 mg/m3))]. Result: periocular wetness, blepharospasm, and hypoactivity (5165 ppm, 9741 ppm, and 13548 ppm): increased shallow respiration, no startle reflex, and narcosis within 2 h. An absence of motoric activity was observed in all animals within 2.5 h (9741 ppm) and 3h (13548 ppm, lacrimination). Clinical signs observed on the same day of exposure were periocular wetness and depressed toe and nail pinch and surface right reflexes at 9741 ppm and 13548 ppm, and perinasal wetness, abdominal breathing, eye blink and surface right reflexes at 13548 ppm. No clinical signs of toxicity were observed during the 14-day postexposure observation period.	**Union Carbide Corporation, 1992**: 28 day inhalation study, rats, (100, 500 and 1000 ppm): the only effect reported was perinasal and periocular encrustation in 1/10 male rats exposed to 500 ppm at 1000 ppm in both males and females. **Un named, 2004**: 90 day, inhalation, rats, test concentration (500, 2000 or 8000 mg/m3), examination includes functional observation: no effects related to neurotoxicity reported up to 8000 mg/m³ air (nominal).	**Candura et al, 1991**: investigated the in vitro effects of five short chain aliphatic alcohols (ethanol, n-propanol and t-butanol) on muscarinic receptor-stimulated phosphoinositide metabolism in cerebral cortical slices from 7 day-old rats. "These results suggest that muscarinic receptor-coupled phosphoinositide metabolism might be a common neurochemical target for the developmental neurotoxicity of short chain aliphatic alcohols."	Physical state: Liquid VP: 28.2 hPa at 25°C Water solubility: "substance is completly miscible in water at 25 °C" log Pow: 0.2 at 25 °C	Narcosis effect reported from the acute oral or inhalation studies. No effcts in the nervous system including clinical signs were reported in the 28 day or 90 day inhalation studies. No studies from literature search related to the neuro developmental effect of n-propane found.

	c) Un named, 1975: ~ OECD TG 403, rats, test concentratioin (26.76 mg/l(approx 10905.4 ppm)), 7 h (after 3.5 the substance was already exhausted). No death occurred. After 3h, pain reflex was lost (narcosis) and accelerated breathing noted in the rats.				
200-467-2 Diethyl ether ether	**Kobayashi, 1985**: Inhalation in mice for 120 min at 2.7, 2.9, 3.2, 3.5, 3.8, 4.6 vol% (males) and 2.7, 2.9, 3.2, 3.5, 3.8, 4.2, 4.6 vol% (females). Result: Convulsions at induction of anethesia. **Schwetz and Becker, 1971**: Adult females and neonates (males and females) 170 minutes via inhalation, test concentration (15vol% and 20vol%). Higher blood concentration of diethyl ether in neonates than adults which reflect the longer exposure period. In comparison to adults, neonates have decreased sensitivity to CNS than adults.	**Un named, 1986**; EPA, 1987: 13 weeks, oral gavage, rat, (500, 2000, 3500 mg/kg bw). Light anesthesia at 2000mg/kg bw and 3500 mg/kg bw. **Dalbey and Feuston, 1996**: OECD TG 413, with Diisopropyl ether (EC no: 203-560-6), inhalation, test concentration (480 ppm, 3300ppm, 7100ppm). no changes in clinical signs. Other repeated dose toxicity studies via inhalation with RA substances reveal no neurotoxicity effects. **Chenoweth et al, 1968**: TK data showed the distribution of diethyl ether in the brain.	not found	Physical state: Liquid VP: 716 hPa at 25 °C Water solubility: 64.9 g/L at 20 °C Log Pow: 0.83	Convulsions from acute inhalation study up to 120 minutes. Acute inhalation study in adults and neonates for 170 minutes showed neonates are less sensitive than adults to diethyl ether. Repeated dose toxicity studies do not report effects related to neurotoxicty. No information found from literature search on the effect of dietheyl ether on neurodevelopmental effect.

203-560-6 Diisopropyl ether	**Machle et al.,1938**: Acute oral toxicity in rabbits at 1.62, 3.3, 5.2, 6.0, 7.2, or 8.2 g/kg bw/day via ora gavage. A lack of coordination and unsteadiness from 5 to 20 minutes, followed by intoxication and light narcosis from 14 to 40 minutes. > 7.2 to 8.2 g isopropyl ether/kg bw, more rapid onset of symptoms and deep narcosis followed. **Machle et al., 1938**: Inhalation toxicity in monkeys, rabbits, and guinea pigs at 0.1, 0.3, 1.0, 3.0, or 6.0% for 180 minutes. signs of anesthesia were reported in all species at 1.0 and 3.0% isopropyl ether (approximately 10000 and 30000 ppm, respectively), and death at 6.0%.	**Unnamed, 1996**: ~ OECD TG 413, rats, test concentration (480 ppm, 3300 ppm, and 7100ppm). No effects reported on the nervous system.	not found	Physical state: Liquid VP: 198.65 hPa at 25 °C Water solubility: 3.11 g/L at 20.2 °C Log Pow: 2.4	Narcosis reported from acute inhalation studies. No report on effects on the NS from subchronic inhalation exposure to 3300 ppm diisopropyl ether. No information found from literature search on the effect of diethyel ether on neurodevelopmental effect.
200-662-2 Acetone	**Bruckner and Peterson, 1981**: Acute inhalation toxicity, test concentration (12,600, 19,000, 25,300, 50,600 ppm). Based on additional tests for CNS depression by measuring impairment of unconditioned performance and reflexes, an approximately linear time-response relationship for depressive effects was observed up to 3 hrs exposure to 30 to 60 mg/L .	**Bruckner and Peterson, 1981**: 2, 4, 8 weeks, test concentration (19,000 ppm or 45,000 mg/m3), rats. no effects reported on the NS. **Dietz et al, 1991**; study name, 1991: ~OECD TG 408, rats, acetone, test concentration (2,500, 5,000, 10,000, 20,000, 50,000 ppm), no functional observation examination dose. No effects in the nervous system reported.	Not found	Physical state: Liquid VP: 58.59 kPa at 294.26 K Solubility: 240 hPa at 20° Log Pow: -0.23	CNS depression reported from acute inhalation study. No effect reported in the nervous system after repated exposure by inhalation or oral route. No information found from literature search on the effect of acetone on neurodevelopment.
204-658-1 n-butyl acetate	**Unnamed, 1987**: ~OECD 403, rats, inhalation, single 4-hour, test concentration (283 - 6867 ppm). Clinical signs includes ocular and respiratory irritation and central nervous system depression. Ataxia and narcosis were observed during and immediately following 6867 ppm.	**Bernard and David, 1996**: 13 weeks, oral, (30, 125, 500 mg/kg bw), rats, no neurobehavioural examination. clinical sings incudes Ataxia and hypoactivity at 500 mg/kg bw. Bernard and David, 1995: 28 day inhalation study, (750, 1500, 3000 ppm), rats, neurobehaviour examination included. Clinical signs includes transient reduced acitivity and reduced response at 3000ppm. No effect in neurobehaviour.	Not found	Physical state: Liquid VP: 11.2 hPa at 20°C Water Solubility: 5.3 g/L at 20°C Log Pow: 2.3	CNS depression reported from acute inhalation study. 13 weeks, oral, study showed ataxia and hypoactivity at 500 mg/kg bw. 28 day inhalation study showed transient reduced acitivity and reduced response at 3000ppm. No effect in neurobehaviour. No information found from literature search on the effect of n-butyl acetate on neurodevelopment.

205-563-8 n-heptane	**Frantik et al., 1994**: heptane, rats and mice, inhalation, 4h male rats and 2h female mice. "Under the conditions of the test, Normal-Heptane was capable of blocking electrically evoked seizures at 2740 ppm (90% confidence interval = 730), underlining the effects on behaviour".	**Unnamed, 1980**: ~OECD TG 413, heptane, rats, inhalation (whole body), test concentration (398, 2970 ppm), 26 weeks. The only treatment-related observations were laboured breathing or rapid breathing and slight prostration during the first week of exposure and anogenital fur and dry rales during weekly observations. The in chamber signs were generally more numerous and severe at the higher concentration and appeared to abate by the second week of the study. The effects observed are consistent with acute CNS depression and generally abated by the second week of study. Frontali et al., 1981: no guideline, heptane, rats, inhalation, 1500 ppm. Under the conditions of this test, inhalation of Normal-Heptane at 1500 ppm did not induce neuropathy in rats. **Simonsen and Lund, 1995** ("Auditory sensitivity study"): n-heptane, male rats, inhalation (whole body), 28 days, 0, 3.3, 16.6 mg/L (re-calculated; corresponding to 0, 800, 4000 ppm). No clinical signs of neuropathy were observed. A loss of auditory sensitivity equal to 10dB at 4000ppm and tested by measurement of auditory brain stem response 2 months after cessation of exposure. **Savolainen and Pfäffli, 1980**: "2 week inhalation neurochemical study", heptane, inhalation, test concentration (4.2, 21 and 62 µM), 2 weeks. "Levels of Normal-Heptane increased in brain and perirenal fat during 2 weeks of exposure. None of the rats showed clinical signs of neurotoxicity. Neurochemical changes seen at week 2, including increased proteolysis and higher RNA brain content, were at control levels after 2 weeks recovery". Ono et al., 1979 and Takeuchi et al., 1980, 1981: Evaluations of peripheral nerve toxicity of n-	Not found	Physical state: Liquid VP: 6.09 kPa at 25 °C Water solubility: "slightly soluble (0.1-100 mg/L)" Log Pow: 4.5	Acute inhalation study showed behavioural effects. Repeated dose toxicity studies in rats with n-heptane via inhalation resulted acute CNS depression in 13 weeks study, loss of auditory sensitivity in 28 day study, transient neurochemical changes but other effects like neuropathy in the 28 day study, and nerve conduction velocity in the motor and sensory nerves or microscopic changes in the peripheral nerves in the 16 weeks study were not reported. No information found from literature search on the effect of n-heptane on neurodevelopment.

		heptane male in rats exposed to 3000 ppm via inhalation 12 hours/day, 7 days/week, for 16 weeks. The conduction velocity of tail nerves was measured to determine the functional status of the peripheral nerves. No abnormal behavioral changes were observed. No statistically significant differences in motor nerve conduction velocity, distal latency or mixed nerve conduction velocity in any region of the tail. No microscopic change in nerves, muscles and neuromass junctions. n-Heptane is not a neurotoxicant in this assay system.			
203-892-1 n-octane	**Unnamed, 1983**: ~OECD TG 403, octane, rats, inhalation, 4h, 24.88 mg/L. "After 15 min of exposure all animals displayed rapid breathing. At 30 min of exposure one male and all females showed tremors and hyperactivity. At one hour these animals became languid with tremors. All animals then were noted to be inactive with rapid respiration for the duration of the exposure. Normal appearance was observed for all animals at Day 1 and subsequently through the 14-day observation period". **Unnamed, 1982**: ~OECD TG 401, 2,2,4-trimethylpentane, rats, oral (gavage), 5000 mg/kg bw. "Clinical observations noted one-hour post exposure in 8 of 10 animals included depression, salivation, wheezing, rough coat, and soft feces. Two female rats appeared normal throughout the study. All animals appeared normal from day 2 through termination of the study".	**Unnamed, 2000**: "Effects of the test compound on cognitive performance were evaluated using a discrete-trial two-choice visual discrimination task", octane, rats, inhalation, 8h, test concentration (300, 900, and 3000 ppm), 3 days. "In conclusion, short-term high-level exposure to n-octane did not induce any toxicologically significant effects on measures of learned performance." **Unnamed, 2000**: FOB and motor activities examined, octane, rats, inhalation, 8h, test concentration (300, 900, and 3000 ppm), 3 days. "In conclusion, short-term high-level exposure to n-octane did not induce any toxicologically significant effects on functional observations and measures of motor activity." **Unnamed, 1998**: ~ OECD TG 413, test substance (Naphtha (petroleum), light alkylate), rats, inhalation (whole body), FOB and motor activity examinations performed, test concentration (668, 2220, and 6646 ppm). No effects related to neurotoxicity reported. **Carpenter et al., 1978**: ~OECD TG 413, nonane, male rats, inhalation, test concentration (1.9, 3.1, 8.4 mg/L). No effects related to neurotoxicity reported.	Not found	Physical state: Liquid VP: 1.86 kPa at 25 °C Water solubility: "slightly soluble (0.1-100 mg/L)" Log Pow: 5.15	Acute toxicity study in rats via oral results transient clinical effects like tremors and hyperactivity with n-octane, and depression and salivation with read across substance 2,2,4-trimethylpentane. Repeated dose toxicity studies in rats with octane or read across substances via inhalation results no effects on cognitive performance, functional observations and motor activity, in 3 days or 13 weeks studies. No information found from literature search on the effect of n-octane on neurodevelopment.

208-759-1 2,2,4-trimethylpentane	**Unnamed, 1982**: ~OECD TG 401, 2,2,4-trimethylpentane, rats, oral (gavage), 5000 mg/kg bw. "Clinical observations noted one-hour post exposure in 8 of 10 animals included depression, salivation, wheezing, rough coat, and soft feces. Two female rats appeared normal throughout the study. All animals appeared normal from day 2 through termination of the study". Unnamed, 1982: ~OECD TG 403, 2,2,4-trimethylpentane, rats, inhalation, 4h, 33.52 mg/L."Exposure-related observations noted in all animals during the exposure period included lying prostrate in the cage and rapid respiration. All animals appeared normal throughout the post exposure period".	**Unnamed, 2001**: "Effects of the test compound on cognitive performance were evaluated using a discrete-trial two-choice visual discrimination task", Alkanes, C7-10-iso-, male rats, inhalation (air), 8h, 3 days, test concentration (300, 900, 3000 ppm). "Visual discrimination performance was also not significantly changed after exposure to iso-octane. A number of statistically significant effects were observed during testing prior to exposure and during the exposure period, but no significant differences between iso-octane-exposed groups and the control group were observed for general measures of responding, measures of stimulus control, measures of disinhibition or measures of psychomotor speed". **Unnamed, 2001**: FOB and locomotor activities examined, octane, rats, inhalation, 3 days, test concentration (300, 900, and 3000 ppm). "In conclusion, short-term high-level exposure to n-octane did not induce any toxicologically significant effects on functional observations and measures of motor activity." no effects related to neurotoxicity reported. **Unnamed, 1998**: ~ OECD TG 413, test substance (Naphtha (petroleum), light alkylate), rats, inhalation (whole body), FOB and motor activity examinations performed, test concentration (668, 2220, and 6646 ppm). No effects related to neurotoxicity reported. **Carpenter et al., 1978**: ~OECD TG 413, nonane, male rats, inhalation, test concentration (1.9, 3.1, 8.4 mg/L). No effects related to neurotoxicity reported.	Not found	Physical state: Liquid VP: 2.8 kPa at 20 °C Water Solubility: 2.2 mg/L at 25 °C Log Pow: 4.08	Acute toxicity study with 2,2,4-trimethylpentane results clinical effects like depression, and salivation via oral; and prostrate via inhalation. Repeated dose toxicity studies in rats with read across substances (Alkanes, C7-10-iso-, octane, Naphtha (petroleum), light alkylate, and nonane) in rats between 3 days to 13 weeks via inhalation results no neurotoxicity effects related to cognitive performance, FOB, and motor activity. No information found from literature search on the effect of 2,2,4-trimethylpentane on neurodevelopment.

203-806-2 cyclohexane	**Unnamed, 2009**: no guideline, Cyclohexane, rats, inhalation, cyclohexane, test concentration (1400 mg/m3, 8000 mg/m3 & 28000 mg/m3), neurobehavioural examination conducted. "Cyclohexane exposure up to 28000 mg/m3 produced minimal acute CNS effects in rats, with small effects on gait and a statistically significant reduction in psychomotor speed in visual discrimination tests". **Unnamed, 2000**: ~EPA OPPTS 870.6500, cyclohexane, male rats, inhalation, test concentration (500, 2000, 7000 ppm). "No mortality, narcosis or obvious signs of behavioural impairment. No obvious signs of intoxication such as lethargy or ataxia when the rats were removed from the exposure chambers". "minor and transient effects on schedule controlled operant behaviour in rats were seen at the 7000 ppm (24,080 mg/m3) (the highest exposure concentration)".	**Frontali et al, 1981**: no guideline, cyclohexane, rats, inhalation, 30 weeks, test concentration (1500, 2500 ppm). "There were no outward manifestations of neuropathy, no effects on body weight gain, and no histopathological changes in nervous tissue". **Unnamed, 2000**: EPA OPPTS 870.3465, cyclohexane, rats, inhalation, 13-14 weeks, test concentration (500, 2000 and 5000 ppm). Transient effects on the alerting response during exposure to 2000 and 7000ppm. No effects on forelimb grip strength, hind-limb grip strength, foot splay, or motor activity were reported.	Not found	Physical state: Liquid VP: 12.7 kPa at 20 °C Water Solubility: 52 mg/L at 23.5 °C Log Pow: 3.44	Acute toxicity study with cyclohexane in rats via inhalation results clinical effects like gait and a statistically significant reduction in psychomotor speed at 28000 mg/m3. Repeated dose toxicity studies in rats with cyclohexane between 3 days to 13 weeks via inhalation results no effects like neuropathy or histopathological changes in the nerve tissue in 30 weeks study; and no neuro-behavioural effects in a 13-14 week study. No information found from literature search on the effect of cyclohexane on neurodevelopment.

203-624-3 methylcyclohexane	**Treon et al., 1943**: no guideline, methylcyclohexane, rabbit, single exposure by oral (gavage), doses (1000 to 10000 mg/kg bw). The cinical sings includes "increased respiratory rate remaining elevated for a few hours, within few minutes after administration, the ears became reddened and slightly cyanotic, ear veins were distended and conjuctival congestion was observed at higher doses. Animals were generally lethargic without exhibiting true narcosis. Convulsions were not observed". **Unnamed, 1979**: no guideline, methylcyclohexane, dog, inhalation (whole body), 4071 ppm, 1h. "concentration of MCH that will not cause chronic or irreversible tissue damage nor produce CNS effects which could impair coordination or prevent a man from self rescue". **Unnamed, 1979**: not guideline, methylcyclohexane, mice, inhalation (whole body), test concentration (26.3 mg/L for first experiment, and 19.1 mg/L for second experiment), 1h. Clinical sings includes hyperactivity during exposure to 19.1 mg/L. At 26.3 mg/L immediate hyperactivity, slight loss of coordination at 12 min, and prostration was noted at 42 and 54 minutes. **Lazarew, 1929**: not guideline, mice, inhalation (whole body), 2h, 30-50 mg/L, methylcyclohexane. "lethargy, narcosis, prostration, clonic convulsions and laboured breathing before death occurred". **Unnamed, 1979**: not guideline, rats, methylcyclohexane, inhalation (whole vapour), 1h, test concentration (26.3 mg/L for first experiment, and 16.7 mg/L for second experiment). Clinical sings includes increased	**Unnamed, 2001**: OECD TG 407, methylcyclohexane, rats, oral (gavage), 28 days, doses (100, 300, 1000 mg/kgbw/day). "Salivation in males at 300 mg/kg bw/day and in both sexes at 1000 mg/kg bw/day within one hour after administration. This change was not observed during recovery period". No changes in FOB examination were reported. **Unnamed, 1985**: no guideline, methylcyclohexane, dog, inhalation (whole body), test concentration (400 and 2000 ppm), neurobehaviour not examined, 12 months. no effects related to neurotoxicity reported. **Unnamed, 1985**: no guideline, methylcyclohexane, mice, inhalation (whole body), test concentration (400, 2000 ppm), 12 months, neurobehaviour not examined. No effects related to neurotoxicity reported. **Unnamed, 1985**: no guideline, methylcyclohexane, rats, inhalation (whole body), test concentration (400, 2000 ppm), 12 months, neurobehaviour not examined. No effects related to neurotoxicity reported.	Not found	Physical state: Liquid VP:"1, 6.18, 10 and 100 kPa at -7.9, 25, 35.5 and 100.5 °C, respectively" Water solubility: 14 mg/L at 25 °C Log Pow: 3.88	Acute toxicity study with methylcyclohexane via inhalation results clinical effects like lethargic without exhibiting true narcosis in rats; no CNS effects in dogs; and hyperactivity, slight loss of coordination and prostration in mice and rats. Repeated dose toxicity studies with methylcyclohexane via inhalation results no effects in neurobehaviour in rats exposed for 28 days. No effects related to neurotoxicity were reported in dogs, mice, and rats exposed for 12 months (however examination like neuro-behavioural examination were not included). No information found from literature search on the effect of methylcyclohexane on neurodevelopment.

	activity during exposure to 16.7 mg/L which is less than at 26.3 mg/L. At 26.3 mg/L immediate hyperactivity, slight loss of coordination at 29min, and prostration at 54 minutes.				
203-777-6 n-hexane	**Unnamed, 1982**: not guideline, rats, inhalation, 4h, 31.86 mg/L, n-hexane. "Other than animals appearing hyperactive in the first hour of observation, no treatment related clinical signs were apparent". **De Martino et al, 1987**: ~ OECD TG 403, n-hexane, rats, inhalation (whole vapour), 24h, 5000 ppm. No effects related neurotoxicity reported.	**Takeuchi et al., 1980**: Not guideline, rats, n-hexane, 3000 ppm 12 hours/day, 7 days/week, 16 weeks. Motor nerve conduction velocity and distal latency were significantly affected after 4 weeks exposure. Examination of neural tissue showed damage to the tibial nerve and dorsal trunk of the tail nerve.	Cheng et al, 2015: In this literature the metabolite of n-hexane (2,5-hexanedione) is indicated as a neuronal toxin in the developing fetus.	Physical state: Liquid VP: 10 kPa at 9.8 °C Water solubility: 0.01 g/L at 25 °C Log Pow: 4	Acute toxicity study with n-hexane via inhalation results clinical effects of hyperactivity in the non-guideline study while no clinical effects related to neurotoxicity were reported in the OECD TG 403 study. Repeated dose toxicity studies with n-hexane via inhalation for 16 weeks results effects on motor nerve conduction velocity and distal latency, and damage to neuronal tissues. Information from literature showed that metabolite of n-hexane (2,5-hexanedione) is developmental neurotoxicant.

201-142-8 Isopentane	**Unnamed, 1982**: OECD TG 423, Cyclopentane, rats, oral, 5000 mg/kg. "Clinical signs observed over the first 24 hours after exposure included depression (sometimes slight), red stains on the nose and/or eyes, rough coat, soft feces, a hunched appearance, and urine stains". **Unnamed, 1994**: not guideline, n-Pentane, male rats and female mice, inhalation (whole body), 4h for male rats and 2h for female mice, test concentration not reported. "The concentration resulting in 37% depression of the shortening of the tonic extension of hindlimbs by 3 seconds in male rats was found to be 21000 ppm, while the concentration resulting in 30% depression of the lengthening of the latency of extension by 0.6 seconds in mice was found to be 23500 ppm." **Unnamed, 1993**: OECD TG 403, Cyclopentane, rats, inhalation (whole body), 4h, 25.30 mg/L. "Hunched posture was observed in all rats (male and female) during hours 1 to 4 of the expsoure period". **Unnamed, 1936**: not guideline, 1-bromopropane, mice, inhalation (whole body), test concentration (4.2 mmol/L, 4.9 mmol/L, 5.4 mmol/L, 5.8 mmol/L, 6.3 mmol/L). "After the mice were removed from the bottle during the "complete anaesthesia" test their recovery time was noted. The average recovery time for the test mice after being complete anaesthesized ranged from 4 to 8 minutes".	**Unnamed, 1998**: OECD TG 413, test concentration (Naphtha (petroleum), light alkylate), rats, inhalation, test concentration (668, 2220, 6646 ppm). "There were no treatment-related effects in mortality, clinical signs, neurotoxicity, body weight, or food consumption."	Not found	Physical state: Liquid VP: 100 kPa at 27.5 °C Water solubility: 0.049 g/L at 25 °C Log Pow: 4	Acute toxicity study results depression in rats with Cyclopentane (oral or inhalation) and with n-pentane, and complete anaesthesia in mice with 1-bromopropane. Repeated dose toxicity studies with Naphtha (petroleum), light alkylate via inhalation in rats for 13 weeks results no effects related to neurotoxicity. No information found from literature search on the effect of isopentane on neurodevelopment.

203-445-0 1-bromopropane	**Unnamed, 1993**: OECD TG 401, 1-bromopropane, rats, oral (gavage), 2000 mg/kg bw. "Clinical signs observed included sedation, lateral decubitus, dyspnoea and piloerection. All signs had disappeared by day 2." **Unnamed, 1997**: ~ OECD 403, 1-bromopropane, inhalation (whole body), 6h, test concentration (100, 50, 35 and 25 mg/L). Clinical signs includes decreased activity, labored breathing, lack of pinch/eye reflex, uncoordination, limited usage of hindlimbs, lying on side, cold to touch, weak, ocular discharge and eyes partly closed.	**Ichihara et al, 1999**: not guideline, 1-bromopropane, rats, inhalation, test concentration (200, 400 and 800 ppm), 12 weeks. Weak kicking on the floor with their hind limbs, poor extension, and poor outspreading of pedal digits, turning up their planta when landing on the plane (up-and-down landing) at 800 ppm. Grip strength of forelimb and hind limb decreased dose-dependently and progressively with exposure period. Forelimb grip strength decreased progressively at 400 ppm or more. Hind limb grip strength significantly decreased at 200 ppm or more after 4 weeks of exposure, but after 8 weeks of exposure, the decrease was significant only in the 800-ppm group. The weight of the cerebrum decreased significantly at 800 ppm but no change in weight of cerebellum and brainstem.	Fueta et al, 2015: The author indicated that the result of the experiment suggested that prenatal exposure to 1-Bromopropane affects neurobehavioral responces in the juvenile period.	Physical state: Liquid VP: 137 mm Hg at 25 °C Water solubility: 2 500 mg/L Log Kow: 2.16	Acute toxicity study in rats with 1-bromopropane via inhalation results sedation, lateral decubitus, dyspnoea and piloerection, and via oral results effects related to CNS depression. Repeated dose toxicity studies in rats with 1-bromopropane for 12 weeks via inhalation results neuro-behavioural effects Information from literature showed that 1-bromopropane affects the neuro-behavioural responses during juvenile period.
200-815-3 Ethylene	**Guest et al, 1981**: not guideline, ethylene, male rats, inhalation (whole body), 5h, 10000 ppm. "No clinical signs or deaths are reported within the first 36 hrs (when the animals were terminated)"	**Unnamed, 2010:** OECD TG 413, rats, inhalation (whole body), test concentration (300, 1000, 3000, and 10000 ppm), neurobehavioural examination performed, 13 weeks. No effects related to neurotoxicity reported.	Not found	Physical state: Gas VP: 2 124 hPa at -90 °C Water Solubility: 131 mg/L at 25 °C Log Pow: 1.13	Acute toxicity study in rats with ethylene via inhalation results no effects related to neurotoxicity. Repeated dose toxicity studies in rats with ethylene via inhalation for 13 weeks results no effects related to neurotoxicity. No information found from literature search on the effect of ethylene on neurodevelopment.

246-690-9 2,4,4-trimethylpentene	**Unnamed, 1972**: no guideline, rats, oral gavage, 2,4,4-trimethylpentene, doses (250, 500, 1 000 or 2 500 mg/kg bw). At 1000 and 2 500 mg/kg bw, a transient reduction in motor activity was reported.	**Unnamed, 1997**: OECD TG 407, 2,4,4-trimethylpentene, rats, oral gavage, test concentration (100, 300, 1000 mg/kg/day). "it is considered that 2,4,4-trimethylpentene did not cause any change to normal nervous system function when administered by gavage at dosages up to 1000 mg/kg/day".	Not found	Physical state: Liquid VP: 5.8 kPa at 25 °C Water solubility: 2.3 mg/L at 20°C Log Kow: 4.9 - 5	Acute toxicity study in rats with 2,4,4-trimethylpentene via inhalation results no effects related to neurotoxicity. Repeated dose toxicity studies in rats with 2,4,4-trimethylpentene via inhalation for 28 days results no effects related to neurotoxicity. No information found from literature search on the effect of 2,4,4-trimethylpentene on neurodevelopment.

201-148-0 2-methylpropan-1-ol	**Unnamed, 1993**: OECD TG 401, rats, 2-methylpropan-1-ol, oral, 2-methylpropan-1-ol, doses (1000, 2000, 2830 and 4000 mg/kg for females; and 2830 mg/kg for males). Clinical sings includes sluggishness, unsteady gait, lacrimation, piloerection, slow breathing, and prostration. **Unnamed, 1994**; EPA, 1995; OECD 2004: inhalation (whole body), rats, 2-methylpropan-1-ol, test concentration (1500, 3000, 6000 ppm). Generalized depression of the central nervous system and labored respiration at 3000 and 6000 ppm. Minimal hypoactivity at 1500ppm. **Un named, 1978**: rats, inhalation (whole body), test concentration (21.1 mg/L for 7 h exposure and 27.7 mg/L for 3 h exposure). Clinical sings includes eyelid closure, aqueous nasal secretion, reduced pain reaction, lateral position, narcosis. **Unnamed, 1993**: OECD TG 403, 2-methylpropan-1-ol, rats, inhalation (whole body), 6h. Clinical sings during exposure includes hypoactivity, lacrimation, narcosis, prostration, abnormal breathing (short, shallow breaths) and wetness of the periocular fur. Prostration, narcosis and negative reflexes (surface righting and toe and tail pinch) were also observed following exposure. All animals recovered by 1 day. **Unnamed, 1979**: rats, inhalation (whole body), 2-methylpropan-1-ol, concentration (ca. 37.74 mg/l), 7h. Clinical sings includes dyspnoea, eyelid closure, loss of the blinking reflex and narcosis, reduced pain reflex.	**Un named, 1990**; un named, 1991; Schilling K. et al., 1997: OECD TG 408, Wistar rats, oral, 2-methylpropan-1-ol, doses (approx. 80, 340, or 1450 mg/kg bw/day). No effects related to neurotoxicity reported. Unnamed, 1985; unnamed, 1986; unnamed 1987: rats (Crj: CD(SD)), oral gavage, 2-methylpropan-1-ol, dose (100, 316, 1000 mg/kg bw/day). Treatment related clinical signs were restricted to the high-dose group and included hypoactivity, ataxia, and salivation. **Unnamed, 1994**: EPA 798.6050 (Neurotoxicity Screening Battery, Sprague-Dawley rats, 2-methylpropan-1-ol, inhalation, test concentration (1500, 3000 and 6000 ppm). Decreased motor activity in 6000 ppm males and, to a lesser extent, females immediately after exposure. A slight transient decrease in alertness in females and a transient, slight, incoordinated gait observed in one male rat exposed to 6000 ppm. **Unnamed, 1996**; **Li etal, 1999**; **EPA 1996**: Sprague-Dawley rats, inhalation 13 weeks, 2-methylpropan-1-ol, test concentration (250, 1000, 2500 ppm). A slight, transient decrease in response to external stimuli during all exposure period and reported by the registrant as general and non-specific depression of the nervous system.	Not found	Physical state: Liquid VP: < 16 hPa at 20 °C Water solubility: 70 g/L at 20 °C Log Pow: 1	Acute toxicity study in rats with 2-methylpropan-1-ol via inhalation or oral routes results CNS depression Repeated dose toxicity studies in rats with 2-methylpropan-1-ol via inhalation for up to 13 weeks resulted clinical effects related to CNS depression. No information found from literature search on the effect of 2-methylpropan-1-ol on neurodevelopment.

201-158-5 Butan-2-ol	**Unnamed, 1986**:~OECD TG 423, butan-2-ol, rats, oral (gavage), doses (950, 1200, 1500, 2000, and 2400 mg/kg bw). All rats at all dose levels had gait and/or posture abnormalities. In the higher dose groups some rats were comatose or prostrate within a few hours of dosing, with some animals being unconscious for 24 hours or more. **Smyth et al, 1954**: ~OECD TG 403, butan-2-ol, male rats, inhalation (whole body). Clinical sings or other effectss related to neurotoxicity not reported.	**Unnamed, 1991; Burleigh-Flayer etal, 1994**: OECD TG 413, isopropanol, rats and mice, inhalation (whole body), 13 weeks, test concentration (100, 500, 1500, 5000 ppm). Clinical signs observed in some of the rats and mice during exposures at 5000 ppm included ataxia, narcosis, lack of a startle reflex, and hypoactivity. During exposures to 1500 ppm, narcosis, ataxia, and hypoactivity were observed in some mice, while only hypoactivity was observed in rats. Neurobehavioral evaluations indicated no changes in the functional observational battery; however, increased motor activity for female rats in the 5000 ppm group was noted at Weeks 9 and 13.	Not found	Physical state: Liquid VP: 12.5 mm HG at 20ºC Water solubility: > 1 000 000 mg/L at 20ºC Log Pow: < 1	Acute toxicity study in rats with butan-2-ol via oral route results gait and/or posture abnormalities. No effects reported after inhalation exposure. Repeated dose toxicity studies in rats with isopropanol via inhalation for 13 weeks resulted clinical effects related to CNS depression. No effects related to neuro-behaviour reported. No information found from literature search on the effect of butan-2-ol on neurodevelopment.

203-539-1 1-methoxy-2-propanol	**Unnamed, 1979**:~OECD TG 401, Sprague-Dawley rats, oral, doses (5000 mg/kg and 2150 mg/kg). Clinical signs includes dyspnea, apathy, abnormal position, stagger, atony, loss of pain reflex, narcosis-like state, spastic gait, scrubby fur, exsiccosis, poor general condition at 5000 mg/kg. **Unnamed, 1961**; **Smyth et al., 1962**:~OECD TG 401, 1-methoxypropan-2-ol, male Carworth Farms Elias rat, oral, doses (8.0 and 4.0 ml/kg). Most deaths occured within 24 hours after dosing and were preceded by a narcotic like state of depression. **Shideman and Procita, 1951**: 1-methoxypropan-2-ol, dog, oral. no effects related to neurotoxicity reported. Unnamed, 1985:~ EU Method B.1, 1-methoxypropan-2-ol, rats, oral, doses (2500, 3150, 3970 and 5000 mg/kg). The commonest clinical signs includes commonest clinical signs being gait abnormalities, chromodacryorrhoea and piloerection. **Unnamed, 1961; Smyth et al., 1962**:~OECD TG 403, 1-methoxypropan-2-ol, female Carworth Farms Elias rat, inhalation (whole body), concentrations (10400ppm (8h), 9974 ppm (4 h/9 litter dessicator), 10000 ppm (4h)). The animals appeared to be poorly coordinated after two hours in the inhalation chamber, inactive at three hours and unconscious at five hours. **Unnamed, 1991**:~OECD TG 403, 1-methoxypropan-2-ol, B6C3F1 mice, inhalation (whole body), concentrations (6000 ppm, and 7000 ppm). Clinical signs includes unresponsiveness to noise with occasional, staggered movement. Upon removal from the chamber, all mice were laterally recumbent	**Un named, 1953; Rowe et a., 1954**:~ OECD TG 408/409, 1-methoxypropan-2-ol, inhalation (whole body), monkey, 6 months, test concentration (Long duration: 800 ppm, 1500 ppm, and 3000 ppm. Short duration: 10000 ppm). Slightly drowsy and unsteady of balance at the end of each exposure to 10000 ppm reported. **Un named, 1953; Rowe et a., 1954**:~ OECD TG 408, 1-methoxypropan-2-ol, rats, inhalation (whole body), concentration (10000 ppm- short duration (0.5h, 1, and 2 h daily), and 1500 ppm, 3000 ppm, and 6000 ppm- long duration (7h/day). Appeared drowsy and unsteady at the end of 10000 ppm for 2 h daily exposed group. Some very slight and minimal evidence on central nervous system effects at 10000 ppm for 1h daily exposed group. Deeply narcosis at 6000 ppm. A mild central nervous system depression at 3000 ppm which rapidly recovered at the end of exposure. **Un named, 1982; Landry et al, 1983:**~ OECD TG 413, rabbits, 1-methoxypropan-2-ol, inhalation (whole body), 13 weeks, test concentration (300 ppm, 1000 ppm, and 3000 ppm). rabbits in the 3000 ppm group appeared to be sedated during the initial exposures and recoved after 1 or 2 weeks of treatment. **Un named, 1953; Rowe et a., 1954**:~ OECD TG 408/409, 1-methoxypropan-2-ol, guinea pig, inhalation (whole body), concentration (1500 ppm, 3000 ppm, and 6000 ppm). Narcotic effect at 6000 ppm was reported. Un named, 1996:~OECD TG 413, 1-methoxypropan-2-ol, mice, inhalation (vapour), 13 weeks, test concentration (300 ppm, 1000 ppm, and 3000 ppm). Sedation was reported in all mice at 3000ppm during the first 3 days of	Not found	Physical state: Liquid VP: 11.7 mmHg at 25 °C Water solubility: > 1 000 000 mg/L at 20 °C Log Pow: < 1	Acute toxicity study in rats and with 1-methoxy-2-propanol via oral and inhalation routes results effects related to CNS depression. Repeated dose toxicity studies in rats/ rabbits/guinea pigs/monkey with 1-methoxy-2-propanol via inhalation between 13 weeks to 2 year resulted clinical effects related to CNS depression which was mostly recovered after the exposure period. No information found from literature search on the effect of 1-methoxy-2-propanol on neurodevelopment.

	with no visible movement, and unresponsive to noise or touch. **Unnamed, 1979**: CAS no: 107-98-2, Sprague-Dawley rat, inhalation (whole body), 7h. Clinical signs includes watery nose secretion, eyelid closure, wiping of the snout, reduced pain reaction, intermittent respiration, lateral position, narcosis, audible respiration noise, staggering gait, scrubby fur, slight corneal opacity.	treatment and recovered afterwards. **Un named, 1998; Spencer and Crissman, 2002**:OECD TG 453, 1-methoxypropan-2-ol, rats, inhalation (whole body), test concentration (300 ppm, 1000 ppm, and 3000 ppm). the sedative effects, characterized by decreased activity and incoordination at 3000 ppm were observed in both sexes during the first week of exposures and recovered after the second week of exposure. **Un named, 1953; Rowe et a., 1954**:~ OECD TG 408/409, 1-methoxypropan-2-ol, rabbit, inhalation (whole body), concentration (800 ppm, 1500 ppm, 3000 ppm, and 6000 ppm). Narcotic effect at 6000 ppm was reported. **Un named, 1998; Spencer and Crissman, 2002**:OECD TG 453, 1-methoxypropan-2-ol, mice, inhalation (whole body), test concentration (300 ppm, 1000 ppm, and 3000 ppm). the sedative effects, characterized by decreased activity and incoordination at 3000 ppm were observed in both sexes during the first week of exposures and recovered after the second week of exposure. **Un named, 1996**:~OECD TG 413, 1-methoxypropan-2-ol, rats, inhalation (vapour), 13 weeks, test concentration (300 ppm, 1000 ppm, and 3000 ppm). Sedation was reported in both sexs at 3000ppm during the first 3 days of treatment and recovered afterwards.			

216-374-5 1-ethoxy-2-propanol	**Unnamed, 1984**:~OECD TG 401, 1-ethoxypropan-2-ol, rats, oral (gavage), 2 ml/kg and 5 ml/kg. Transitory salivation, inactivity, unsteady gait, rapid respiration, partial ptosis, hunched posture and prostration were seen at 5 ml/kg. Unnamed, 1984: ~OECD TG 403, 1-ethoxypropan-2-ol, rats, inhalation (nose only), 4h, test concentration (9.59 g/m3 or 2213 ppm). Animal movements were uncoordinated when removed from the exposure tubes. 3/6 appeared sedated. All animals recovered within approximately 75 minutes after being returned to fresh air. **Unnamed, 1984**: 1-ethoxypropan-2-ol, mice, inhalation (nose only), 10 min, test concentration (2.81, 3.38, 4.34, 4.66, 5.52, or 7.16 g/m3). No information reported regarding to the clinical sings.	**Unnamed, 1984**: ~OECD TG 407, 1-ethoxypropan-2-ol, rats, oral, 2 ml/kg/day: All animals survived the study. Slight loss of condition, including coat staining and lack of grooming was observed.	Not found	Physical state: Liquid VP: 965 -1448Pa at 25 °C Water solubility: 897 at 20 °C Log Pow: 0 (QSAR)	Acute toxicity study in rats and with 1-ethoxy-2-propanol via oral results clinical effects such as inactivity, unsteady gait, partial ptosis, hunched posture and prostration. Repeated dose toxicity studies in rats with 1-ethoxy-2-propanol via oral for 28 days results slight loss of condition. No information found from literature search on the effect of 1-ethoxy-2-propanol on neurodevelopment.

213-611-4 2-methoxy-2-methylbutane	**Unnamed, 1989**:~OECD TG 401, 2-methoxy-2-methylbutane, rats, oral (gavage), doses (2000, 2500 and 3000 mg/kg). Clinical sings includes ataxia, emaciation, prostration, hypothermia, hypoactivity, dyspnoea, hypopnea, wet and dry rales, both clear and red nasal and oral discharge, staining of the fur and piloerection and unthrifty coat. **Unnamed, 1991**:~OECD TG 403, 2-methoxy-2-methylbutane, rats, inhalation (whole body), 4h, 5400 mg/m³. Clinical sings includes rales seen immediately after exposure in all animals.	**Unnamed, 1995**:~OECD TG 407, 2-methoxy-2-methylbutane, rats, oral (gavage), test concentration (0.125, 0.5, 1.0 g/kg bw/day), 29 days. "The majority of animals did not exhibit unusual clinical signs during the experiment". **Unnamed, 1997**:EPA OTS 798.2450 (90-Day Inhalation Toxicity), 2-methoxy-2-methylbutane, Fischer 344 rats, inhalation (whole body), 13 weeks, test concentration (250, 1500, 3500 ppm). In the high dose group, most animals were prostrate during the exposure of TAME. Also the animals in the mid dose group were prostrate or lethargic during the first month of the exposure. The latter half of the study few animals of this group had laboured breathing and lethargy. After the single 6-hour exposure (3500 ppm) to the satellite group of 10 rats/dose/sex dose related effects on the central nervous system and neuromuscular junction after one hour interval were described. The effects included depression of the central nervous system and neuromuscular junction impairment. The effects were no longer evident after 6 or 24 hours acute exposure and they were not seen after repeated exposure to TAME. In the 1500 ppm dose group, these effects were only seen in male rats. There were no neuropathological changes at any exposure level. The NOAEL for acute neurobehavioral effects of TAME was 250 ppm in males and 1500 ppm in females. Mean absolute brain weight decreased (singnificant, 4-5%) in both sexes at termination of exposure, and brain-to-body weight ratio increased (significantly) at 3500ppm. After the recovery period this changes remained in males.	Not found	Physical state: Liquid VP: 9 100 Pa at 25 °C Water solubility: 10.4 g/L at 20 °C Log Pow: 1.55	Acute toxicity study in rats with 2-methylpropan-1-ol via inhalation or oral routes results CNS depression. Repeated dose toxicity studies in rats with 2-methylpropan-1-ol via inhalation for 13 weeks resulted clinical effects related to CNS depression. No information found from literature search on the effect of 2-methylpropan-1-ol on neurodevelopment.

201-159-0 Butanone	**Unnamed, 1986**: OECD TG 423, butan-2-ol, rats, oral (gavage), doses (950, 1200, 1500, 2000, and 2400 mg/kg). All rats at all dose levels had gait and/or posture abnormalities. The higher dose groups some rats were comatose or prostrate within a few hours of dosing, with some animals being unconscious for 24 hours or more.	**Unnamed, 1981**: ~OECD TG 413, rats, butanone, inhalation (whole body), test concentration (1254, 2518, 5041 ppm). In high-dose female rats, "significantly depressed" brain weight was reported. "No changes in neurological function was observed".	Not found	Physical state: Liquid VP: 126 hPa at 25 °C Water solubility: "very soluble (> 10000 mg/L)" Log Pow: 0.3	Acute toxicity studies in rats with butanone via oral results clinical effects like gait and/or posture abnormalities and became comatos at the highest dose. Repeated dose toxicity study in rats with butanone via inhalation reported no effects related to neurotoxicity. No information found from literature search on the effect of butanone neurodevelopment.
201-185-2 methyl acetate	**U.S. EPA/OPTS, 1994**: methyl acetate, oral (gavage), 50 mg/kg bw, male rat. "Methyl acetate did not cause lethal effects". Unnamed, 1962: ~OECD TG 401, methyl acetate, male rats, oral (gavage). "practically nontoxic".	**Unnamed, 1999**: OECD TG 412, neurobehavioual ecxamination included, methyl acetate, rats, inhalatioin (nose only), test concentration (75 ppm 350 ppm 2000 ppm). No effects related to neurotoxicity reported.	Not found	Physical state: Liquid VP: 228 hPa at 20 °C Water solubility: 243.5 g/L at 20 °C Log Pow: 0.18	Acute toxicity studies in rats with methyl acetate via oral reported no effects related to neurotoxicity. Repeated dose toxicity study in rats with methyl acetate via inhalation results no effects related to neurotoxicity. No information found from literature search on the effect of methyl acetate neurodevelopment.

205-500-4 ethyl acetate	**Munch, 1972**: ethyl acetate, oral, acute, rabbits. The oral narcotic dose (ND50) for ethyl acetate in rabbits was reported to be 4493 mg/kg. **Flury and Wirth, 1933**: Acute, ethyl acetate, mice, inhalation. "An inhalation threshold concentration for narcosis of 18 mg/L for ethyl acetate in the mouse was reported. Deep narcosis occurred after 190 -240 minutes of exposure".	**Christoph et al, 2003**: EPA test guideline, ethyl acetate, Crl:CDBR rats, inhalation, 13 weeks, test concentration (350, 750 or 1500 ppm), functional observational battery and motor activity tests performed. Exposure to 750 or 1500 ppm diminished behavioral responses to unexpected auditory stimuli during the exposure session and appeared to be an acute sedative effect which rapidly reversed following cessation of exposure. No other effects were reported except for reduced motor activity in the 1500 ppm females, an effect that was not present after the 4 -week recovery period. The subchronic NOEC for neurotoxicity is considered to be 750 ppm based on transient reduction in motor activity in female rats. Unnamed, 1995: ethyl acetate, rats, inhalation, 2 weeks, concentrations (1500, 3000, or 6000 ppm), unctional observational battery and motor activity tests performed. Hypoactivity, blepharospasm, and lack of startle reflex were noted during exposure to 3000 and 6000 ppm ethyl acetate. Changes in MA and FOB parameters were small and difficult to attribute to ethyl acetate due to the small group sizes.	Not found	Physical state: Liquid VP: 9.83kPa at 20 °C Water solubility: 83.1g/l at 20 °C Log Pow: 0.68	Acute toxicity inhalation studies in mice and rabbits treated with ethyl acetate results narcosis. Repeated dose inhalation toxicity study in rats with ethyl acetate results effect reported as acute sedative effect. No information found from literature search on the effect of ethyl acetate neurodevelopment.
203-561-1 Isopropyl acetate	**De Ceaurriz et al, 1983**: acute, Isopropyl acetate, 4h, mice. Isopropyl acetate was shown to reduce the total duration of immobility measured over a 3-min period in a concentration-related manner. **Munch, 1972**: isopropyl acetate, oral, acute, rabbits. "The median narcotic dose of orally administered isopropyl acetate is 50 mmols per kg (3064 mg/kg) The ND 50 is defined as the quantity producing stupor, loss of voluntary movements in half of the animals".	**Unnamed, 1994**: no guideine, propan-2-ol, rats, inhalation, 13 weeks, test concentration (500, 1500 and 5000 ppm), functional observation battery was conducted. "Ataxia, narcosis, hypoactivity, lack of startle reflex at 1500 and/or 5000 ppm. Narcosis only occurred in the first 2 weeks at 5000 ppm and not observed after Week 2. The narcosis noted before week 2 was not observed following week 2 suggesting some adaptation to isopropanol. There was no histologic neuropathology in peripheral and central nervous systems".	Not found	Physical state: Liquid VP: 6.03kPa at 20 °C Water solubility: "very soluble (> 10000 mg/L)" Log Kow: 1.03	Acute toxicity study in mice treated with isopropyl acetate via inhalation results immobility. Repeated dose toxicity studies in rats with propan-2-ol via inhalation for 13 weeks results CNS depression. No information found from literature search on the effect of isopropyl acetate on neurodevelopment.

203-686-1 propyl acetate	**Frantik et al, 19994**: no guideline, propyl acetate, inhalation, 4h for rats, and 2h for mice. For rats "37% of the maximum tonic extension of the hindlimbs; corresponding to 6600 ppm". For mice "30% of the maximum velocity of tonic extension; corresponding to 6200 ppm".	**Unnamed, 1996**; **David et al, 1998**: ~ US EPA Pesticide Assessment Guidelines, n-Butyl acetate, rats, inhalation, 5d/week for 13/14 weeks, test concentrations (500, 1500 and 3000 ppm), functional observation battery and motor activity tests conducted. Exposure to 3000 ppm exhibited less movement, decreased alertness, and slower response. The mean total motor activity counts and total ambulations were higher during Week 4 than at other times. No sings of neurobehavioural effects.	Not found	Physical state: Liquid VP: 47.9 hPa at 25 °C Solubility: Log Pow: 1.4	Acute toxicity study in mice treated with propyl acetate via inhalation results effect on tonic extension of the hindlimbs. Repeated dose toxicity studies in rats with propan-2-ol via inhalation for up to 13 weeks results effects related to CNS depression. No effects on neuro-behaviour reported. No information found from literature search on the effect of propan-2-ol on neurodevelopment.
259-370-9 2-ethoxy-1-methylethyl acetate	**Unnamed, 1985**:OECD TG 401, 2-ethoxy-1-methylethyl acetate, rats, oral (gavage), 5 g/kg bodyweight. Clinical sings shortly after exposure includes piloerection, hunched posture, abnormal gait (waddling), lethargy, pallor of the extremities and increased salivation. **Unnamed, 1985**:~OECD TG 403, 2-ethoxy-1-methylethyl acetate, Wistar rats, inhalation (whole body), > 4h, 6.99 mg/l. "Lacrimation, abnormal body posture and abnormal respiratory pattern were the main clinical signs evident during exposure. These signs were considered to be consistent with exposure to a mildly irritating vapour".	**Unnamed, 1984**:~OECD TG 407, 1-ethoxypropan-2-ol, rats, oral (gavage), 2 ml/kg/day. Clinical sings reported includes slight loss of condition, including coat staining and lack of grooming. **Unnamed, 1986**:~OECD TG 413, 1-ethoxypropan-2-ol, Wistar rat, inhalation (whole body), 6 hours, test concentration (nominal: 0.624, 1.658, 11.19 mg/l). Clinical signs includes a reduced 'startle response' was reported in the high dose animals during exposure. **Unnamed, 1986**:~OECD TG 412, 2-ethoxy-1-methylethyl acetate, Crl: (WI) BR Strain rat, inhalation (whole body), 6 hours, test concentration (nominal: 100, 300, 1200ppm). The only treatment effect observed was a reduced response to external stimuli during exposure and restored after end of exposure.	Not found	Physical state: Liquid VP: 202.6 Pa at 25 °C Water solubility: 69.6 g/L at 18 °C Log Pow: 0.76	Acute toxicity study in mice treated with 2-ethoxy-1-methylethyl acetate via oral clinical effects like hunched posture, abnormal gait (waddling), lethargy, pallor of the extremities and increased salivation. Repeated dose toxicity studies in rats with 2-ethoxy-1-methylethyl acetate results slight loss of coordination in 28 day study via oral, transient reduced response to external stimuli in 28 day study via inhalation, and reduced 'startle response' in 13 weeks study. No information found from literature search on the effect of 2-ethoxy-1-methylethyl acetate neurodevelopment.

203-692-4 n-Pentane	**Frantik et al., 1994** (male rats and female mice for 4 hours and 2 hours, respectively): At 21000 ppm, 37% depression of the shortening of the tonic extension of hind limbs by 3 seconds in male rats, while at 23500 ppm, 30% depression of the lengthening of the latency of extension by 0.6 seconds in mice. **Stoughton, 1936**: acute Toxicity of n-pentane in mice via inhalation. 1st test series ("light anesthesia" test: 3.0, 3.5, and 4.2 mmol): mice lightly anaesthetized from 1.3 to 10 minutes. No mice died during the first test series. 2nd test series ("complete anaesthesia" test: 4.2, 4.5, and 4.9 mmol/L): 8 mortalities at 4.5 mmol/L, and 7 mortalities at 4.9 mmol/L. The average recovery time for the survivor was 4 to 8 minutes. The LC50 was not calculated.	**Takeuchi et al., 1981; Schreiner, 1998; Gamer, 1998** (90 day studies via inhalation): each contains a neurotoxicity screening component. No neurotoxicty effect reported. **Takeuchi et al., 1980** (6 weeks via inhalation at 3000 ppm): no effect in prolong distal latency or disturb the conduction velocity of the motor nerve and mixed nerve in the rat's tail. No changes in the peripheral nerve, the neuromuscular junction, and muscle fibre. No changes in body weight or behaviour.	Not found	Physical state: Liquid VP: 58.59 kPa at 294.26 K Log Kow: 3.45 Water solubility: 38.5 mg/L at 20 °C	Narcosis observed from acute inhalation studies. No neurotoxicity effects were observed in the 90 day and 6 weeks inhalation studies. No neurodevelopmental effects found from the literature search.

250-610-8 Isoheptane	No acute study provided on isoheptane. **Unnamed, 1982**: The acute oral LD50value in rats was greater than 5000 mg/kg for iso-octane (CAS No. 540-84-1). Clinical observations noted one-hour post exposure in 8 of 10 animals included depression, salivation, wheezing, rough coat, and soft faeces. Two female rats appeared normal throughout the study. All animals appeared normal from day 2 through termination of the study. **Unnamed, 1982**: Ten male and female Sprague-Dawley rats were exposed to n-heptane (CAS No. 142-82-5) via whole body inhalation at nominal concentration of 29290 mg/m³ for 4 hours (similar to OECD 403). A slight reduction of mean male body weights was noted on day 2 post-exposure but males recovered by day 4. All animals appeared normal throughout the study and at terminal necropsy with the exception of one female observed with enlarged mandibular lymph nodes on the right side. The LC50was greater than the nominal concentration of 29300 mg/m³. **Unnamed, 1982:** The dermal LD50 value of iso-octane (CAS No. 540-84-1), as determined in rabbits, was greater than the limit dose of 2000 mg/kg. All rabbits appeared normal throughout the study. Very slight dermal erythema was noted in all animals on day 1 after dosing, which persisted in one male and one female on day 3. All erythema had cleared by day 7. Very slight oedema was noted in two males and one female on day 1 and cleared by day 3. Epidermal scaling was noted in one female on day 10.	There are no inhalation repeated dose toxicity data available on isoheptane in the registration dossier. **A) Ono et al., 1979 and Takeuchi et al., 1980, 1981**: Evaluations of peripheral nerve toxicity of n-pentane, n-hexane and n-heptane male rats were exposed to 0 or 3000 ppm 12 hours/day, 7 days/week, for 16 weeks. The conduction velocity of tail nerves was measured to determine the functional status of the peripheral nerves. n-heptane: statistically significantly depressed ($p<0.01$) body weight gain after 8 weeks of exposure but gradually increased throughout the experiment (but not statistically significantly lower). No abnormal behavioral changes were observed. No statistically significant differences in motor nerve conduction velocity, distal latency or mixed nerve conduction velocity in any region of the tail. No microscopic change in nerves, muscles and neuromass junctions. n-Heptane is not a neurotoxicant in this assay system. n-hexane: induced neuropathy. NOAEC: > 3000 ppm (12470 mg/m³). Note: No information included for n-pentane in this report. **B) Unnamed, 1980**: n-heptane administered to rats via whole body inhalation at 398 and 2970 ppm for 26 weeks with a subsequent 2-week recovery period conducted similar to OECD 413. The only treatment-related observations were laboured breathing or rapid breathing and slight prostration during the first week of exposure and anogenital fur and dry rales during weekly observations. The in chamber signs were generally more numerous and severe at the higher concentration and appeared to abate by	not found	Physical state: liquid VP: 8.9 kPa log Kow: 3.7 Water Solubility: 2.5 mg/L	No studies (acute or repeated dose studies) to investigate the neurotoxic effect of n-heptane. The registrant assigned STOT SE 3 (H336) based on read-across within a category approach. n-heptane or Naphta (petroleum), light alkylate (CAS no: 64741-66-8) was not a neurotoxicant while in n-hexane induced neuropathy in 16 weeks inhalation study at 3000 ppm. No literature was found on isoheptane showing effects in the developmental nervous system.

		the second week of the study. The effects observed are consistent with acute CNS depression and generally abated by the second week of study. NOAEC: 2970 ppm corresponding to 12200 mg/m³. **C) Simonsen and Lund, 1995** ("Auditory sensitivity study"): n-heptane, male rats, inhalation (whole body), 28 days, 0, 3.3, 16.6 mg/L (re-calculated; corresponding to 0, 800, 4000 ppm). No clinical signs of neuropathy were observed; body weight gain reduced after termination of exposure and becomes statistically significant at 16.6 mg/L. A loss of auditory sensitivity equal to 10dB at 4000ppm and tested by measurement of auditory brain stem response 2 months after cessation of exposure Systemic: NOAEC 3.3 mg/L, LOAEC 16.6 mg/L; Auditory: NOAEL 3.3 mg/L, LOAEC 16.6 mg/L. D) Unnamed, 1998: ~OECD TG 413, CAS no: 64741-66-8, Sprague-Dawley rats, inhalation (whole body), test concentration (0, 668, 2220, and 6646 ppm (0, 2.4, 8.1, and 24.3 g/m3)). NOAEC for subchronic toxicity and neurotoxicity (neurobehavioural studies and neuropathological studies) = 6646 ppm (no effects except adaptive response of liver weight at 6646 ppm).			

203-625-9 Toluene	Non-human information 1) Acute Oral toxicity **A) Withey and Hall, 1975**: EU B1; rats; doses: 4000, 4560, 5200, 5930, or 6760 mg/kg of toluene. Highest dose resulted hind-limb paralysis and petechial bleeding, especially from the urinary tract, eyes and nose. The LD50 was calculated to be 5580 mg/kg. **B) Kimura et al, 1971**: Similar oral LD50 values; approximately 5600 mg/kg and 6400 mg/kg for younger and older adult rats, respectively. 2) Acute inhalation toxicity **A) BASF, 1980**: Concentrations: 6.08, 20.00, 23.98, 38.87 and 61.80 mg/L; 4 hours; 10males/females rats): LC50 > 20 mg/L (28.1 mg/L in males and females; 25.7 mg/L in males and 30 mg/L in females). According to the EU RAR (2003), animals showed watery discharge from eyes and nose, unrest, increased respiration, rocking gait, narcosis, startling movements and hyperaemia of the ears and extremities. In the highest exposure group, salivation was observed. In the group exposed to 6.08 mg/L of toluene, no adverse clinical signs were observed. All surviving rats appeared normal after 3 days following the exposure. **B) Páez-Marínez et al, 2003**: toluene, mice. The authors conclude that toluene has anxiolytic-like activity, as evidenced by its effects on burying behaviour and passive avoidance behaviour. It increased the number of electric shocks received by the animals	Non-human information 1) Oral **Huff, 1990**: Two key studies in 10 rats and mice, respectively; 312, 625, 1250, 2500, or 5000 mg toluene/kg in corn oil by gavage for 13 weeks. Deaths at 5000 mg/kg (in all rats and mice), 2500 mg/kg (8male and 1 female rat; and 4/sex mice), and 1250 mg/kg 91 mouse). Clinical signs (rats or mice): prostration, hypoactivity, ataxia, piloerection, lachrymation, and excessive salivation at 5000 and 2500 mg/kg groups. There were no treatment related effects in the haematological and serum chemical analyses or urinalyses in either species. In rats at 1250 and 2500 mg/kg there were differences in the weight of a number of organs and neuropathological changes in the brain, consisting of neuronal cell necrosis in the dentate gyrus and Ammons horn of the hippocampus. In addition to the hippocampal lesions, necrosis and/or mineralisation were present in the granular layer of the cerebellar cortex. In mice, relative brain and testis weight, and absolute kidney weight was increased in male mice at 5000 mg/kg. Myocardial degeneration was found in 3 male and 2 female mice from this group. The NOAEL for repeat dose oral toxicity is considered to be 625 mg/kg in rats and mice. 2) Inhalation **A) Huff, 1990**: 15 week study in rats, inhalation, concentration (100, 625, 1250, 2500, or 3000 ppm toluene 6.5 hours/day for 5 days/week). Death reported at 3000 ppm (8 males during week 2). Lower body weights, adverse clinical signs and differences in absolute or relative	Da-Silvia et al, 1990: Toluene vapor (800 mg/m3) 6 h daily from gestation days 14 to 20, and 6 to 11 in rats and hamsters resulted effect on exploratory behaviour: Male rat offspring exposed to toluene displayed shorter latencies than male controls to choose one side of a T maze in a spontaneous alternation test. Hamsters exposed to toluene performed worse in a rotating rod test.	Physical state: Liquid VP: 3088.9Pa at 21.1°C and 4130.0Pa at 26.6°C. Water solubility: 573-587 mg/l at 25°C Log Kow: 2.73	Acute exposure to toluene in human volunteers show that dizziness and sleepiness are experienced at air levels < 20 mg/L for 4h and rocking gait and narcosis were observed in rats at this same concentration). After repeated dose exposure toluene case adverse effects including impairment of auditory function and morphological evidence of cell loss in the rat cochlea, neuron loss in the central nervous system of animals and in humans neuropsychological effects, auditory dysfunction and effects on colour vision have been reported. Toluene resulted neurobehavioural effects in the rats and hamster offsprings and the effect becomes more stronger in the hamster offspring.

	during conditioned defensive burying testing, which was interpreted by the authors as indicative of a possible adverse effect on learning. **C) Unnamed, 1980**: Concentrations (Nominal: 7, 31.6, 52.2, 78.3, 104.4 mg/L; Analysed: 6.08, 20.00, 23.98, 38.87, 61.80 mg/L), ~OECD 43, toluene, rats, inhalation (whoe body), 4h. Animals showed watery discharge from eyes and nose, unrest, increased respiration, rocking gait, narcosis, startling movements and hyperaemia of the ears and extremities. In the highest exposure group, salivation was observed. In the group exposed to 6.08 mg/L of toluene, no adverse clinical signs were observed. All surviving rats appeared normal after 3 days following the exposure. 3) Acute toxicity: dermal **Smyth et al, 1969**: A single acute dermal toxicity study on toluene in the rabbit has been found. Fur was removed from the entire trunk by clipping and the dose held in contact with the skin for 24 hours (retained by impervious plastic film). The 4 animals per group were immobilised during the contact period, after which the film was removed and the rabbits caged for a 14 day observation period. The LD50 value was reported to be 14.1 mL/kg, corresponding to 12267 mg/kg (using a density of 0.87). No information on clinical signs or mortality pattern was provided. Human information The acute effects of toluene inhalation exposure have been investigated in a number of human experimental studies in volunteers. These studies indicate that toluene produces a	organ weights (particularly liver and kidney) at 3000, 2500 and 1250 ppm. Plasma cholinesterase activity decreased as exposure concentration increased. NOAEC = 625 ppm. **B)** No effects in neurobehavioural, or other NS effects reported in the other provided repeated dose toxicity studies in rats (**NTP, 1990; Gibson and Hardisty (1983**), mice (**Huff, 1990**). **C) Unnamed,1984**: Special studies addressing neurotoxicity and ototoxicity demonstrate that toluene is ototoxic in the rat and can produce neurochemical and pathological changes. However, the data are insufficient to determine NOAEC values. Human information After repeated dose exposure via inhalation in humans toluene causes a number of adverse effects including neuropsychological effects, auditory dysfunction and disturbances of colour vision. **Key data** published since the EU RAR (2003) addressing these effects are those of **Seeber et al (2004) and Schaper et al (2003, 2004).** There was no evidence that long-term exposure to toluene at 26 ppm for 21 years had any effects on cognitive function (**Seeber et al, 2004**). There was no evidence of ototoxicity resulting from occupational exposure to toluene below 50 ppm (mean exposure 26 ppm / 98 mg/m3) in a longitudinal study over 5 years (**Schaper et al, 2003**). Similarly no effect of human occupational exposures to toluene on colour vision were found in a follow up study over 4 years with three repeated examinations (**Schaper et al, 2004**). These studies			

	number of subjective sensations such as headache, dizziness, feeling of intoxication, irritation and sleepiness and decreases in acute neurobehavioural performance at concentrations = 75 ppm **(EU RAR, 2003)**. **Muttray et al (2005)** exposed twenty healthy men to a constant level of 50 ppm toluene. The Pupillographic Sleepiness Test (PST) was performed before and after 4.5 hours of exposure. Acute symptoms were assessed with the Swedish Performance Evaluation System (SPES) self-assessment questionnaire, once before and 3 times during exposure. There was no effect of toluene exposure on PST or tiredness but scores for unpleasant smell and irritation to the throat were increased. A NOAEC of 50 ppm (188 mg/m3) can be determined for acute neurobehavioural effects in humans.	demonstrate that 26 ppm (98 mg/m3) is a NOAEC for human adverse effects. This value will be taken forward in the risk characterisation.			

ANNEX 5

A5.1 Articles, reports and other documents searched and studied for this review

Articles, reports and other documents searched and studied for this review are presented in a table, shown below. Where relevant, highlights of the study are given in brief. Notice that not all information necessarily has been directly used in the review; it may have been selected to derive background information on specific subjects.

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Note: Each item in the table below is followed by the complete reference.

A5.1.1 Table with Articles, reports and documents (complete references included)

Compound	Type of study	Background / Aim /Study design	Results / Conclusions	Reference
oluene (CAS 108-88-3)	***In vivo***	Pregnant rats (Wistar); Inhalation: 0 (n=7) or 4,500 mg toluene/m3 (n=13) (0 or 1,200 ppm); 6 h/day; gestation day 7 to PND 18. Developmental and neurobehavioral effects in the offspring were investigated using a test battery including assessment of functions similar to those in the proposed OECD TG for Developmental Neurotoxicity Study, i.e., physical development, reflex ontogeny, motor function, motor activity, sensory function, and learning and memory. Study of postnatal development and behavior after pre- and postnatal exposure to 1200 ppm toluene.	**Main effects:** **Developmental toxicity** (lower birth weights, delayed reflex development) **Developmental neurotoxicity** (increased activity level, and impaired cognitive functions in offspring of exposed dams). **Results exposed offspring** **Neurobehavior**: **Development** of exposed offspring: Body weights reduced until PND 10; Reflexes: some show delayed ontogeny. **Neurobehavior (**12-14 offspring /sex/group). Motor activity: increased with about 100% (males, females); Morris water maze (learning ability spatial navigation): at the age of 3 months (female offspring), time to locate hidden platform after this was moved to new position in the pool increased; swim length similarly increased (around 50%). Effect not related to impaired swimming ability (swim speed similar to control values)! These Morris water maze data repeat finding of impaired learning after platform relocation in female offspring exposed prenatally to 1,800 ppm toluene found in the study by **Hougaard et al., 1997**. **Conclusion**: Exposure to 1200 ppm toluene during brain development caused long-lasting developmental neurotoxicity i.e., increased motor activity in both sexes and impaired cognitive function in female offspring. The exposure did not cause maternal toxicity or decreased viability of the offspring.	**Hass U, Lund SP, Hougaard KS, Simonsen L (1999)** Developmental neurotoxicity after toluene inhalation exposure in rats. Neurotoxicol Teratol 21:349–357.

Toluene (CAS 108-88-3)	***In vivo***	Development and neurobehavioral effects of prenatal exposure to toluene were studied after exposing pregnant rats (Mol:WIST) to 1800 ppm of the solvent for 6 h daily on days 7–20 of gestation Study of prenatal exposure on postnatal development and neurobehavioral effects in offspring The battery of tests used in this study was developed to evaluate developmental neurotoxicity of industrial chemicals and includes assessment of postnatal growth, reflex ontogeny, neuromotor abilities (Rotarod), activity level (Open field), reactivity, habituation and prepulse-inhibition (Acoustic startle), sensory function (Auditory brainstem response) and learning and memory ability (Morris water maze).and it is in compliance with OECD Test Guideline 426 (Developmental Neurotoxicity Study):	**Main effects:** **Developmental toxicity** (reduced body weights until PND 10) **in absence of maternal toxicity** (Maternal body weight gain was slightly but non-significantly suppressed in exposed dams during the period of exposure). **Developmental neurotoxicity** (impaired learning abilities demonstrated at age 2-3 months, most marked in female offspring) **Results exposed offspring** **Development** Body weights significantly lower until PND 10; **Neurobehavior**: no effects on motor function (rotarod), activity level (open field), acoustic startle, and prepulse inhibition; Auditory brain stem response: small effects in male-exposed offspring; Morris water maze (learning / memory function): Acquisition learning: (most marked in females) some indications of impaired cognitive functions, which was confirmed during further testing, especially in reversal and new learning.	**Hougaard KS, Hass U, Lund SP, Simonsen L. (1999)** Effects of prenatal exposure to toluene on postnatal development and behavior in rats. Neurotoxicol. Teratol. 21: 241–250. The LOAEC for long-lasting developmental neurotoxicity induced by prenatal exposure to toluene is 1800 ppm; a NOAEC cannot be set .
Toluene	***In vivo***	The present study was undertaken in order to investigate if toluene induced oxidative stress in brains from rats exposed prenatally to 1800 ppm toluene 6 hr/day at days 7–20 during the pregnancy. 35–42 days after birth the rats were killed and synaptosomal fractions were prepared for the experiments	**Results:** Synaptosomes from rats exposed prenatally to toluene exhibited an increased level of oxidative stress when incubated with toluene *in vitro* compared to synaptosomes from unexposed offspring. Also the cell membrane was affected, as the calcium leakage was more increased from exposed synaptosomes than from unexposed. The membrane fluidity increased significantly when synaptosomes were incubated with toluene for 10 min. *in vitro* but the change in fluidity was identical in both groups of offspring **Conclusion**: The results indicate that prenatal exposure to toluene induces long-lasting changes in oxidative status and membrane function.	**Edelfors S, Hass U, Hougaard KS.(2002)** Changes in markers of oxidative stress and membrane properties in synaptosomes from rats exposed prenatally to toluene. Pharmacol Toxicol.90:26-31

Toluene (203-625-9) Physical state: Liquid VP: 3088.9Pa at 21.1°C and 4130.0Pa at 26.6°C. Water solubility: 573-587 mg/l at 25°C Log Kow: 2.73	***In vivo***	Pregnant rats and hamsters; 800 mg/m3 toluene; 6 h /day; GD 14 to 20 (rats); GD 6 to 11 (hamsters). Growth, neuromotor development and performance of the offspring in behavioral tasks were assessed	**Results:** (exposed offspring) **Rats**: number of litters with low birth weight pups increased; Behavior: T-maze: shorter latencies to choose one side of a T maze in a spontaneous alternation test (males). **Hamsters**: Rotarod: worse performance in a rotating rod test. **Conclusion**: Results confirm toluene fetotoxicity in rats and suggest an effect on exploratory behavior which may be related to hormonal changes in early life. Neuromotor effects of exposure of hamsters to toluene in utero deserve further investigation.	**da-Silva VA , Malheiros LR , Bueno FM. (1990)** Effects of toluene exposure during gestation on neurobehavioral development of rats and hamsters. Brazilian Journal of Medical and Biological Research 23: 533-537.
Toluene	***In vivo***	Pregnant rats (Sprague-Dawley); 0, 8000 or 12,000 ppm toluene; 15min twice daily; GD 8 to GD 20. *This study used a rat model to determine how prenatal exposure to abuse levels of toluene would affect performance in a spatial learning and memory task, the Morris Water Maze (MWM).*	**Results exposed offspring**: MWM: acquisition: prenatal toluene-exposed animals not differing; probe trial and reversal learning: impaired performance (12,000 ppm; PND 44) **General conclusion**: Study indicates that prenatal exposure to repeated inhaled abuse patterns of high concentrations of toluene can impair spatial memory function that persists into adolescence. *Male and female offspring (N=104) observed in the MWM, for 5days beginning on postnatal day (PN) 28 and again on PN44.*	**Callan SP , Hannigan JH , Bowen SE. (2015)** Prenatal toluene exposure impairs performance in the Morris Water Maze in adolescent rats. Neuroscience. doi: 10.1016/j.neuroscience.2015.08.050. [Epub ahead of print] http://dx.doi.org/10.1016/j.neuroscience.2015.08.050

Toluene	***In vivo***	Pregnant rats (Wistar); 1500 ppm toluene, 6 hr/day, GD 7 to 20; **or** chronic mild stress, GD 9 to 20 as single exposure ; **or** in combination. Behavioral, immunohistopathological, molecular, biological, and neurochemical methods were applied to investigate the offspring for developmental neurotoxicity and level of apoptosis in the brain.	**Results:** TUNEL staining and DNA-laddering: marked decrease number of apoptotic cells from PND 22 to 27 (cerebellum and hippocampus). Peak in the number of TUNEL positive cells (cerebellum; PND 22). No sign. influence of exposure except that DNA-laddering increased by toluene exposure (cerebellum, PND 27). Caspase-3 activity decreased with age (cerebellum and hippocampus). Stress and toluene, when singly exposed, increased activity in cerebellum (PND 6) whereas co-exposure to stress and toluene did not. Stress increased caspase-3 activity (hippocampus, PND 22). **There was overall consistency between the results obtained by the three supplementary methods regarding the influence of exposure and age on apoptotic activity in cerebellum and hippocampus.** *New methods to quantitate the relative level of apoptosis measured as DNA-laddering and the caspase-3 activity in tissue are presented.*	**Ladefoged O, Hougaard KS, Hass U, Sørensen IK, Lund SP, Svendsen GW, Lam, HR. (2004)** Effects of combined prenatal stress and toluene exposure on apoptotic neurodegeneration in cerebellum and hippocampus of rats. Basic & Clinical Pharmacology & Toxicology 94, 169–176.

Toluene	***In vivo***	Pregnant rats (Wistar) **Study 1**.Inhalation. 1200 ppm toluene; 6 h/day; gestation day 7 to PND 18. *Sperm analysis* in the adult male offspring at PND 110 **Study 2.**Inhalation. 1800 ppm toluene; 6 h/day; gestation day 7-20. Male offspring were killed at PND 11, 21 or 90: *Testis toxicity*: Paired testes weight, histopathology and immune-expression of vimentin in Sertoli cells used as markers. *In the brain*: number of apoptotic cells in hippocampus and cerebellum (TUNEL assay)	**Results:** **Reproductive parameters**: Neither pre- and postnatal exposure to 1200 ppm toluene nor prenatal exposure to 1800 ppm induced significant effects on the reproductive parameters investigated **Neuronal apoptosis**: However, prenatal exposure to 1800 ppm toluene did increase neuronal apoptosis in the cerebellum of weaned male rats, possibly by delaying postnatal migration of granule cells to their final destination, or by toluene-induced retardation of generalised fetal growth. **Results exposed offspring (males)** **Study 1**. Toluene did not affect the semen quality of exposed rats. **Results exposed offspring (males)** **Study 2.** **Development** of exposed offspring: Body weights still reduced on PND 11, but on PND 21 and 90 tending to controls. Testes weights (absolute and relative) reduced in all three age groups (not sign.); histopathology and immune-expression of vimentin in Sertoli cells: not affected **Neurodevelopment** Hippocampus: almost no apoptosis observed in any age group and no differences in apoptotic neurodegeneration between exposed and exposed males at PND 11, 21 or 90. Cerebellum: marked increase number apoptotic cells at PND 21 compared with the other age groups. Toluene induced significant increase in number of apoptotic cells in cerebellar granule layer at PND 21 (mean increased from 37 in control group to 71 in toluene-exposed group). *Thus, the granular cell layer in cerebellum is a highly relevant tissue with which to study toluene-induced apoptosis, because of the continuous migration of neurons and high frequency of neuronal apoptosis during the weaning period.*	**Dalgaard M, Hossaini A, Hougaard K, Hass U, Ladefoged O**. (2001) Arch Toxicol 75: 103-109. doi:10.1007/s002040000209

Toluene	***In vivo***	Timed-pregnant rat (Sprague Dawley); Inhalation. 12,000 or 8,000 ppm toluene, or 0 ppm (controls); 30 min twice daily (60 min total daily exposure); gestation days 8-20. Model for solvent abuse during pregnancy Pups were assessed from postnatal day (PN) 4 to PN21 using a developmental battery measuring growth (i.e., body weight), maturational milestones (e.g., eye opening & incisor eruption), and biobehavioral development (e.g., negative geotaxis & surface righting).	**Results:** Development of exposed offspring: Body weights reduced 12,000 ppm or 8,000 ppm beginning at PND 4 (12,000 ppm) and PND 12 (8,000 ppm) until PND 21; increases in an index of poor perinatal outcome, specifically a composite of malformations, defined "runting" and neonatal death; No significant delays in reaching maturational milestones. **Conclusion**: A comparison of the present, conservative results with findings in previous studies implies that binge patterns of toluene exposure in pregnant rats modeling ,human solvent abuse can result in developmental and morphological deficits in offspring. While there was no significant toluene-induced shift in physical maturation as assessed by developmental "milestones" (i.e., pinnae detachment & incisor eruption), there was significant reduction in postnatal growth up to weaning. Unlike in previous study, the changes that occurred did so in the presence of obvious maternal effects, assessed as dam gestational weight gain. These results do not exclude the possibility that maternal toxicity as well as teratogenic effects of toluene may contribute to outcomes. The results suggest that abuse of inhaled organic solvents like toluene may result in similar early developmental outcomes in humans	**Bowen SE, Hannigan JH (2013)** Binge Toluene Exposure in Pregnancy and Pre-weaning Developmental Consequences in Rats. Neurotoxicol Teratol . 38: 29–35. doi:10.1016/j.ntt.2013.04.002.
Toluene	***In vivo***	Rats, toluene (0, 500, and 1500 ppm); 6 months duration *After an exposure-free period, neurobehavioural, morphometric, pathological, and biochemical examinations were performed.*	**Results**: No loss of neurons; Mean nuclear volume and mean perikaryonal volume and variation of the values of these parameters increased in exposed groups (500 ppm). Noradrenaline (NA), dopamine (DA), and 5-hydroxytryptamine (5-HT) levels sign. changed in various brain regions; no neurobehavioural or gross pathological changes found. **Conclusion**: this investigation failed to reveal overt toluene-induced CNS-neurotoxicity, however, certain irreversible effects were found which further add to the accumulating evidence of the **chronic CNS-neurotoxicity** of toluene. Although hippocampal weigh was not reduced at 500 ppm an increase in perikaryonal volume and nuclear volume was found at this level, indicating that 500 ppm is not a clear NOAEC.	**Ladefoged O, Strange P, Møller A, Peter Arlien-Søborg P. (1991)** Irreversible Effects in Rats of Toluene (Inhalation) Exposure for Six Months. Pharmacology & Toxicology 68: 384 – 390. DOI: 10.1111/j.1600-0773.1991.tb01257.x

Toluene vapor	***In vivo***	Pregnant rats and hamsters; 800 mg/m3; 6 h/day; GD 14 to 20 (rats) and GD 6 to 11 (hamsters)	**Results exposed offspring**: Rats: T-maze (Exploratory behavior): shorter latencies to choose one side of a T maze in a spontaneous alternation test (male offspring) Hamsters: Rotarod: worsened performance in a rotating rod test.	**Khattak S, K-Moghtader G, McMartin K, Barrera M, Kennedy D, Koren G (1999).** Pregnancy outcome following gestational exposure to organic solvents. A prospective controlled study. *JAMA*; 281:1106-1109. doi:10.1001/jama.281.12.1106.
Toluene	***In vivo***	Rat ; 1,500 ppm	The effect observed at 1,500 ppm (neuron loss) is considered to be irreversible and biologically important. Possibly transient reduction in the volume of hippocampal structures during postnatal development; **neuron loss in the regio inferior of the hippocampus;** cognitive impairment in Morris maze at least shortly after exposure, and changes in neurotransmitters and other neurochemical parameters.	**Korbo L, Ladefoged O, Lam HR, et al. (1996).** Neuronal loss in hippocampus in rats exposed to toluene. Neurotoxicogy 17: 359–366.
Toluene	***In vivo/in vitro***	Neonatal rats' ; Toluene 250, 500, 750 mg/kg; PND 4 to 10. Toluene has become increasingly popular as a drug of abuse. Inhaling toluene leads to a feeling of euphoria and several reports have shown that children born to women who had abused toluene during pregnancy present a syndrome (toluene embryopathy or fetal solvent syndrome) that is characterized by CNS effects (e.g. microencephaly), growth retardation and facial dysmorphologies. The characteristics of the fetal solvent syndrome are very similar to those observed in the fetal alcohol syndrome	**Results:** ***in vivo*** **:**Brain and body weights: sign. reduced; Brain levels GFAP: sign. reduced; Brain neuron-specific enolase: not changed. ***in vitro*****:** pharmacologically relevant concentrations of toluene (250-1,000 microM) significantly inhibit proliferation of rat cortical astrocytes without causing overt cytotoxicity. **Conclusion**: Results indicate that toluene does not cause selective microencephaly; however, it affects brain weight, and appears to target developing astrocytes, possibly by inhibiting their proliferation.	**Burry M, Guizzetti M, Oberdoerster J, Costa LG. (2003)** Developmental Neurotoxicity of Toluene: in vivo and in vitro Effects on Astroglial Cells. Developmental Neuroscience 25:14-19.

C7 and C9 aliphatic (n-heptane, n-nonane), naphthenic (methylcyclohexane, 1,2,4-trimethylcyclohexane (TMCH)) and aromatic (toluene, 1,2,4-trimethylbenzene (TMB)) hydrocarbons	***In vitro*; rat brain synaptosome fraction**. .	Studying effects of C7 and C9 aliphatic (n-heptane, n-nonane), naphthenic (methylcyclohexane, 1,2,4-trimethylcyclohexane (TMCH)) and aromatic (toluene, 1,2,4-trimethylbenzene (TMB)) hydrocarbons on the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in rat brain synaptosome fraction; pos.control: MeHg.	**Results**: Exposure of the synaptosomes to the hydrocarbons produced a concentration-dependent linear increase in the formation of the fluorescence of 29,79-dichlorofluorescein (DCF) as a measure of the production of ROS and RNS. The naphthenic hydrocarbon TMCH showed the strongest potential for ROS and RNS formation in rat brain synaptosomes, followed by TMB, toluene, n-nonane, n-heptane, and methylcyclohexane, respectively. **Conclusion**: C7 and C9 aliphatic, naphthenic, and aromatic hydrocarbons stimulated formation of ROS and RNS in rat brain synaptosomes. The naphthenic hydrocarbon TMCH stimulated formation of ROS and RNS in the synaptosomes through Ca2+-dependent activation of PLA2 and nNOS, and through increased transition permeability of the MTP. Exposure of humans to the naphthenic hydrocarbon TMCH may stimulate formation of free radicals in the brain, which may be a key factor leading to neurotoxicity.	**Myhre O, Fonnum F. (2001)** The effect of aliphatic, naphthenic, and aromatic hydrocarbons on production of reactive oxygen species and reactive nitrogen species in rat brain synaptosome fraction: the involvement of calcium, nitric oxide synthase, mitochondria, and phospholipase A. Biochemical Pharmacology 62: 119–128
Volatile solvents (toluene)	**Review**	The abuse of volatile organic solvents (inhalants) leads to diverse sequelae at levels ranging from the cell to the whole organism. This paper reviews findings from the last 10 years of animal models investigating the behavioral and mechanistic effects of solvent abuse.	**Summary:** In research with animal models of inhalant abuse, NMDA, GABAA, glycine, nicotine, and 5HT3 receptors appear to be important targets of action for several abused solvents with emerging evidence suggesting that other receptor subtypes and nerve membrane ion channels may be involved as well. The behavioral effects vary in magnitude and duration among the solvents investigated. The behavioral effects of acute and chronic inhalant abuse include motor impairment, alterations in spontaneous motor activity, anticonvulsant effects, anxiolytic effects, sensory effects, and effects on learning, memory and operant behavior (e.g., response rates and discriminative stimulus effects). In addition, repeated exposure to these solvents may produce tolerance, dependence and/or sensitization to these effects.	**Bowen, S. E., Batis, J.C., Paez-Martinez, N., Cruz, S.L.(2006)** The Last Decade of Animal solvent Abuse Research: Mechanistic and Behavioral Studies. Neurotoxicology and Teratology http://dx.doi.org/10.1016/j.ntt.2006.09.005

Toluene	**Review**	It has long been known that individuals will engage in voluntary inhalation of volatile solvents for their rewarding effects. However, research into the neurobiology of these agents has lagged behind that of more commonly used drugs of abuse such as psychostimulants, alcohol and nicotine. This imbalance has begun to shift in recent years as the serious effects of abused inhalants, especially among children and adolescents, on brain function and behavior have become appreciated and scientifically documented.	**Summary**: In this review, it is discussed the physicochemical and pharmacological properties of toluene, a representative member of a large class of organic solvents commonly used as inhalants. This is followed by a brief summary of the clinical and pre-clinical evidence showing that toluene and related solvents produce significant effects on brain structures and processes involved in the rewarding aspects of drugs. This is highlighted by tables highlighting toluene's effect on behaviors (reward, motor effects, learning, etc.) and cellular proteins (e.g. voltage and ligand-gated ion channels) closely associated the actions of abused substances. **Conclusion**: These sections demonstrate not only the significant progress that has been made in understanding the neurobiological basis for solvent abuse but also reveal the challenges that remain in developing a coherent understanding of this often overlooked class of drugs of abuse.	**Cruz SL, Rivera-García MT, Woodward JJ. (2014)** Review of toluene action: clinical evidence, animal studies and molecular targets. J Drug Alcohol Res. 3: x-x. doi:10.4303/jdar/235840.

Toluene	**Review** (human and animal studies; fetal solvent .	In this paper, it is consider the impact of **toluene abuse** on our society. It describe the specific neurobehavioral deficits in toluene **leukoencephalopathy;** review the spectrum of neuroimaging findings in patients with this disorder; summarize the **teratogenic effects of toluene in both humans and animal models**; and offer possible explanations for the range of neuropathological damage seen in brains of individuals who **chronically abuse toluene**.	**Summary**: Toluene, a common organic solvent that for some individuals is a substance of abuse, has been recognized as a neurotoxin that damages cerebral white matter. As the primary solvent in spray paints, thinners, lacquers, and glues, toluene is inhaled for its capacity to cause euphoria. Toluene abusers with long-term, intense exposure are at risk for toluene leukoencephalopathy, a syndrome characterized by dementia, cerebellar ataxia, corticospinal tract dysfunction, brainstem signs, and cranial neuropathies. Dementia is the most disabling clinical feature, with a characteristic pattern of deficits consisting of inattention, apathy, memory dysfunction, visuospatial impairment, and preserved language, but more subtle neurobehavioral impairment can often be detected early in the disorder before dementia develops. MRI demonstrates cerebral and cerebellar atrophy, and the severity of cognitive dysfunction correlates with the degree of cerebral white matter hyperintensity on T2-weighted images. Toluene leukoencephalopathy has become a prototypical example of white matter dementia, and, more generally, underscores the importance of cerebral white matter in neurobehavioral function. Neuropathological examination shows cerebral and especially cerebellar myelin loss, perivascular macrophages stuffed with coarse or linear PAS-positive debris, and trilaminar inclusions by EM. Extent of myelin and axonal loss in patients is highly variable, possibly due to extent of exposure, age of onset of toluene abuse, coexistent abuse of other substances, or polymorphisms in the gene encoding the enzyme aldehyde dehydrogenase. Although toluene leukoencephalopathy is well documented in inhalant abusers, the potential of low-level occupational or household toluene exposure to cause brain damage is uncertain. Long term exposure to low levels of toluene has been speculated to cause neurobehavioral dysfunction, but the threshold of exposure above which leukoencephalopathy occurs is debated. Toluene abuse in pregnancy causes a constellation of teratogenic features known as fetal solvent syndrome, which is similar to the fetal alcohol syndrome. Advanced neuroimaging techniques such as diffusion tensor imaging, magnetization transfer imaging, and magnetic resonance spectroscopy promise to improve the early recognition of leukoencephalopathy in individuals exposed to toluene when the potential for reversibility is maximal. Animal studies suggest that astrocytes are activated by toluene, paralleling the reactive gliosis seen in human cases with lesser degrees of myelin damage. Despite these animal studies, however, many questions regarding the specific effects of toluene on myelin and other cellular components of the CNS remain to be answered.	**Filley ChM, Halliday W, Kleinschmidt De Masters BK. (2004)** The effects of toluene on the Central Nervous System. Journal of Neuropathology and Experimental Neurology 63: 1-12

Alcohol, Toluene	**Review**	Developmental neurotoxicity can be ascribed to in utero exposure to exogenous substances or to exposure of the fetus to endogenous compounds that accumulate because of genetic mutations. One of the best recognized human neuroteratogens is ethanol. The Fetal Alcohol Syndrome (FAS) is characterized by growth deficiency, particular facial features, and central nervous system (CNS) dysfunctions (mental retardation, microencephaly and brain malformations). Abuse of toluene by pregnant women can lead to an embryopathy (fetal solvent syndrome, (FSS)) whose characteristics are similar to FAS. Phenylketonuria (PKU) is a genetic defect in phenylalanine (Phe) metabolism. Offspring of phenylketonuric mothers not under strict dietary control are born with maternal PKU (mPKU), a syndrome with similar characteristics as FAS and FSS. This paper briefly reviews some of the key features of FAS, mPKU and toluene embryopathy, as well available evidence to address the hypothesis that alterations in astrocyte development may represent a common mode of action to explain at least some of the CNS effects found in these similar syndromes.	**Results**: Alcohol, and its metabolites, as well as toluene, freely cross the placenta, suggesting a direct toxic effect on the developing fetus. While ethanol and toluene may share the common feature of interfering with the cell membrane, this may not be the case with Phe and/or its metabolites. Rather than common mechanisms of teratogenesis, these agents may have different mechanisms of developmental pathogenesis with a common phenotypic endpoint. Alternatively, these agents may share similar 'modes of action', by targeting similar cell types or cellular processes, albeit with different biochemical or molecular mechanisms. Substantial *in vivo* and *in vitro* evidence indicates that ethanol can also affect developing astrocytes. In particular, ethanol can inhibit the proliferation of glial cells, an effect that would lead to a decreased number of astrocytes and contribute to the observed microencephaly. Though no information is available on astroglial cell loss upon developmental toluene exposure or in mPKU, limited *in vitro* studies suggest that toluene as well as Phe and its metabolites PEA (phenylethylanine) and PAA (phenylacetic acid) may inhibit proliferation of astrocytes. Such effect may on one hand contribute to the ensuing microencephaly. Furthermore, an effect on glial cells may in turn affect the development of neurons, given the essential role of astrocytes in fostering the development and survival of neurons **Conclusion**: Though the hypothesis of a central role for glial cells in the developmental neurotoxicity of FAS, mPKU and FSS remains speculative at this time, due mostly to the limited information available for the two latter syndrome, it may offer a working hypothesis to design further studies on possible common modes of action.	**Costa LG, Guizzetti M, Burry M, Oberdoerster J (2002)** Developmental neurotoxicity: do similar phenotypes indicate a common mode of action? A comparison of fetal alcohol syndrome, toluene embryopathy and maternal phenylketonuria. Toxicology letters 127: 197-205

Toluene	**Hazard Summary**-Created in April 1992; Revised in July 2012	The main sources of information for this fact sheet are EPA's Integrated Risk Information System (IRIS), which contains information on inhalation chronic toxicity of toluene and the RfC, oral chronic toxicity and the RfD, and the carcinogenic effects of toluene, and the Agency for Toxic Substances and Disease Registry's (ATSDR's) Toxicological Profile for Toluene.	**Summary**: The central nervous system (CNS) is the primary target organ for toluene toxicity in both humans and animals for acute (short-term) and chronic (long-term) exposures. CNS dysfunction and narcosis have been frequently observed in humans acutely exposed to elevated airborne levels of toluene; symptoms include fatigue, sleepiness, headaches, and nausea. CNS depression has been reported to occur in chronic abusers exposed to high levels of toluene. Chronic inhalation exposure of humans to toluene also causes irritation of the upper respiratory tract and eyes, sore throat, dizziness, and headache. Human studies have reported developmental effects, such as CNS dysfunction, attention deficits, and minor craniofacial and limb anomalies, in the children of pregnant women exposed to high levels of toluene or mixed solvents by inhalation. **Conclusion**: EPA has concluded that there is inadequate information to assess the carcinogenic potential of toluene.	https://www3.epa.gov/airtoxics/hlthef/toluene.html Toluene | Technology Transfer Network Air Toxics Web site | **US EPA.** Technology Transfer Network - Air Toxics Web Site Toluene 108-88-3; Hazard Summary-Created in April 1992; Revised in July 2012.
Industrial chemicals / developmental neurotoxicants	**Review**	In 2006,a systematic review was done and identified five industrial chemicals as developmental neurotoxicants: lead, methylmercury, polychlorinated biphenyls, arsenic, and toluene. Since 2006, epidemiological studies have documented six additional developmental neurotoxicants—manganese, fluoride, chlorpyrifos, dichlorodiphenyltrichloroethane, tetrachloroethylene, and the polybrominated diphenyl ethers. It was postulate that even more neurotoxicants remain undiscovered. To control the pandemic of developmental neurotoxicity, it was proposed a global prevention strategy. Untested chemicals should not be presumed to be safe to brain development, and chemicals in existing use and all new chemicals must therefore be tested for developmental neurotoxicity. To coordinate these efforts and to accelerate translation of science into prevention, it was proposed the urgent formation of a new international clearinghouse.	**Results:** Industrial chemicals known to cause developmental neurotoxicity in human beings in 2006 and 2013, according to chemical group: *Metals and inorganic compounds*: Arsenic and arsenic compounds, lead, and methylmercury(2006), Fluoride and manganese (2013); *Organic solvents* (Ethanol) toluene (2006), Tetrachloroethylene (2013); *Pesticides:* None (2006), Chlorpyrifos and DDT/DDE* (2013); *Other organic compounds:* Polychlorinated biphenyls (2006), Brominated diphenyl ethers (2013). *Total*: 6 (including ethanol) (2006); 6 (2013). *DDT=dichlorodiphenyltrichloroethane and DDE=dichlorodiphenyldichloroethylene **Conclusion**: The updated findings presented in this Review confirm and extend the 2006 conclusions. During the 7 years since the previous report, the number of industrial chemicals recognised to be developmental neurotoxicants has doubled. Exposures to these industrial chemicals in the environment contribute to the pandemic of developmental neurotoxicity.	**Grandjean P, Landrigan PJ. (2014)** Neurobehavioural effects of developmental toxicity. Lancet Neurol 13: 330–38. **Grandjean P, Landrigan PJ (2006)** Developmental neurotoxicity of industrial chemicals. The Lancet. DOI:10.1016/S0140-6736(06)69665-7

Xylene (CAS-no 1330-20-7)	***In vivo***	Pregnant rats (Mol:WIST) were exposed to 500 ppm technical xylene 6 h per day on gestation days 7-20. The dose level was selected so as not to induce maternal toxicity or decrease the viability of offspring	**Results:** In the exposed offspring, a delay in the ontogeny of the air righting reflex, a lower absolute brain weight, and impaired performance in behavioral tests for neuromotor abilities (Rotarod) and for learning and memory (Morris water maze) were found. Generally, the effects were most marked in the female offspring.	**Hass U, Lund SP, Simonsen L, Fries AS. (1995).** Effects of prenatal exposure to xylene on postnatal development and behavior in rats. Neurotoxicol Teratol. 17:341-9.
Xylene (CAS-no 1330-20-7)	***In vivo***	The persistence of neurobehavioral effects in female rats (Mol:WIST) exposed to 500 ppm technical xylene (dimethylbenzene, CAS-no 1330-20-7) for 6 hours per day on days 7-20 of prenatal development was studied. The dose level was selected so as not to induce maternal toxicity or decreased viability of offspring. Investigations of learning and memory abilities were performed using a Morris water maze. This task requires rats to spatially navigate, using distal extramaze cues to locate a small platform under the surface of the water in a large pool.	**Results:** At the age of 16 weeks, the exposed offspring showed impairments when the platform was relocated in the pool. Impaired performances after platform relocation were also observed in exposed offspring at 28 and 55 weeks of age, although the difference was not statistically significant at 55 weeks. **Conclusion:** These data could indicate that the effect was partly reversible, although over a long time period. However, another explanation could be that the animals became more practised at solving the problem (finding the platform) as continued testing occurred and therefore were able to compensate for the neurotoxic effect of the prenatal xylene exposure	**Hass U, Lund SP, Simonsen L. (1997).** Long-lasting neurobehavioral effects of prenatal exposure to xylene in rats. Neurotoxicology. 18:547-51.
Isopropanol (200-661-7) Physical state: Liquid VP: 44 hPa at 20 °C Water Solubility: "Compound is soluble, however, unable to determine degree of solubility from the word "miscible"". Log Pow: 0.05	***In vivo***	Rats, Isopropanol at 200, 700, 1200 mg/kg/day via oral gavage from GD 6 to PND 21. Neurobehavioral and neuropathological parameters were measured in offsprings	**Conclusion**: The author concluded that "no evidence of developmental neurotoxicity associated with isopropanol exposure as high as 1200 mg/kg/day".	**Bates HK, McKee RH, Bieler GS. Gardiner TH, Gill MW, Strother DE, Masten LW. (1994).** Developmental neurotoxicity evaluation of orally administered isopropanol in rats. Fundam. Appl. Toxicol. 22: 152-158.

n-propanol (200-746-9) Physical state: Liquid VP: 28.2 hPa at 25°C Water solubility: "substance is completly miscible in water at 25 °C" log Pow: 0.2 at 25 °C	***In vitro***	*In vitro* effects of five short chain aliphatic alcohols (ethanol, n-propanol and t-butanol) on muscarinic receptor-stimulated phosphoinositide metabolism in cerebral cortical slices from 7 day-old rats.	**Conclusion**: "These results suggest that muscarinic receptor-coupled phosphoinositide metabolism might be a common neurochemical target for the developmental neurotoxicity of short chain aliphatic alcohols."	**Candura SM, Balduini W, Costa LG (1991).** Interaction of short chain aliphatic alcohols with muscarinic receptor-stimulated phosphoinositide metabolism in cerebral cortex from neonatal and adult rats. *Neurotoxicology*; 12(1):23-32.
1-bromopropane (203-445-0) Physical state: Liquid VP: 137 mm Hg at 25 °C Water solubility: 2 500 mg/L Log Kow: 2.16	***In vivo***		**Conclusion**: The author indicated that the result of the experiment suggested that prenatal exposure to 1-Bromopropane affects neurobehavioral responses in the juvenile period	**Fueta Y, Kanemitsu M, Egawa S, Ishidao T, Ueno S, Hori H (2015).** Prenatal Exposure to 1-Bromopropane Suppresses Kainate-Induced Wet Dog Shakes in Immature Rats. *J UOEH*; 37(4):255-261. doi:10.7888/juoeh.37.255.
Trichloroethylene (201-167-4)	***In vivo***	Rats, 1,1,1-TCE (trichloroethylene), (75, 250, 750 mg/kg/day), oral gavage, from GD 6 to GD 10. Neurobehavioral and neuropathological parameters were measured in offsprings	**Result**: no effect related to neurotoxicity in the offspring	**EPA, 1998**

Trichloroethylene (201-167-4)	***In vivo***	1,1-TCE (trichloroethylene), Oral (drinking water) in 6 week old male offspring of dams exposed gestationally to 0, 0.01, and 0.1mg/ml	**Result:** a) Increased locomotor activity at 0.01 and 0.1 level. b) Alters brain redox homeostasis in the cerebellum (reduced concentration of GSH, GSSG, the glutathione redox (GSH/GSSG) ratio, and the percentage of oxidized glutathione equivalents in the cerebellum). c) Alters transsulfuration and transmethylation metabolites in plasma. d) Positive association between oxidative endpoint GSH in plasma and cerebellum e) Nitrotyrosine increased in cerebellum and plasma. f) increased plasma inflammatory biomarkers. g) increases population of memory CD4+ T cells in adult mice. h) Prenatal TCE exposure alters CD4+ T cell cytokine production **Conclusion**: The result suggests that the prenatal period is a critical stage of life by which the developing CNS and immune system are susceptible to long-lasting changes mediated	**Blossom SJ, Melnyk SB, Li M, Wessinger WD, Cooney CA (2016).** Inflammatory and oxidative stress-related effects associated with neurotoxicity are maintained after exclusively prenatal trichloroethylene exposure. *Neurotoxicology*; pii: S0161-813X(16)30002-X. doi:10.1016/j.neuro.2016.01.002 .
Trichloroethylene (201-167-4) Physical state: Liquid VP: 9.9 kPa at 25 °C Water solubility: 1.1 g/L at 20 °C Log Kow: 2.53	***In vivo***		**Conclusion:** The author indicated that chlorinated hydrocarbon class that includes trichloroethylene have effects on motor, sensory, or cognitive function that are detectable using functional measures such as behavior. In addition, there is evidence that each of these chemicals are developmental toxicants if exposure occurs during critical periods of development	**Evangelista de Duffard AM, Duffard R (1996).** Behavioral toxicology, risk assessment, and chlorinated hydrocarbons. *Environ Health Perspect suppl*; 104:353-360.

n-butanol (200-751-6) Physical state: Liquid VP: 9.31 hPa at 25 °C Water solubility: 66 g/L at 20 °C Log Pow: 1 at 25 °C	***In vivo***	Rats exposed to n- butanol via oral (drinking water) at 0.24, 0.8 and 4% (0.3; 1.0 and 5.0 g/kg/day) for 8 weeks before and during gestation.	**Result:** Internal hydrocephalus was the most frequent anomaly found in the foetuses of female rats exposed to n-butanol at mid and high dose level. Pathological changes in the CNS includes (dilation of subarachnoid space, cerebral ventricles,) at all dose levels. No detectable toxic effect in the parental females. But produced congenital defects including the CNS of their offspring.	**Sitarek K, Berlińska B, Barański B (1994).** Assessment of the effect of n-butanol given to female rats in drinking water on fertility and prenatal development of their offspring. *Int J Occup Med Environ Health*; 7(4):365-370.
n-hexane (203-777-6) Physical state: Liquid VP: 10 kPa at 9.8 °C Water solubility: 0.01 g/L at 25 °C Log Pow: 4	***In vivo***		In this literature the metabolite of n-hexane (2,5-hexanedione) is indicated as a neuronal toxin in the developing fetus.	**Cheng X, Luo R, Wang G, Xu CJ, Feng X, Yang RH, Ding E, He YQ, Chuai M, Lee KK, Yang X (2015).** Effects of 2,5-hexanedione on angiogenesis and vasculogenesis in chick embryos. *Reprod Toxicol*; 51:79-89. doi:10.1016/j.reprotox.2014.12.006.

Methyl ethyl ketone (MEK)	Review Comment	Environmental causes of microcephaly are the result of deleterious exposures which occur during pregnancy and which interfere with normal proliferation and migration of neural cells the fundamental building blocks of the central nervous system. Environmental factors commonly associated with microcephaly include: ethanol ingestion; inhalation of mixed solvent vapor; certain pharmaceutical drugs; maternal infection, and radiation. Certain chemicals, such as methyl ethyl ketone [MEK], have the capacity to produce microcephaly because they target the developing fetal brain and interfere with proper, timely, and adequate proliferation and migration of the neural cells.	**Summary**: MEK exposed brains showed significant retardation of growth and development of the cerebellar cortex. The implications of this finding are profound and compelling. Damage to the cortex interferes with intellectual functioning, reasoning, and judgment. The weight of evidence is that the human species is at least as vulnerable as the rat or mouse to the effects of in utero MEK exposure In normal fetal development in humans, the most significant brain growth occurs in the second half of pregnancy. The biological foundation for the existence of an association between in utero MEK exposure and damage to the developing fetal brain, is MEK's neurotoxic properties, its affinity for lipid rich tissue including the brain, its ready skin penetration, and its ability to cross the placenta, and the fetal liver's inability to detoxify it. **Conclusion**: MEK is a fetotoxic chemical that induces developmental delay, including retarded brain development in humans and *in utero* exposure to MEK can readily cause microcephaly and mental retardation.	**Alexander Law Group. 1994.** methyl ethyl ketone, fetal neurotoxicity, microcephaly and mental retardation: in utero brain damage caused by toxic solvent exposure http://www.alexanderinjury.com /library-toxic-18
Methyl iso-butyl ketone (MiBK)	***In vivo***	Methyl iso-butyl ketone (MiBK), a commercial solvent, was selected by the US Environmental Protection Agency (US EPA) for testing under the Multi-Substance Rule for the Testing of Neurotoxicity (US EPA, 1993) using schedule-controlled operant behavior (SCOB) to determine if subchronic exposure to MiBK vapor had the potential to alter behavior as an indicator of neurotoxicity. Food-restricted and ad libitum-fed Sprague-Dawley male rats were exposed to 0, 250, 750, or 1500 ppm MiBK for 6 h/day, 5 d/wk for 13 weeks. SCOB testing of food-restricted animals, using a multiple fixed ratio (FR)/fixed interval (FI) schedule (FR20:FI120), was conducted prior to each exposure to maintain the operant behavior; the data from Weeks -1, 4, 8, and 13 were evaluated for evidence of neurotoxicity. SCOB testing was also evaluated for two weeks following the cessation of exposures. Ad libitum-fed animals were included to assess systemic effects using routine indicators such as changes in body weight, food consumption, and organ weight	**Results:** No significant differences were seen in fixed-ratio run rate, FR pause duration, fixed-interval response rate, and index of curvature values at any concentration. Animals exposed to 750 and 1500 ppm MiBK exhibited clinical signs associated with transient reduced activity levels, but only during exposure. No signs of reduced activity were observed immediately after exposure for either group. No other treatment-related abnormalities were observed during exposure. Food-restricted animals did not demonstrate any increased or decreased sensitivity to the CNS depressive effects of MiBK relative to the ad libitum-fed animals. No treatment-related body weight differences were observed within either the food-restricted groups or the ad libitum-fed groups, although body weights of the former were clearly depressed compared with those of the latter. Relative and absolute liver, and relative kidney weights were significantly greater for the 750 and 1500 ppm ad libitum-fed animals. No differences in kidney weight were observed for food-restricted animals, but absolute and/or relative liver weights were significantly higher for all the treated food-restricted groups. **Conclusion**: The results of this study indicate that repetitive exposures to high concentrations of MiBK vapors do not result in adverse effects on operant behavior in the rat.	**David RM, Bernard LG, Banton MI, Tyler TR, Topping DC, Gill MW, O'Donoghue JL (1999)** The effect of repeated methyl iso-butyl ketone vapor exposure on schedule-controlled operant behavior in rats. Neurotoxicology.20:583-93.

Methyl isobutyl ketone (MIBK)	**Two generation reproduction study.**	Rats(Sprague-Dawley), exposed to MIBK 0, 500, 1000, or 2000 ppm, 6 h daily, for 70 days prior to mating. F(0) and F(1) females were exposed from mating through gestation day 20 and from postnatal day 5; F(2) litters were maintained through postnatal day 21.	**Results:** There was a dose-related increase in adult animals with no or a decreased response to a sound stimulus at 1000 and 2000 ppm; however, no adverse clinical signs occurred 1 h after exposure, suggesting this was a transient sedative effect. Clinical signs of central nervous system (CNS) depression in the pups were observed and one F(1) pup died after initial exposure to 2000 ppm on postnatal day 22;subsequently exposure was delayed until postnatal day 28. Decreased body weight gain and slight decreased food consumption were observed during the first 2 weeks of exposure in both generations at 2000 ppm. There were no adverse effects on male and female reproductive function or landmarks of sexual maturation. Increased F(0) and F(1) liver weights with associated centrilobular hypertrophy occurred in rats at 2000 ppm, indicative of an adaptive response. Increased male kidney weights at all exposure concentrations, associated with hyaline droplets, were indicative of male rat-specific nephropathy. Other than acute sedative effects, the no-observed-adverse-effect level (NOAEL) for parental systemic effects (excluding male rat kidney) was 1000 ppm, based on transient decreased body weight and food consumption; for reproductive effects, 2000 ppm, the highest concentration tested; and for neonatal toxicity, 1000ppm (based on acute CNS depressive effects).	**Nemec et al., 2004:** Inhalation two-generation reproductive toxicity study of methyl isobutyl ketone in rats, International Journal of Toxicology, 23:127-43.
Organic solvents	**Meta-analysis**	Organic solvents are widely used, but conflicting reports exist concerning paternal exposure and adverse pregnancy outcomes. This study was a meta-analysis to assess the risks of spontaneous abortions (SAs) and major malformations (MMs) after paternal exposure to organic solvents. Medline, Toxline, Reprotox, and Embase from 1966 to 2003 were searched. Two independent reviewers searched for cohort and case-control studies in any language on adult human males exposed chronically to any organic solvent. Two non-blinded independent extractors used a standardized form for data extraction; disagreements were resolved through consensus discussion.	**Results:** Forty-seven studies were identified; 32 exclusions left 14 useable studies. Overall random effects odds ratios and 95% confidence intervals (CI95%) were 1.30 (CI95%: 0.81–2.11, N.1,248) for SA, 1.47 (CI95%: 1.18–1.83, N.384,762) for MMs, 1.86 (CI95%: 1.40–2.46,N.180,242) for any neural tube defect, 2.18 (CI95%: 1.52–3.11,N.107,761) for anencephaly, and 1.59 (CI95%: 0.99–2.56, N.96,517; power.56.3%) for spina bifida. **Conclusions**: Paternal exposure to organic solvents is associated with an increased risk for neural tube defects but not SAs (spontaneous abortions).	**Logman JFS, de Vries LE, Hemels MEH, Khattak S. (2005)** Paternal organic solvent exposure and adverse pregnancy outcomes: A Meta-Analysis. American Journal of Industrial Medicine 47: 37-44.

Organic solvents	**A prospective, observational, controlled study** Occupational exposure	Numerous women of childbearing age are exposed occupationally to organic solvents. Previous retrospective studies have reported conflicting results regarding teratogenic risk. **Patients**: 125 pregnant women exposed occupationally to organic solvents; seen during first trimester; between 1987 and 1996. Each pregnant woman exposed, matched to a pregnant woman exposed to a nonteratogenic agent on age (±4 years), gravidity (±1), and smoking and drinking status. ***Objective**: To evaluate pregnancy and fetal outcome following maternal occupational exposure to organic Solvents.*	**Results**: Significantly more major malformations occurred among fetuses of women exposed to organic solvents than controls (13 vs 1; relative risk, 13.0; 95% confidence interval, 1.8-99.5). Twelve malformations occurred among the 75 women who had symptoms temporally associated with their exposure, while none occurred among 43 asymptomatic exposed women (P<.001). (One malformation occurred in a woman for whom such information was missing.) More of these exposed women had previous miscarriage while working with organic solvents than controls (54/117 [46.2%] vs 24/125 [19.2%]; P<.001). However, exposed women who had a previous miscarriage had rates of major malformation that were similar to exposed women who had no previous miscarriage. **Conclusions**: Occupational exposure to organic solvents during pregnancy is associated with an increased risk of major fetal malformations. This risk appears to be increased among women who report symptoms associated with organic solvent exposure. Women's exposure to organic solvents should be minimized during pregnancy. Symptomatic exposure appears to predict higher fetal risk for malformations.	**Khattak S, K-Moghtader G, McMartin K, Barrera M, Kennedy D, Koren G. (1999)** Pregnancy Outcome Following Gestational Exposure to Organic Solvents. A Prospective Controlled Study. *JAMA* 281: 1106-1109. doi:10.1001/jama.281.12.1106.

Hydrocarbon solvents	**Review**	The present document summarizes information on the physical/chemical properties and toxicological hazards of hydrocarbon solvents and provides examples of the ways in which the information on hazard characterization can be used for hazard classification and to set occupational exposure limits.	**Summary:** Because of the compositional complexity, hydrocarbon solvents are now identified by a nomenclature ("the naming convention") that describes them in terms of physical/chemical properties and compositional elements. Despite the compositional complexity, most hydrocarbon solvent constituents have similar toxicological properties, and the overall toxicological hazards can be characterized in generic terms. To facilitate hazard characterization, the solvents were divided into 9 groups (categories) of substances with similar physical and chemical properties. Hydrocarbon solvents can cause chemical pneumonitis if aspirated into the lung, and those that are volatile can cause acute CNS effects and/or ocular and respiratory irritation at exposure levels exceeding occupational recommendations. Otherwise, there are few toxicologically important effects. The exceptions, n-hexane and naphthalene, have unique toxicological properties, and those solvents containing constituents for which classification is required under the Globally Harmonized System (GHS) are differentiated by the substance names. Toxicological information from studies of representative substances was used to fulfill REACH registration requirements and to satisfy the needs of the OECD High Production Volume (HPV) initiative. **Conclusion**: As shown in the examples provided, the hazard characterization data can be used for hazard classification and for occupational exposure limit recommendations.	**Mckee RH, Adenuga MD, Carrillo JC (2015)** Characterization of the toxicological hazards of hydrocarbon solvents. Crit Rev Toxicol, 45: 273–365
Inhalant volatile solvent (abuse)	**Review**	The deliberate misuse of volatile substances poses a poorly recognized risk for considerable morbidity and mortality in **adolescent** populations worldwide. Inhalant abuse is the deliberate inhalation of vapors with the intention to alter one's consciousness or become intoxicated.	**Summary**: This review discusses the prevalence of inhalant abuse in the United States, summarizes the various types of substances used, highlights the major physiologic effects of inhalants, and briefly discusses associated risk behaviors, prevention and medical management. references to mechanisms underlying solvent-induced neurotoxicity.	**Kurtzman TL, Otsuka KN, Wahl RA. (2001)** Inhalant Abuse by Adolescents. Review article. Journal of Adolescent Health 28: 170-180.

Inhalant abuse	**Review** adolescents	Inhalants have the potential to result in serious organ system dysfunction or even sudden death. Inhalants remain one of the most commonly abused substances by adolescents in the United States today. This article reviews the most recent epidemiologic data on inhalant abuse, summarizes the **types of substances** used and their **mechanism of actions**, and discusses clinical toxicity and medical management.	**Results:** Toxicity: Neurologic As the primary site of action of inhalants, the CNS is also the most vulnerable to their toxic effects. Because of their highly lipophilic nature, the inhalants act quickly to produce CNS depression. Acutely, the CNS depression is manifested by slurred speech, diplopia, ataxia, disorientation, and visual hallucinations. Further CNS depression can lead to coma, seizures, and respiratory arrest. Chronic volatile abuse can have equally severe CNS consequences. Toluene, in particular, has been linked to severe, permanent cortical atrophy and widespread cerebellar damage (Filley et al., 1990). Less commonly, toluene can cause cranial nerve damage manifested by opsoclonus, optic atrophy, tinnitus, and sensorineural hearing loss Williams, 1988). Inhalation of glues and paints containing hexane, methyl- N -butyl ketone, and methylisobutyl ketone results in a classic sensorimotor peripheral neuropathy with a "glove and stocking" distribution Tenenbein et al., 1984. **Perinatal and teratogenic**: Inhalants are highly lipophilic and, therefore, readily cross the placenta. Chronic toluene abuse, in particular, has been associated with an embryopathy similar to fetal alcohol syndrome (Lazar et al., 1983). A neonatal withdrawal syndrome has been described with chronic inhalant abuse (Tenebein et al., 1996). Finally, abuse of toluene and some halogenated hydrocarbons during pregnancy can increase the risk of spontaneous abortion and premature delivery (Khattak et al., 1999). **Conclusion**: Despite data that suggest that the abuse of volatile substances may be decreasing among American youth, it remains a serious health risk for today's adolescents. Inhalant abuse remains widespread. The medical complications from both acute and chronic abuse include severe and potentially fatal multiorgan system toxicities. Inhalant abuse should be considered a risk factor for abuse and addiction of other substance over a lifetime. The recognition and appropriate treatment of inhalant abuse remains a major challenge facing today's pediatricians and emergency physicians.	**Lorenc JD (2003**) Inhalant abuse in the pediatric population: a persistent challenge. Therapeutics and toxicology. Opin Pediatr 15: 204–209.

Organic solvents and pesticides	**Review**	Exposure to neurotoxic chemicals is of particular concern when it occurs during early development. The immature brain is highly vulnerable prenatally and is therefore at risk due to occupational exposures incurred by pregnant women. A systematic search of the literature has been performed with emphasis on epidemiological studies on female workers and the neurodevelopment of their children.	**Summary**: This review covers the background information and the modest epidemiological evidence on occupational exposures of female workers to industrial chemicals and the consequences in regard to the child's neurodevelopment. The majority of the occupational studies identified aimed to assess organic solvents and organophosphate pesticide effects in the offspring, **and consistent neurobehavioral impairments were reported.** The evidence suffers from a variety of shortcomings and sources of imprecision. These problems would tend to cause an underestimation of the true extent of the risks. The overall experimental and epidemiological evidence suggests that the substantial vulnerability of the developing nervous system to low concentrations of neurotoxic chemicals should lead to a strengthened emphasis on protection of pregnant workers and women in general against substances that may cause harm to the fetus. A precautionary principle in regard to neurodevelopmental toxicity should therefore be applied in occupational health, and this issue should also attract more research, preferably with a focus on exposure assessment and valid outcome measures in prospective study designs. While preventive measures should not be delayed, research is needed to improve our understanding of the mechanisms involved and help in identifying the best means of protecting future generations against a silent pandemic of developmental neurotoxicity.	**Julvez J, Grandjean P. (2009)** Neurodevelopmental toxicity risks due to occupational exposure to industrial chemicals during pregnancy. Industrial Health 47: 459-68

Pesticides	**Review**	This brief review highlights some of the main neurotoxic pesticides, their effects, and mechanisms of action.	**Summary**: This brief overview highlights the most relevant aspects of neurotoxicity associated with exposure to pesticides. Iinsecticides are most often responsible for neurotoxic effects in humans, as the nervous system represents their biological target in insects. In addition to implications for acute toxicity, exposure to pesticides raises concerns for possible long-term effects and developmental effects. Whether chronic exposure to low doses of certain pesticides may contribute to the etiology of some neurodegenerative diseases (most notably Parkinson's disease) and/or to other less defined behavioral alterations, remains a topic of public concern, and more research is needed. Furthermore, the possibility that developmental exposure to pesticides (both in utero and neonatally) may contribute to developmental disorders in children, such as attention deficit hyperactivity disorder, autism, or learning disabilities, needs to be further investigated. Finally, a most challenging endeavor would be that of ascertain whether developmental exposure to pesticides may result in "silent neurotoxicity", i.e. may cause nervous system damage that would be manifest as a clinical condition only later in life. For example, damage to nigrostriatal dopaminergic neurons early in life would be expected to result in clinical manifestations of Parkinson's disease as the individual ages, by adding the early insult to the normal age-related loss of neurons.	**Costa LG, Giordano G, Guizzetti M, Vitalone A (2008)** Neurotoxicity of pesticides: a brief review. Frontiers in Bioscience 13: 1240-9

Methylmercury, lead, polybrominated diphenyl ether and organophosphate inseticides	**Review**	Both prenatal and early postnatal exposure to neurotoxic chemicals may have deleterious influences on the structure and functions of the nervous system. It has been estimated that one out of six children has a developmental disability, and in most cases these disabilities affect the nervous system [3]. These include learning disabilities, autism-related disorders, attention deficit hyperactivity disorder, developmental delays, cerebral palsy, or sensory deficits. Such disorders are usually difficult to correct either pharmacologically or byother types of intervention and are thus permanent, causing enormous damage and costs to families and society. While there are many etiological causes to such developmental disabilities, it has been estimated that around 3%may be due exclusively to exposure to neurotoxicants, and another 25% may arise from the interaction of individual genetic susceptibility and environmental chemicals [141]. It has been often underlined that even more subtle changes, such as a decrease of a few IQ points, may not have a significant impact on a single individual, but are of great significance for society,as would be the case of Pb [46]. Authors suggest testing guidelines for developmental neurotoxicity may have to be revisited, as situations like those mentioned in this article (e.g., for BDE-209 and bisphenol-A for which guideline developmental neurotoxicity testing provided no evidence of adverse effects, in contrast to other findings) complicate a proper health risk evaluation.	**Summary**: The developing central nervous system is often more vulnerable to injury than the adult one. Of the almost 200 chemicals known to be neurotoxic, many are developmental neurotoxicants. Exposure to these compounds *in utero* or during childhood can contribute to a variety of neurodevelopmental and neurological disorders. Two established developmental neurotoxicants, methylmercury and lead, and two classes of chemicals, the polybrominated diphenyl ether flame retardants and the organophosphorus insecticides, which are emerging as potential developmental neurotoxicants, are discussed in this paper. **Conclusion**: Developmental neurotoxicants may also cause silent damage, which would manifest itself only as the individual ages, and may contribute to neurodegenerative diseases such as Parkinson's or Alzheimer's diseases. Guidelines for developmental neurotoxicity testing have been implemented, but there is still room for their improvement and for searching and validating alternative testing approaches.	**Giordano G, Costa LG (2012)** Developmental neurotoxicity: some old and new issues. International Scholarly Research Network Toxicology. doi:10.5402/2012/814795

Polycyclic Aromatic Hydrocarbons (PAHs)	***In vivo***	Rats (wistar). Three experimental groups per period of exposure were formed. A group exposed to a lower concentration of PAHs (2 µg/kg/day during gestation and 1.5 µg/kg/day during lactation), a group exposed to a 100-fold higher concentration of PAHs (reached 200 µg/kg/day during gestation and 150 µg/kg/day during lactation.), and a control group. Developing organisms can be exposed also to PAHs due to the ability of these compounds to pass through the placental barrier as well as through the breast milk. In the present study, short-term effects of exposures to the same PAH mixture during gestation, or during gestation and lactation, were assessed by evaluating motor and sensory development of rat pups, and by measuring cerebral cytochrome oxidase activity (a marker of energetic metabolism) in different brain areas. Brain levels of PAHs and some monohydroxylated metabolites were also evaluated in pups at birth and at 21 days of postnatal life.	**Results:** No significant short-term modifications of behavioral development and of cerebral metabolism were observed following an early PAH exposure whatever the dose and the period of exposure. Surprisingly, the same brain levels of concentration of PAHs and metabolites were observed in control and exposed pups in both studies **Conclusion**: The measurement of the PAH brain levels of concentration raised the difficulty to overcome environmental contamination of control animals and the choice of such controls in experimental studies which focus on the neurotoxicity of exposure to low levels of pollutants. Nevertheless, the neurotoxicity of early exposure to ubiquitous chemicals like PAHs remains under question and needs to be studied further, especially regarding the long-term effects of such exposure. For instance, significant behavioral changes relating to locomotor activity and anxiety and concomitant variations of the regional cytochrome oxidase activity were reported at the adult stage in animals exposed to the mixture of PAHs during gestation and lactation (Crépeaux et al., 2012) whereas no significant behavioral disturbances have been observed in the same animals over the first 4 weeks of life (the present study). In contrast, no long-term effects have been observed in animals exposed early to PAHS only during the 3 weeks of gestation (Crépeaux et al., 2013),underlining the importance of the period of exposure as a critical window for brain and behavior development.	**Crépeaux G, Grova N, Bouillaud-Kremarik P, Sikhayeva N, Salquèbre G, Rychen G, Soulimani R, Appenzeller B, Schroeder H.(2014)** Short-term effects of a perinatal exposure to a 16 polycyclic aromatic hydrocarbon mixture in rats: assessment of early motor and sensorial development and cerebral cytochrome oxidase activity in pups. Neurotoxicology 43: 90-101
Polychlorinated biphenyls (PCB)	**Review**	This review focus on the current knowledge of the structure and function of ryanodine receptors (RyRs) in muscle and nerve cells and how PCBs and related non-coplanar structures alter these functions. The molecular and cellular mechanisms by which noncoplanar PCBs and related structures alter local and global Ca2+ signaling properties and the possible short and long-term consequences of these perturbations on neurodevelopment and neurodegeneration are reviewed	**Summary:** Chronic low level polychlorinated biphenyls (PCB) exposures remain a significant public health concern since results from epidemiological studies indicate PCB burden is associated with immune system dysfunction, cardiovascular disease, and impairment of the developing nervous system. Of these various adverse health effects, developmental neurotoxicity has emerged as a particularly vulnerable endpoint in PCB toxicity. Arguably the most pervasive biological effects of PCBs could be mediated by their ability to alter the spatial and temporal fidelity of Ca2+ signals through one or more receptor mediated processes. The observation that RyRs play a critical role in diverse tissue types and in numerous cellular processes raises an interesting challenge in light of emerging data identifying RyRs as a direct molecular target in PCB neurodevelopmental toxicity.	**Pessah IN, Cherednichenko G, Lein PJ (2010).**Minding the calcium store: ryanodine receptor activation as a convergent mechanism of PCB toxicity. Pharmacol Ther. 125: 260–85

Polybrominated diphenyl ethers (PBDEs)	***In Vitro***	Polybrominated diphenyl ethers (PBDEs) are persistent and bioaccumulative flame retardants, which are found in rising concentrations in human tissues. They are of concern for human health because animal studies have shown that they possess the potential to be developmentally neurotoxic. Because there is little knowledge of the effects of PBDEs on human brain cells, their toxic potential for human neural development *in vitro* was investigated. Moreover, authors studied the involvement of thyroid hormone (TH) disruption in the effects caused by PBDEs In this study, two PBDE congeners were used: BDE-47 and BDE-99 (0.1–10 µM), which are most prominent in human tissues. As a model of neural development, authors employed primary fetal human neural progenitor cells (hNPCs), which are cultured as neurospheres and mimic basic processes of brain development *in vitro*: proliferation, migration, and differentiation.	**Results**: PBDEs do not disturb hNPC proliferation but decrease migration distance of hNPCs. Moreover, they cause a reduction of differentiation into neurons and oligodendrocytes. Simultaneous exposure with the TH receptor (THR) agonist triiodothyronine rescues these effects on migration and differentiation, whereas the THR antagonist NH-3 does not exert an additive effect. **Conclusion**: PBDEs disturb development of hNPCs in vitro via endocrine disruption of cellular TH signaling at concentrations that might be of relevance for human exposure.	**Schreiber T, Gassmann K, Götz C, Hübenthal U, Moors M, Krause G, Merk HF, Nguyen N, Scanlan TS, Abel J, Rose CR, Fritsche E (2010)** Polybrominated diphenyl ethers induce developmental neurotoxicity in a human *in vitro* model: evidence for endocrine disruption. Environmental Health Perspectives 118: 572-8

Polybrominated diphenyl ethers (PBDEs) and their hydroxylated (OH-) or methoxylated forms	**Review**	In this review authors extend knowledge from previous reviews of underlying mechanisms of adverse effects on the nervous system by exposure to PBDEs (Costa and Giordano 2007; Fonnum and Mariussen 2009) and also focus on the contribution of OH-PBDEs to PBDE-induced neurotoxicity.	**Summary:** Many rodent studies reported behavioral changes after developmental, neonatal, or adult exposure to PBDEs, and other studies documented subtle structural and functional alterations in brains of PBDE-exposed animals. Functional effects have been observed on synaptic plasticity and the glutamate–nitric oxide–cyclic guanosine monophosphate pathway. In the brain, changes have been observed in the expression of genes and proteins involved in synapse and axon formation, neuronal morphology, cell migration, synaptic plasticity, ion channels, and vesicular neurotransmitter release. Cellular and molecular mechanisms include effects on neuronal viability (via apoptosis and oxidative stress), neuronal differentiation and migration, neurotransmitter release/uptake, neurotransmitter receptors and ion channels, calcium (Ca2+) homeostasis, and intracellular signaling pathways. Bioactivation of PBDEs by hydroxylation has been observed for several endocrine end points. This has also been observed for mechanisms related to neurodevelopment, including binding to thyroid hormone receptors and transport proteins, disruption of Ca2+ homeostasis, and modulation of GABA and nicotinic acetylcholine receptor function. **Conclusion**: The increased hazard for developmental neurotoxicity by hydroxylated (OH□)PBDEs compared with their parent congeners via direct neurotoxicity and thyroid disruption clearly warrants further investigation into a) the role of oxidative metabolism in producing active metabolites of PBDEs and their impact on brain development; b) concentrations of parent and OH-PBDEs in the brain; and c) interactions between different environmental contaminants during exposure to mixtures, which may increase neurotoxicity.	**Dingemans MML, van den Berg M, Westerink RHS (2011)** Neurotoxicity of brominated flame retardants: (In)direct effects of parent and hydroxylated Polybrominated Diphenyl Ethers on the (developing) nervous system. Environmental Health Perspectives 119: 900-7

Various compounds (Ethanol, PCB, PBDE, Chlorpyrifosoxone, Dieldrin, Manganese, Arsenic, Mercury, Lead, Valproic acid)	**Review *(in vitro)***	Developmental neurotoxicity (DNT) is also one of the most difficult forms of toxicity to pinpoint, as it is not necessarily related to cell loss. A change in the overall proportions of neural cells in the nervous system suffices to alter its function significantly. Similarly, changes in positioning, organization and connectivity of any given number of neurons affect their network function. The resultant forms of DNT, such as behavioral problems and speech impairments are difficult to model in animals and in vitro systems (4). Following the National Research Council (NRC) initiative on toxicology for the 21st century (21c-Tox) new *in vitro* methods have been developed and may be assembled to a tiered battery of tests capable of improving the predictive value of toxicity testing with respect to human health (8). The advent of embryonic stem cells (ESCs) allows for the first time to develop novel test systems modeling early human neural development *in vitro*, thus enabling this aspect of DNT testing (9, 10). It has been shown for murine embryonic stem cells (mESCs) that the differentiating cells followed the main stages and transitions of *in vivo* embryonic development (11) While similar formal proof is lacking for human embryonic stem cells (hESCs), it is assumed that similar to mESC, hESC recapitulate human embryonic/fetal neural development. To successfully use hESC-derived cells for *in vitro* assays for DNT, careful assessment of the test systems is crucial, which is discussed in this review.	**Summary:** Development of *in vitro* systems, such as those based on embryonic stem cell differentiation, depends on the selection of adequate test and training compounds. It is recommend the use of two classes of positive controls, the “gold standard compounds” for which developmental neurotoxicity (DNT) has been proven in man, and the “pathway compounds” that are known to disrupt signaling pathways and key processes relevant for neuronal differentiation. The concept of toxicity endophenotypes (TEP) is introduced as changes in neuronal connectivity resulting from exposure to developmental toxicants. Thus, TEPs provide the scientific rationale for modeling DNT with simple *in vitro* models of key neurodevelopmental events. In this context, it is discussed scientific and technical aspects of the test compound selection process. It is suggested to include compounds with unspecific toxicity, besides negative control compounds, and the authors recommend tandem approaches to determine relative toxicities instead of absolute measures. Finally, authors discuss how to avoid pitfalls by distinguishing between unspecific forms of cytotoxicity and specific developmental neurotoxicity. A compilation of compound lists corresponding to the above-discussed principles supplement this review.	**Kadereit S, Zimmer B, van Thriel C, Hengstler JG, Leist M (2012)** Compound selection for *in vitro* modeling of developmental neurotoxicity. Frontiers in Bioscience 17: 2442-60

Aflatoxin B1	***In ovo***	*In ovo* exposure of RIR eggs. The concentrations of 5ng/5µl 20%alcohol/egg (Low dose, LD) and 10ng/5µl 20%alcohol/egg (High dose, HD) of AFB1 in treatment group and 5µl 20% alcohol/egg of alcohol were injected in vehicle control group in air sac of eggs at '0' day of incubation. Parameters: Estimation of total protein; Estimation of glucose; Analysis of neurotransmitters (Acetylcholine esterase activity (AChE)); Glutamate; Tryptophan; Monoamine Oxidase A (MAO-A) and B (MAO-B):	**Results**: Present study has identified developmental neurotoxicity of 5ng and 10ng aflatoxin B1 on day '0' *in ovo* exposure of RIR eggs. AFB1 exposure to developing chick is associated with significant elevation of total protein and depletion of glucose in brain and serum. The activity of AChE, MAO-A and MAO-B were significantly decreased by in ovo AFB1 exposure. Moreover, AFB1 caused significant depletion of glutamate level where as tryptophan level was increased in AFB1 treatment groups. **Conclusion**: *In ovo* exposure to AFB1 may cause alterations in various neurotransmitters which may be resulted into brain deformities in developing embryo.	**Parmar H, Sharma P, Anerao I, Roy H (2016)**. Aflatoxin B1 induced developmental neurotoxicity in RIR egg. Advances in Applied Science Research 7 :23-7
Anesthetics	**Review**	Studies on rodents and subhuman primates suggest that prolonged exposure to general anesthetics may induce widespread neuronal cell death and neurological sequelae; seriously questioning the safety of pediatric anesthesia	**Summary:** This review presents recent developments in this rapidly emerging field. There is mounting and convincing preclinical evidence in rodents and nonhuman primates that anesthetics in common clinical use are neurotoxic to the developing brain in vitro and cause long-term neurobehavioral abnormalities in vivo. Prior to the publication of animal data and after the publication of animal data, there are several human cohort studies that demonstrate the association of poor neurodevelopmental outcome in neonates, who underwent major surgery during their neonatal period. This review summarizes our present understanding of some of the key components responsible for anesthesia-induced neuroapoptosis and offers some of neuroprotective strategies that could be beneficial as adjunct therapy in preventing anesthesia-induced death of developing neurons in the neonates. A randomized literature search was carried out using search words apoptosis, general anesthetics, and developing brain from 1979 to 2011 for effects of general anesthetics on developing brain in PUBMED and relevant published literature reviewed. **Conclusion**: The potentially alarming issue of anesthesia-induced neuronal damage in the immature brain is gathering a lot of interest among practicing anesthesiologists. By improving our understanding of the mechanism by which anesthesia induces neuronal damage in the immature brain, we can devise the more effective preventive strategies to use the existing anesthetic drugs to their full advantage, without the risk of neurotoxic side effects.	**Reddy SV (2012)** Effect of general anesthetics on the developing brain .J Anaesthesiol Clin Pharmacol.28:

Volatile anesthetic	**Review**	This review will provide an update on current data regarding volatile anesthetic induced neurotoxicity and neuroprotection in neonatal and infant populations. In addition, this paper will discuss ongoing studies and the trajectory of further research over the coming years.	**Summary:** The use of volatile anesthetics, a group of general anesthetics, is an exceedingly common practice. These anesthetics may have neuroprotective effects. Over the last decade, anesthetic induced neurotoxicity in pediatric populations has gained a certain notoriety based on pre-clinical cell and animal studies demonstrating that general anesthetics may induce neurotoxicity, including neuroapoptosis, neurodegeneration, and long-term neurocognitive and behavioral deficits. With hundreds of millions of people having surgery under general anesthesia worldwide, and roughly six million children annually in the U.S. alone, the importance of clearly defining toxic or protective effects of general anesthetics cannot be overstated. Yet, with our expanding body of knowledge, we have come to learn that perhaps not all volatile anesthetics have the same pharmacological profiles; certain ones may have a more favorable neurotoxic profile and may actually exhibit neuroprotection in specific populations and situations. Thus far, very few clinical studies exist, and have not yet been convincing enough to alter our practice. **Conclusion**: The neuroprotective and neurotoxic effects of volatile anesthetics in humans are not clear. No studies have been performed to determined volatile anesthetic induced neuroprotection in children. Clinical data thus far has remained too weak to either support or refute claims of anesthetic mediated neurotoxicity in children.	**Chiao S, Zuo Z (2014)** A Double-Edged Sword: Volatile Anesthetic Effects on the Neonatal Brain. Brain Sci. 4: 273-94

Anesthetics	**Review**	The purpose of this review is to summarize current knowledge of detailed biochemical evidence for the role of c-aminobutyric acid type A receptors (GABAA–Rs) in the mechanisms of general anesthesia.	**Results:** With the knowledge that all general anesthetics positively modulate GABAA-R-mediated inhibitory transmission, site-directed mutagenesis comparing sequences of GABAA-R subunits of varying sensitivity led to identification of amino acid residues in the transmembrane domain that are critical for the drug actions *in vitro*. Using a photo incorporable analogue of the general anesthetic, R(+)etomidate, authors identified two transmembrane amino acids that were affinity labelled in purified bovine brain GABAA-R. Homology protein structural modelling positions these two residues, aM1-11' and bM3-4', close to each other in a single type of intersubunit etomidate binding pocket at the b/a interface. This position would be appropriate for modulation of agonist channel gating. Overall, available information suggests that these two etomidate binding residues are allosterically coupled to sites of action of steroids, barbiturates, volatile agents,and propofol, but not alcohols. Residue a/bM2-15' is probably not a binding site but allosterically coupled to action of volatile agents, alcohols, and intravenous agents,and a/bM1-(-2') is coupled to action of intravenous agents. **Conclusions:** Establishment of a coherent and consistent structural model of the GABAA-R lends support to the conclusion that general anesthetics can modulate function by binding to appropriate domains on the protein. Genetic engineering of mice with mutation in some of these GABAA-R residues are insensitive to general anesthetics *in vivo*, suggesting that further analysis of these domains could lead to development of more potent and specific Volatiledrugs.	**Olsen RW, Li GD (2011)** GABAA receptors as molecular targets of general anesthetics: identification of binding sites provides clues to allosteric modulation. J Can Anesth 58:206–15
Dearomatized white spirit	***In vivo***	The purpose of the study was to investigate the potential developmental neurotoxicity of the widely used organic solvent, white spirit. Rats (Mol:WIST) were exposed to 0 or 800 ppm dearomatized white spirit for 6 hr per day on gestation days 7-20. Developmental and neurobehavioural effects in the offspring were investigated using a test battery including assessment of physical development, reflex ontogeny, motor function, motor activity and, learning and memory.	**Results:** No significant effects were recorded on motor function and the activity in Open Field. In the initial learning period (age 1 month), the performance in a Morris water maze was similar in exposed and control animals. When testing for memory at the age of 2 months, the exposed male offspring used more time to locate the hidden platform. After platform relocation, impaired cognitive function was revealed in the exposed females. At the age of 5 months, learning and memory deficits were observed in exposed offspring. The differences were not related to poorer swimming capabilities, because swim speeds were similar to control values **Conclusion**: The results show that prenatal exposure to 800 ppm white spirit caused long-lasting learning and memory deficits in rats.	**Hass U, Ladefoged O, Lam HR, Ostergaard G, Lund SP, Sinonsen L.(2001)** Behavioural effects in rats after prenatal exposure to dearomatized white spirit. Pharmacol Toxicol. 89:201-7.

Anesthetics	Review Comment	This review contains varied articles that all relate in some way to the possibility of neuronal toxicity arising from the use of inhaled and certain IV anesthetics in the immature and developing animal.	**Summary:** There are at least two possible general mechanisms of pro-apoptotic and/or neurotic injury. The first is that receptor blockade by anesthetic drugs decreases "trophic" stimulation at a critical point in time, leading to induction of cell death programs (analogous to the effects of growth factor deprivation in a variety of cell types). The second is that the transient receptor blockade caused by anesthetic drugs results in subsequent receptor upregulation (protein expression and/or receptor activity). When the blocking anesthetic is removed, the affected neuron is subsequently subjected to significantly increased NMDA activity, resulting in increased excitatory amino acid neurotoxicity. The mechanisms remain largely unsolved, although the available evidence weakly suggests the second. Regardless of the exact mechanisms, the available data raise the specter that standard clinical anesthesia practice could promote brain injury in the human fetus, infant, and young child. These reports taken together led the United States Food and Drug Administration (FDA) to convene an Advisory Committee Meeting in April of 2007 (meeting transcript available at http://www.fda.gov/ohrms/docets/ac/07/transcripts/2007/4285t1.pdf). :Increased research on mechanisms of anesthetic action are needed to discover drugs with greater specificity or mechanisms of action that do not involve -aminobutyric acid and NMDA receptors. A translational research effort using currently available methods in genomics and proteomics should be initiated. The goal would be to identify sensitive and specific biomarkers for neurocognitive injury in human neonates and infants. **Conclusion:** Anesthetic-induced developmental neurotoxicity must be framed within the appropriate context. Based on the current knowledge and lack of appropriate alternatives, there is no scientific basis to recommend changes in clinical practice.	**McGowan Jr FX, Davis PJ (2008)** Anesthetic-Related Neurotoxicity in the Developing Infant: Of Mice, Rats, Monkeys and, Possibly, Humans. Anesth Analg 106: 1599-602

Anesthetics	**Review**	Anesthesia kills neurons in the brain of infantile animals, including primates, and causes permanent and progressive neurocognitive decline. The anesthesia community and regulatory authorities alike are concerned that is also true in humans.	**Summary**: This review summarizes the current knowledge about the risks of pediatric anesthesia to long-term cognitive function. This review gives also an overview of some of the mechanisms that have been proposed for anesthesia-induced cognitive decline and discusses possible treatment options. Moreover, early results of comparative animal studies of anesthetic neurotoxicity are also discussed **Conclusion:** Knowledge of developmental anesthetic neurotoxicity is rapidly accumulating but clarity about the mechanisms or the significance of this phenomenon for human pediatric anesthesia is not emerging. A change in clinical anesthetic practice is unwarranted, based on the currently available human literature and should probably not be based on animal studies. Most importantly, a change in clinical practice requires a superior alternative to current practice, and no evidence guides us as to what this might be. More research is urgently needed to determine whether anesthesia impairs brain function in humans, what the specific deficit is, and how it can be prevented and/or treated. This will require both human trials and good translational animal models and mechanistic studies. The SmartTots initiative, a joint effort of the International Anesthesia Research Society and the Food and Drug Administration, through funding such research, may go a long way toward meeting this important goal**.**	**Stratmann G (2011)** Neurotoxicity of Anesthetic Drugs in the Developing Brain. Anesthesia and the Pediatric Brain 113: 1170-9

Anesthetic	**Review**	Some drugs used for sedation and anesthesia produce histopathologic central nervous system changes in juvenile animal models. These observations have raised concerns regarding the use of these drugs in pediatric patients. Authors summarized the findings in developing animals and describe the steps that the Food and Drug Administration (FDA) and others are taking to assess potential risks in pediatric patients.	**Results**: Numerous animal studies in rodents indicate that NMDA receptor antagonists,including ketamine, induce neurodegeneration in the developing brain. The effects of ketamine are dose dependent. The data suggest that limiting exposure limits the potential for neurodegeneration. There is also evidence that other general anesthetics, such as isoflurane, can induce neurodegeneration in rodent models, which may be exacerbated by concurrent administration of midazolam or nitrous oxide. There are very few studies that have examined the potential functional consequences of the neurodegeneration noted in the animal models. However, the studies that have been reported suggest subtle, but prolonged, behavioral changes in rodents. Although the doses and durations of ketamine exposure that resulted in neurodegeneration were slightly larger than those used in the clinical setting, those associated with isoflurane were not. There are insufficient human data to either support or refute the clinical applicability of these findings. **Conclusion**: Animal studies suggest that neurodegeneration, with possible cognitive sequelae, is a potential long-term risk of anesthetics in neonatal and young pediatric patients. The existing nonclinical data implicate not only NMDA-receptor antagonists, but also drugs that potentiate -aminobutyric acid signal transduction, as potentially neurotoxic to the developing brain. The potential for the combination of drugs that have activity at both receptor systems or that can induce more or less neurotoxicity is not clear; however, recent nonclinical data suggest that some combinations may be more neurotoxic than the individual components. The lack of information to date precludes the ability to designate any one anesthetic agent or regimen as safer than any other. Ongoing studies in juvenile animals should provide additional information regarding the risks.	**Mellon RD, Simone AF, Rappaport BA (2007)** Use of Anesthetic Agents in Neonates and Young Children. Pediatric Anesthesia 104: 509-20.

Anesthetics	**Review**	This review summarizes the remarkable developments which have occurred in general anaesthetic research over the past decade demonstrating that, rather than acting nonspecifically to disrupt lipid membranes, general anaesthetics target certain CNS proteins in a highly selective manner.	**Summary:** Early theories of general anaesthetic action focused on nonspecific disruption of neuronal cell membranes. Compelling evidence now demonstrates that certain general anaesthetics act in a highly specific manner upon central nervous system proteins. Key amino acids within the a subunit of GABAA receptors may contribute to an anaesthetic binding pocket for volatile anaesthetic agents. Genetically engineered mice harbouring single amino acid mutations at critical sites within GABAA receptor subunits lack certain components of i.v. anaesthetic activity *in vivo.* GABAA receptors containing specific subunits appear to mediate the sedative (b2 subtype) and anaesthetic (b3 subtype) activity of i.v. anaesthetics.	**Weir CJ (2006)** The molecular mechanisms of general anaesthesia: dissecting the GABAA receptor. Continuing Education in Anaesthesia, Critical Care & Pain. 6: 49-53

DNT	**Review**		**Summary:** Prospective studies are particularly relevant, since they permit the capture of time varying exposures and other relevant covariates for children's health. The short interval between many exposures and outcomes (e.g. in utero exposures and infant birth size) further supports the use of prospective studies. A draft OECD Test Guideline 426, Developmental Neurotoxicity Study, has been developed based on the United States guideline (OECD, 2003a). Developmental neurotoxicity studies are designed to develop data on the potential functional and morphological hazards for the nervous system arising in the offspring from exposure of the mother during pregnancy and lactation. The OECD draft test guideline is designed as a separate study, but the observations and measurements can also be incorporated into a twogeneration study. The neurological evaluation consists of assessment of reflex ontogeny, motor activity, motor and sensory function, and learning and memory; and evaluation of brain weights and neuropathology during postnatal development and adulthood. The behavioural testing includes assessment of the individual animal for a number of relevant behavioural functions, but none of the tests assesses two or more animals together. This means that some behavioural end-points of potential relevance (e.g. sexual behaviour, play behaviour, social interaction among animals, and aggression) are not assessed using the current test guidelines. Challenges remain regarding the impact of the environment on children's health during development. One such challenge is the need to identify critical windows, including those before, during, or shortly after conception, for the spectrum of health end-points relevant for child health. Recruitment of couples prior to first attempting pregnancy offers promise for identifying new critical windows and the ability to assess maternally, paternally, and parentally mediated effects on child health. Use of home fertility monitors, in light of our inability to measure conception, may help to time conception and, hence, exposures in relation to conception and gestation.	**Hass U** Methodologies to assess health outcomes in children 168-216.

DNT	**Review**	The need for developmental neurotoxicity testing has been recognized for decades and guidelines are available, as the USEPA guideline and the OECD draft TG 426. Regulatory testing of industrial chemicals for developmental neurotoxicity is required to some extent, especially for pesticides in the US. Until recently, however, developmental neurotoxicity testing of industrial chemicals has not been a clear regulatory requirement in EU, probably due to the lack of an accepted OECD TG. The revised EU Technical Guidance Document for Risk Assessment (EU-TGD) has now included the OECD draft TG 426 in the testing strategy for new and existing substances, and biocides. Hopefully, this will lead to an improved database for risk assessment of potential developmental neurotoxicants. However, the regulatory authorities and toxicologists will also be faced with the challenge that decisions have to be made concerning e.g. when testing should be requested, how testing should be performed, as well as evaluation of the results and the regulatory consequences. In this paper, these three issues are discussed based on the recommendations given in the EU-TGD.	**Conclusion:** The revised EU-TGD has now included the OECD draft TG 426 Developmental Neurotoxicity Study in the testing strategy for new and existing substances, and biocides. Hopefully, this will lead to an improved database for risk assessment of potential developmental neurotoxicants. A number of existing chemicals will probably meet the criteria for requiring developmental neurotoxicity testing, however, limitations in the data available for a number of chemicals indicates that the triggering schema may not be sufficient to elicit testing of all chemicals that may be developmental neurotoxicants. This causes concern as there is a regulatory need for identifying chemicals that may induce neurotoxicity during development. Choice of appropriate testing methods is crucial for the identification of developmental toxicants and the overall design of the study as well as the functional end points included in the OECD draft guideline are relevant and should ideally allow the identification of potential developmental neurotoxicants	**Hass U (2003)** Current status of developmental neurotoxicity: regulatory view. Toxicology Letters 140-141 :155-9

DNT			**Summary:** Developmental neurotoxicity constitutes effects occurring in the offspring primarily as a result of exposure of the mother during pregnancy and lactation. To exert their effect, these chemicals or their metabolites must pass the placenta and/or the blood–brain barrier. In experimental animals, exposure to neurotoxic chemicals during critical periods of brain development has induced permanent functional disturbances in the CNS. Although available data suggest that proper animal models exist, only few chemicals have been tested. Neurotoxicity testing is not required by national authorities for classification of chemicals. Epidemiological evidence is very limited, but severe irreversible effects have been observed in humans following in utero exposures to a few known developmental neurotoxicants. The large number of chemicals with a potential for developmental neurotoxicity in humans stresses the importance of generating basic kinetic data on these chemicals based on relevant experimental models. First of all, data are needed on their ability to pass the placenta and the developing blood–brain barrier, to accumulate, and to be metabolized in the placenta and/or the fetus. These kinetic data will be essential in establishing a scientifically based hazard evaluation and risk assessment.	**Andersen HR, Nielsen JB, Grandjean P (2000)** Toxicologic evidence of developmental neurotoxicity of environmental chemicals. Toxicology 144: 121-7

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ANNEX 6

A6.1 Information on developmental neurotoxicity derived from Annexes 4 and 5

A6.1.1 Information on developmental neurotoxicity, derived from Annex 4

Toluene:

Da-Silva et al, 1990:

Full-text article not online (too old).

Period of exposure: Pregnant rats and hamsters were exposed to toluene vapor (800 mg/m3) 6 h daily from gestation days 14 to 20, and 6 to 11, respectively.

When DNT effects measured: Full-text article not online (too old).

Neurobehavioral deficits:
Rats: T-maze: shorter latencies to choose one side of a T maze in a spontaneous alternation test (males).
Hamsters: Rotarod: worse performance in a rotating rod test.

Trichloroethylene:

Evangelista de Duffard, 1996:

No DNT for this substance, written about DNT for other substances.

EPA, 1998:

Full-text article not yet found.

Period of exposure: Rats, 1,1,1-TCE (trichloroethylene), (75, 250, 750 mg/kg/day), oral gavage, from GD 6 to GD 10.

When DNT effects measured: Full-text article not yet found.

Neurodegeneration: No effects of neuropathological parameters.

Neurobehavioral deficits: No effects of neurobehavioral parameters.

Blossom et al, 2016:

Period of exposure: Prenatal exposure, from pregnancy until birth.

When DNT effects measured: 6 week old male offspring of dams … were evaluated.

Neurodegeneration:
b) Alters brain redox homeostasis in the cerebellum (reduced concentration of GSH, GSSG, the glutathione redox (GSH/GSSG) ratio, and the percentage of oxidized glutathione equivalents in the cerebellum). c) Alters transsulfuration and transmethylation metabolites in plasma.
d) Positive association between oxidative endpoint GSH in plasma and cerebellum
e) Nitrotyrosine increased in cerebellum and plasma.
f) increased plasma inflammatory biomarkers.
g) increases population of memory CD4+ T cells in adult mice.
h) Prenatal TCE exposure alters CD4+ T cell cytokine production

Neurobehavioral deficits:
a) Increased locomotor activity at 0.01 and 0.1 level.

n-butanol:

Sitarek et al, 1994:

Full-text article not online (too old).

Period of exposure: Rats exposed to n- butanol via oral (drinking water) at 0.24, 0.8 and 4% (0.3; 1.0 and 5.0 g/kg/day) for 8 weeks before and during gestation.

When DNT effects measured: during the fertility of female rats and their foetal development.

Neurodegeneration:
Internal hydrocephalus was the most frequent anomaly found in the foetuses of female rats exposed to n-butanol at mid and high dose level.
Pathological changes in the CNS includes (dilation of subarachnoid space, cerebral ventricles,) at all dose levels.
No detectable toxic effect in the parental females. But produced congenital defects including the CNS

of their offspring.

Isopropanol:

Bates et al, 1994:

Period of exposure: Isopropanol at 200, 700, 1200 mg/kg/day via oral gavage from GD 6 to PND 21.

When DNT effects measured: Weaned pups were assessed for day of testes descent or vaginal opening and for motor activity on PNDs 13, 17, 21, 47, and 58; auditory startle on PNDs 22 and 60; and active avoidance on PNDs 60-64. These pups were euthanized and examined on PND 68.
Pups were perfused *in situ* on PND 22 and PND 68 and tissues from the central and peripheral nervous systems were examined for possible histopathologic lesions.

Neurodegeneration: Neuropathological parameters were measured.

Neurobehavioral deficits: Neurobehavioral parameters were measured.

The author concluded that "no evidence of developmental neurotoxicity
associated with isopropanol exposure as high as 1200 mg/kg/day".

n-propanol:

Candura et al, 1991:

Full-text article not online (too old).

Period of exposure: 90 min of incubation, among others.

When DNT effects measured: after 90 min of incubation, among others.

Other neurotransmitters or receptors: These results suggest that muscarinic receptor-coupled phosphoinositide metabolism might be a common neurochemical target for the developmental neurotoxicity of short chain aliphatic alcohols.

n-hexane:

Cheng et al 2015, refers to Cheng et al 2012:

Period of exposure: Whole embryo: The chick embryos were exposed to different concentrations of 2,5-HD or PBS (control) at Hamburger and Hamilton (HH) stage 10 (Hamburger and Hamilton, 1992). Assays with cultures: In well plates, treatment with 2,5-HD lasted 24h.

When DNT effects measured: Whole embryo: After treatment, the embryos were incubated for a further 10 h or 4 days and then harvested for analysis.
Assays with cultures: Dissociated cortical neurons were prepared from 11-day (E11) chick embryos.

Apoptosis: MTT cell viability and MMP assays demonstrated that exposure to 2,5-HD dramatically reduced neuron viability and enhanced apoptosis.

Neurodegeneration: We established that in the presence of 2,5-HD, the dorsal neural tubes were malformed during the closure of the neural folds. In addition, exposure to 2,5-HD could also inhibit neural differentiation as revealed by immunofluorescent staining for neurofilament (NF). We also demonstrated that the impaired neurodevelopment was attributed to negative effect of 2,5-HD on neurite development and positive effect on apoptosis in developing neurons. Specifically, we found 2,5-HD treatment resulted in fewer neurons and the neurites projecting from the neurons were significantly shorten when compared with control cultures. In addition, MTT and mitochondrial membrane potential (MMP) assays revealed neuron cell viability was reduced by exposure to 2,5-HD in a dose-dependent fashion. In sum, our results suggest that chronic exposure to 2,5-HD is harmful to the developing embryo, especially in the context of neurodevelopment.

1-bromopropane:

Fueta et al, 2015:

Period of exposure: Seven dams were exposed to 1-BP vapor at a concentration of 700 ppm (6 h/day) for 20 days from GDs 1 to 20 in an exposure chamber, whereas the other seven dams were provided fresh air in the same type of chamber.

When DNT effects measured: PBS or KA was intraperitoneally injected to the F1 rats at PND 14, after which the F1 rats were placed in a clear plastic cage, and the scratching and WDS were observed by video-recording for 180 min in a room for the behavioral observation.

Neurotransmitters/receptors: In our previous study of the developmental neurotoxicity of 1-BP, prenatal exposure to 1-BP altered hippocampal excitability and the gene expression of the Na+

channel and glutamate receptor subunits on postnatal day (PND) 14.
In article there is theorizing about the mechanisms.

Neurodegeneration: Our results indicate that prenatal 1-BP exposure may disturb the susceptibility to KA (kainate) or the functions of neural networks related to the WDS (wet dog shakes).

Neurobehavioral deficits: In conclusion, we demonstrate here that prenatal exposure to 1-BP suppresses WDS induced by the administration of a low dose of KA.

A6.1.2 Information on developmental neurotoxicity, derived from Annex 5

Toluene:

Hass et al, 1999:

Period of exposure: Pregnant rats (Wistar); Inhalation: 0 (n=7) or 4,500 mg toluene/m3 (n=13) (0 or 1,200 ppm); 6 h/day; gestation day 7 to PND 18.

When DNT effects measured: See table 1 of this article:
Physical development: day 3, 10, 14, 31-pos.
Reflex ontogeny: days 2-3, 13-14, 15-16
Motor function: days 22-24
Motor activity: days 28, 29
Learning and memory: 9 weeks, 13 weeks
Hearing function: 4 months

Neurobehavioral deficits:
Delayed ontogeny of reflexes, and increased motor activity in the open field was registered in the exposed offspring. Impaired cognitive function was revealed in the exposed female offspring at the age of 3.5 months, i.e., they used more time to locate the hidden platform in the Morris water maze after platform relocation.

Hougaard et al, 1999:

Period of exposure: Development and neurobehavioral effects of prenatal exposure to toluene were studied after exposing pregnant rats (Mol:WIST) to 1800 ppm of the solvent for 6 h daily on days 7–20 of gestation.

When DNT effects measured: See table 1 of this article:
Mother–pup interaction: PNDs 3,6,9
Physical development: PND 2-pos., 10-pos., 13-pos., 31-pos.
Reflex development: PND 2-pos., 12-pos., 15-pos.
Motor function: PND 22–24
Habituation and prepulse inhibition: PND 23±1, PND64±1
Motor activity: PNDs 28 and 29
Learning and memory: PNDs 42–46, PNDs 70–71, PNDs 72–74
Sensory function: 3 Months

Neurobehavioral deficits:
Neurobehavioral evaluation of the pups revealed no effects on motor function (rotarod), activity level (open field), acoustic startle, and prepulse inhibition. Measurements of hearing function using auditory brain stem response revealed small effects in male-exposed offspring. Performance in a Morris water maze during initial learning gave some indications of impaired cognitive functions,

which was confirmed during further testing, especially in reversal and new learning. Effects on cognitive functions seemed most marked in female offspring.

Edelfors et al, 2002:

Period of exposure: The present study was undertaken in order to investigate if toluene induced oxidative stress in brains from rats exposed prenatally to 1800 ppm toluene 6 hr/day at days 7-20 during the pregnancy.
When DNT effects measured: 35-42 days after birth the rats were killed and synaptosomal fractions were prepared for the experiments.

Apoptosis: Apoptosis is related to oxidative stress, membrane fluidity, membrane leakage of calcium. Synaptosomes from rats exposed prenatally to toluene exhibited an increased level of oxidative stress when incubated with toluene in vitro compared to synaptosomes from unexposed offspring. Also the cell membrane was affected, as the calcium leakage was more increased from exposed synaptosomes than from unexposed. The membrane fluidity increased significantly when synaptosomes were incubated with toluene for 10 min. in vitro but the change in fluidity was identical in both groups of offspring. The results indicate that prenatal exposure to toluene induces long-lasting changes in oxidative status and membrane function.

Neurodegeneration:
In this part of the present study, the brains of the pups from toluene-exposed dams were smaller than the brains from the control pups. In contrast, the ratio of synaptosome protein to brain weight was identical in the two groups of animals indicating that the brain tissue has developed normally but slower.
The results indicate that prenatal exposure to toluene induces long-lasting changes in oxidative status and membrane function.

Callan et al, 2015:

Period of exposure: Pregnant Sprague-Dawley rats were exposed to 0, 8000 or 12,000ppm (ppm) of toluene for 15min twice daily from gestation day 8 (GD8) through GD20.

When DNT effects measured: Male and female offspring (N=104) were observed in the MWM for 5days beginning on postnatal day (PN) 28 and again on PN44.

Neurobehavioral deficits:
While prenatal toluene-exposed animals did not differ in initial acquisition in the Morris Water Maze (MWM), rats prenatally exposed to 12,000ppm toluene displayed performance deficits during a probe trial and in reversal learning on PN44. Overall, this study indicates that prenatal exposure to repeated inhaled abuse patterns of high concentrations of toluene can impair spatial memory function that persists into adolescence.

Ladefoged et al, 2004:

Period of exposure: Pregnant Wistar rats were exposed to 1500 ppm toluene 6 hr/day from gestational day 7-20 or to chronical mild stress from gestational day 9-20 as single exposure or in combination.

When DNT effects measured: The number of apoptotic cells in cerebellum postnatal day 22, 24, and 27 and in hippocampus (postnatal day 22, 24, and 27) were counted after visualization by the TUNEL staining or measured by DNA-laddering technique. Caspase-3 activity was determined in cerebellum (postnatal day 6, 22, 24, and 27) and in hippocampus (postnatal day 6 and 22).

Apoptosis:
TUNEL staining and DNA-laddering technique showed a marked decrease in number of apoptotic cells from postnatal day 22 to 27 in both cerebellum and hippocampus. Apparently, a peak in the number of TUNEL positive cells was identified in cerebellum at postnatal day 22. There was no statistically significant influence of exposure except that DNA-laddering in cerebellum at postnatal day 27 was increased by toluene exposure. Caspase-3 activity decreased in cerebellum and hippocampus with age. At postnatal day 6 stress and toluene, when singly exposed, increased activity in cerebellum whereas co-exposure to stress and toluene did not. Stress increased caspase-3 activity in hippocampus postnatal day 22. There was overall consistency between the results obtained by the three supplementary methods regarding the influence of exposure and age on apoptotic activity in cerebellum and hippocampus.

Neurodegeneration:
The absolute brain weight of animals used for the histopathological and molecular biological investigations was statistically significantly lower in offspring of dams exposed to both toluene and stress (P=0.023) compared to controls (table 2), whereas the relative brain weight (brain weight in percent of animal weight) was statistically significantly higher (P=0.014) (table 2). This may indicate affected brain development.

Neurobehavioral deficits:
In summary, no exposure-related effects on learning and memory were registered in the female offspring.

Dalgaard et al, 2001:

Period of exposure: Pregnant rats were exposed to 1800 ppm from GD 7 to GD 20.

When DNT effects measured: The male offspring were killed at PND 11, 21 or 90.

Apoptosis:
In the hippocampus, almost no apoptosis was observed in any age group, and there were no differences in apoptotic neurodegeneration between male rats exposed to 1800 ppm and control animals at PND 11, 21 or 90. Generally, a marked increase in number of apoptotic cells was observed in cerebellar granule cells at PND 21 compared with the other age groups. Toluene induced a

statistically significant increase in the number of apoptotic cells in the cerebellar granule layer at PND 21. The mean was increased from 37 in the control group to 71 in the toluene-exposed group. Thus, the granular cell layer in cerebellum is a highly relevant tissue with which to study toluene-induced apoptosis, because of the continuous migration of neurons and high frequency of neuronal apoptosis during the weaning period.
Prenatal exposure to 1800 ppm toluene did increase neuronal apoptosis in the cerebellum of weaned male rats, possibly by delaying postnatal migration of granule cells to their final destination, or by toluene-induced retardation of generalised fetal growth.

Neurodegeneration:
The brain weight was not significantly decreased in male rats prenatally exposed to 1800 ppm toluene.

Bowen and Hannigan, 2013:

Period of exposure: In this study, timed-pregnant Sprague Dawley rats were exposed from Gestation Day (GD) 8 to GD20 to 12,000 or 8000 parts per million (ppm) toluene, or 0ppm (controls) for 30min twice daily, 60min total daily exposure.

When DNT effects measured: Pups were assessed from postnatal day (PN) 4 to PN21 using a developmental battery measuring growth (i.e., body weight), maturational milestones (e.g., eye opening & incisor eruption), and biobehavioral development (e.g., negative geotaxis & surface righting).

Neurobehavioral deficits:
Prenatal treatment effected grip strength. No significant role of prenatal treatment for other behavioral assessments.

Ladefoged et al, 1991:

DNT not measured.

Khattak et al, 1990:

Not in Pubmed.

Period of exposure: Pregnant rats and hamsters; 800 mg/m3; 6 h/day; GD 14 to 20 (rats) and GD 6 to 11 (hamsters).

Neurobehavioral deficits:
Rats: T-maze (Exploratory behavior): shorter latencies to choose one side of a T maze in a spontaneous alternation test (male offspring).
Hamsters: Rotarod: worsened performance in a rotating rod test.

Korbo et al, 1996:

Full-text article not online (too old).

DNT probably not measured.

Burry et al, 2003:

Period of exposure: The present study examines the effects of toluene administration (250, 500 and 750 mg/kg) in neonatal rats from postnatal day 4 to 10.
Cell cultures: Cells were treated with toluene…for 24 h.

When DNT effects measured:
On PND 11, pups were sacrificed by decapitation and body and whole brain weights recorded.
Animals treated with 750 mg/kg toluene from PND 4 to 10 were sacrificed 2 h after the last dose.
Cell cultures: not clear.

Apoptosis:
This treatment resulted in a significant reduction of levels of glial fibrillary acidic protein, but not of neuron-specific enolase in rat brain. In vitro experiments demonstrate that pharmacologically relevant concentrations of toluene (250-1,000 microM) significantly inhibit proliferation of rat cortical astrocytes without causing overt cytotoxicity.

Neurodegeneration:
This treatment resulted in a significant decrease in both brain and body weights.
These results indicate that toluene does not cause selective microencephaly; however, it affects brain weight, and appears to target developing astrocytes, possibly by inhibiting their proliferation.

C7 and C9 aliphatic (nheptane, nnonane), naphthenic (methylcyclohexane, 1,2,4-trimethylcyclohexane (TMCH)) and aromatic (toluene, 1,2,4-trimethylbenzene (TMB)) hydrocarbons:

Myhre and Fonnum, 2001:

DNT not measured.

Volatile solvents (toluene):

Bowen et al, 2006:

Is a review.

Period of exposure:
Clinical cases: Very high levels of maternal solvent exposure typical of abuse.
Gospe and Zhou 1998: Exposed pregnant rats to a single daily dose of toluene by gavage (650 mg/kg) from gestation day (GD) 6 through GD19.
Thiel and Chahoud 1997: Exposed pregnant Wistar rats to toluene (300 ppm to 1200 ppm for 6 h/day) from GD9 through GD21.
Hougaard and coworkers, in a series of papers: Exposed Wistar rats to 1200 or 1500 ppm toluene for 6 h/ day from GD7 to GD18 or GD20.
Jones and Balster 1997: Approximated an abuse pattern in pregnant mice with inhalations up to 2000 ppm of toluene limited to three 60-min periods per day for a 5-day period within the human equivalent of mid second trimester (GD12 to GD17).
A recent study of Bowen: We exposed pregnant rats to repeated 15-min bouts of very high toluene concentrations (8000 ppm or 12,000 ppm) twice a day from GD8 through GD20 to mimic solvent abuse during pregnancy.
Recent studies: Early postnatal stages when synaptogenesis occurs.

When DNT effects measured:
Clinical cases: At birth.

Neurotransmitters/receptors:
Recent studies: Found that this solvent alters several responses related
to the glutamatergic system.

Neurodegeneration:
Clinical cases: The affected infants were typically premature and/or growth retarded and microcephalic with severe facial dysmorphology (e.g., deep-set eyes, small face, low set ears, micrognathia), and spatulate fingertips and small fingernails. Fetal solvent syndrome.
Gospe and Zhou 1998: Found age-dependent reductions in brain development. Young offspring had 15% reductions in forebrain cell numbers which were normalized by weaning. Using a slightly longer exposure schedule, the same group reported that brains from toluene-exposed pups had a lower amount of neurons in the cortical layer.

Neurobehavioral deficits:
Thiel and Chahoud 1997: Reported that even the highest concentration of toluene did not significantly affect behavior.
Hougaard and coworkers, in a series of papers: found lower pup birth weight, deficits in surface righting at PN3 and in auditory startle at PN13, and significant impairment in acquisition and

retention in the Morris-water maze. This effect was more pronounced in male than in female pups. However, there were inconsistent effects on open field activity and no effects on rotarod performance or pre-pulse inhibition of startle.
Jones and Balster 1997: There were small, transient toluene-induced reductions in pup weight, surface righting, inversion righting, and grip strength.
A recent study of Bowen: This treatment significantly increased the rate of negative postnatal outcome including altered negative geotaxis behavior. These deficits in growth were more persistent after our repeated 15-min binge exposures to high concentrations than were seen with much longer exposures (6 h) to lower concentrations (1800 ppm) in other studies even though the apparent total daily exposures were fairly equivalent.

Toluene:

Cruz et al, 2014:

Is a review.

Period of exposure:
Table 2 of this article: Prenatal exposure.
Clinical: During pregnancy.
Follow-up studies of children: Exposed during gestation to solvents.
Preclinical: Prenatal exposure to toluene.
Of particular interest in understanding the long-term effects of inhalant use: Exposure to these agents during adolescence.

Neurobehavioral deficits:
Table 2 of this article: In open field test, increased locomotor activity. In postnatal test battery (surface righting, air righting, auditory startle), delayed reflexes. In waiting-for-reward task, impulsivity-like. In open field test (amphetamine induced locomotion), sensitization to hyperlocomotion.
Clinical: The fetus can be affected with developmental disorders, physical malformations or even death. A fetal solvent syndrome (FSS), analogous to the fetal alcohol spectrum disorder (FASD), has been described. Thus, infants born from mothers who misused toluene-based products can have smaller heads, a thin upper lip, lower set ears, and other signs similar to what has been described for FASD.
Follow-up studies of children: Show growth retardation, language impairment, and cerebellar dysfunction.
Preclinical: As in humans, has been associated with malformations, growth retardation, delayed reflexes, and attention deficit in pups.
Of particular interest in understanding the long-term effects of inhalant use: How this impacts normal brain development, cognition, and behavior in adults. This is especially relevant for frontal

cortical areas that undergo significant maturation during the time that many solvent users are experimenting with these agents.

Filley et al, 2004:

In this review, fetal solvent syndrome is briefly discussed.

Period of exposure:
Gospe and Zhou 1998: Prenatal toluene exposure.

Neurodegeneration:
Gospe and Zhou 1998: Permanent reduction in forebrain myelination.

Alcohol, toluene:

Costa et al, 2002:

Is a review.

Neurodegeneration:
The similarities of clinical features have led to the suggestion that fetal alcohol syndrome (FAS), (maternal phenylketonuria (mPKU)) and fetal solvent syndrome (FSS) may share a common pathogenesis. Yet, what these common mechanisms could be remains elusive.
Alcohol, and its metabolites, as well as toluene, freely cross the placenta, suggesting a direct toxic effect on the developing fetus. With regard to neurons, there is ample evidence that ethanol causes loss of neurons upon in vivo exposure, as well as apoptotic neuronal death in vitro. A loss of neurons has been reported also upon developmental exposure to toluene, however, no information on the direct toxicity of toluene on neuronal cells is available.
Substantial in vivo and in vitro evidence indicates that ethanol can also affect developing astrocytes. In particular, ethanol can inhibit the proliferation of glial cells, an effect that would lead to a decreased number of astrocytes and contribute to the observed microencephaly. Though no information is available on astroglial cell loss upon developmental toluene exposure, limited in vitro studies suggest that toluene may inhibit proliferation of astrocytes. Such effect may on one hand contribute to the ensuing microencephaly. Furthermore, an effect on glial cells may in turn affect the development of neurons, given the essential role of astrocytes in fostering the development and survival of neurons.
Though the hypothesis of a central role for glial cells in the developmental neurotoxicity of FAS, (mPKU) and FSS remains speculative at this time, due mostly to the limited information available for the two latter syndrome, it may offer a working hypothesis to design further studies on possible common modes of action.

Toluene:

EPA - Health Effects Notebook for Hazardous Air Pollutants – Toluene, 2012:

Period of exposure: Pregnant women.

Neurobehavioral deficits:
CNS dysfunction, attention deficits, minor craniofacial and limb anomalies, and developmental delay were observed in the children of pregnant women exposed to toluene or to mixed solvents during solvent abuse. Growth retardation and dysmorphism were reported in infants of another study. However, these studies were confounded by exposure to multiple chemicals.

Industrial chemicals / developmental neurotoxicants:

Grandjean and Landrigan, 2014:

Is a review.

Many examples of DNT chemicals associated with neurodegeneration or neurobehavioral deficits are discussed in this article. Only organic solvents are discussed here.

Neurobehavioral deficits:
In US children 0–5 years of age, IQ points may be lost to an unknown extent, by exposure to organic solvents.
Maternal consumption of alcohol during pregnancy, even in very small quantities, has been linked to a range of neurobehavioural adverse effects in offspring, including reduced IQ, impaired executive function and social judgment, delinquent behaviour, seizures, other neurological signs, and sensory problems.
The occupational health literature suggests that solvents can act as neurotoxicants, but the identification of individual responsible compounds is hampered by the complexity of exposures. In a French cohort study of 3000 children, investigators linked maternal occupational solvent exposure during pregnancy to deficits in behavioural assessment at 2 years of age. The data showed dose-related increased risks for hyperactivity and aggressive behaviour. One in every five mothers in this cohort reported solvent exposures in common jobs, such as nurse or other hospital employee, chemist, cleaner, hairdresser, and beautician. In Massachusetts, USA, follow-up of a well-defined population with prenatal and early childhood exposure to the solvent tetrachloroethylene (also

called perchlor ethylene) in drinking water showed a tendency towards deficient neurological function and increased risk of psychiatric diagnoses.
Experimental studies have reported Parkinson's disease as a result of developmental exposures to the solvent trichloroethylene.

Grandjean and Landrigan, 2006:

Is a review.

Neurobehavioral deficits:
Fetal alcohol syndrome is qualitatively different from the syndrome in adults. It was originally described in infants of mothers with a serious drinking habit, and involves cognitive and behavioural deficits and changes in facial features. Permanent neurotoxic damage in the mother is not a prerequisite for irreversible effects in the child. At low consumption, subtle but permanent neurotoxicity, including decreased IQ scores, has been seen.
Less reliable documentation is available for other solvents widely used in industry. Because of its anaesthetic effects, toluene has been abused by sniffing, and case reports have reported that infants of mothers who sniffed toluene in pregnancy had abnormally low scores on developmental tests and showed delayed development of speech and motor function. Additional evidence of cognitive deficits in children comes from small studies of mothers who reported occupational exposure to solvents, including toluene, during pregnancy. The women were apparently exposed within permissible workplace limits aimed at prevention of neurotoxicity in the workers themselves.

Xylene (CAS-no 1330-20-7):

Hass et al, 1995:

Period of exposure: Pregnant rats (Mol:WIST) were exposed to 500 ppm technical xylene 6 h per day on gestation days 7-20. The dose level was selected so as not to induce maternal toxicity or decrease the viability of offspring.

When DNT effects measured: See table 1 of this article:
Preweaning
Ear unfolding PNDs 2 to positive
Surface righting PNDs 2 to positive
Homing response PNDs 6 and 7
Incisor eruption PNDs 10 to positive
Auditory startle PNDs 12 to positive
Eye opening PNDs 13 to positive

Air righting PNDs 15 to positive
Postweaning
Rotarod PNDs 24-26
Open field PNDs 27 and PND34(±2)
Sexual maturation PNDs 30 to positive
Morris water maze 3-4 months

Examined for macroscopical changes on PND 28±2.

Neurobehavioral deficits and neurodegeneration:
In the exposed offspring, a delay in the ontogeny of the air righting reflex, a lower absolute brain weight, and impaired performance in behavioral tests for neuromotor abilities (Rotarod) and for learning and memory (Morris water maze) were found. Generally, the effects were most marked in the female offspring.

Hass et al, 1997:

Full-text article not online.

Period of exposure:
The persistence of neurobehavioral effects in female rats (Mol:WIST) exposed to 500 ppm technical xylene (dimethylbenzene, CAS-no 1330-20-7) for 6 hours per day on days 7-20 of prenatal development was studied. The dose level was selected so as not to induce maternal toxicity or decreased viability of offspring.

Neurobehavioral deficits:
Investigations of learning and memory abilities were performed using a Morris water maze. This task requires rats to spatially navigate, using distal extramaze cues to locate a small platform under the surface of the water in a large pool. At the age of 16 weeks, the exposed offspring showed impairments when the platform was relocated in the pool. Impaired performances after platform relocation were also observed in exposed offspring at 28 and 55 weeks of age, although the difference was not statistically significant at 55 weeks. These data could indicate that the effect was partly reversible, although over a long time period. However, another explanation could be that the animals became more practised at solving the problem (finding the platform) as continued testing occurred and therefore were able to compensate for the neurotoxic effect of the prenatal xylene exposure. Further studies are planned to investigate whether neurobehavioral effects resulting from prenatal xylene exposure can interact with neurophysiological aging processes.

Isopropanol (200-661-7):

Bates et al, 1994:

Period of exposure: Isopropanol at 200, 700, 1200 mg/kg/day via oral gavage from GD 6 to PND 21.

When DNT effects measured: Weaned pups were assessed for day of testes descent or vaginal opening and for motor activity on PNDs 13, 17, 21, 47, and 58; auditory startle on PNDs 22 and 60; and active avoidance on PNDs 60-64. These pups were euthanized and examined on PND 68.
Pups were perfused in situ on PND 22 and PND 68 and tissues from the central and peripheral nervous systems were examined for possible histopathologic lesions.

Neurodegeneration: Neuropathological parameters were measured.

Neurobehavioral deficits: Neurobehavioral parameters were measured.

The author concluded that "no evidence of developmental neurotoxicity associated with isopropanol exposure as high as 1200 mg/kg/day".

n-propanol (200-746-9):

Candura et al, 1991:

Full-text article not online (too old).

Period of exposure: 90 min of incubation, among others.

When DNT effects measured: After 90 min of incubation, among others.

Other neurotransmitters or receptors: These results suggest that muscarinic receptor-coupled phosphoinositide metabolism might be a common neurochemical target for the developmental neurotoxicity of short chain aliphatic alcohols.

1-bromopropane (203-445-0):

Fueta et al, 2015:

Period of exposure: Seven dams were exposed to 1-BP vapor at a concentration of 700 ppm (6 h/day) for 20 days from GDs 1 to 20 in an exposure chamber, whereas the other seven dams were provided

fresh air in the same type of chamber.

When DNT effects measured: PBS or KA was intraperitoneally injected to the F1 rats at PND 14, after which the F1 rats were placed in a clear plastic cage, and the scratching and WDS were observed by video-recording for 180 min in a room for the behavioral observation.

Neurotransmitters/receptors: In our previous study of the developmental neurotoxicity of 1-BP, prenatal exposure to 1-BP altered hippocampal excitability and the gene expression of the Na+ channel and glutamate receptor subunits on postnatal day (PND) 14.
In article there is theorizing about the mechanisms.

Neurodegeneration: Our results indicate that prenatal 1-BP exposure may disturb the susceptibility to KA (kainate) or the functions of neural networks related to the WDS (wet dog shakes).

Neurobehavioral deficits: In conclusion, we demonstrate here that prenatal exposure to 1-BP suppresses WDS induced by the administration of a low dose of KA.

Trichloroethylene (201-167-4):

EPA, 1998:

Full-text article not found.

Period of exposure: Rats, 1,1,1-TCE (trichloroethylene), (75, 250, 750 mg/kg/day), oral gavage, from GD 6 to GD 10.

When DNT effects measured: Full-text article not yet found.

Neurodegeneration: No effects of neuropathological parameters.

Neurobehavioral deficits: No effects of neurobehavioral parameters.

Blossom et al, 2016:

Period of exposure: Prenatal exposure, from pregnancy until birth.

When DNT effects measured: 6 week old male offspring of dams … were evaluated.

Neurodegeneration:
b) Alters brain redox homeostasis in the cerebellum (reduced concentration of GSH, GSSG,

the glutathione redox (GSH/GSSG) ratio, and the percentage of oxidized glutathione equivalents in the cerebellum). c) Alters transsulfuration and transmethylation metabolites in plasma.
d) Positive association between oxidative endpoint GSH in plasma and cerebellum
e) Nitrotyrosine increased in cerebellum and plasma.
f) increased plasma inflammatory biomarkers.
g) increases population of memory CD4+ T cells in adult mice.
h) Prenatal TCE exposure alters CD4+ T cell cytokine production

Neurobehavioral deficits:
a) Increased locomotor activity at 0.01 and 0.1 level.

Evangelista de Duffard, 1996:

No DNT for this substance, written about DNT for other substances.

n-butanol:

Sitarek et al, 1994:

Full-text article not online (too old).

Period of exposure: Rats exposed to n-butanol via oral (drinking water) at 0.24, 0.8 and 4% (0.3; 1.0 and 5.0 g/kg/day) for 8 weeks before and during gestation.

When DNT effects measured: during the fertility of female rats and their foetal development.

Neurodegeneration:
Internal hydrocephalus was the most frequent anomaly found in the foetuses of female rats exposed to n-butanol at mid and high dose level.
Pathological changes in the CNS includes (dilation of subarachnoid space, cerebral ventricles,) at all dose levels.
No detectable toxic effect in the parental females. But produced congenital defects including the CNS of their offspring.

n-hexane:

Cheng et al 2015, refers to Cheng et al 2012:

Period of exposure: Whole embryo: The chick embryos were exposed to different concentrations of 2,5-HD or PBS (control) at Hamburger and Hamilton (HH) stage 10 (Hamburger and Hamilton, 1992).
Assays with cultures: In well plates, treatment with 2,5-HD lasted 24h.

When DNT effects measured: Whole embryo: After treatment, the embryos were incubated for a further 10 h or 4 days and then harvested for analysis.
Assays with cultures: Dissociated cortical neurons were prepared from 11-day (E11) chick embryos.

Apoptosis: MTT cell viability and MMP assays demonstrated that exposure to 2,5-HD dramatically reduced neuron viability and enhanced apoptosis.

Neurodegeneration: We established that in the presence of 2,5-HD, the dorsal neural tubes were malformed during the closure of the neural folds. In addition, exposure to 2,5-HD could also inhibit neural differentiation as revealed by immunofluorescent staining for neurofilament (NF). We also demonstrated that the impaired neurodevelopment was attributed to negative effect of 2,5-HD on neurite development and positive effect on apoptosis in developing neurons. Specifically, we found 2,5-HD treatment resulted in fewer neurons and the neurites projecting from the neurons were significantly shorten when compared with control cultures. In addition, MTT and mitochondrial membrane potential (MMP) assays revealed neuron cell viability was reduced by exposure to 2,5-HD in a dose-dependent fashion. In sum, our results suggest that chronic exposure to 2,5-HD is harmful to the developing embryo, especially in the context of neurodevelopment.

Methyl ethyl ketone (MEK):

Mentioned review comment “methyl ethyl ketone, fetal neurotoxicity, microcephaly and mental retardation: in utero brain damage caused by toxic solvent exposure” not found in scientific literature.

Methyl iso-butyl ketone (MiBK):

David et al, 1999:

Full-text article not found.

In this article, DNT is not measured.

Nemec et al, 2004:

Period of exposure:
MIBK was administered to 30 Sprague-Dawley rats/sex/group via whole-body inhalation at concentrations of 0, 500, 1000, or 2000 ppm, 6 h daily, for 70 days prior to mating. F(0) and F(1) females were exposed from mating through gestation day 20 and from postnatal day 5; F(2) litters were maintained through postnatal day 21.

Neurobehavioral deficits:
There was a dose-related increase in adult animals with no or a decreased response to a sound stimulus at 1000 and 2000 ppm; however, no adverse clinical signs occurred 1 h after exposure, suggesting this was a transient sedative effect. Clinical signs of central nervous system (CNS) depression in the pups were observed and one F(1) pup died after initial exposure to 2000 ppm on postnatal day 22; subsequently exposure was delayed until postnatal day 28. Decreased body weight gain and slight decreased food consumption were observed during the first 2 weeks of exposure in both generations at 2000 ppm.
Other than acute sedative effects, the no-observed-adverse-effect level (NOAEL) for parental systemic effects (excluding male rat kidney) was 1000 ppm, based on transient decreased body weight and food consumption; for reproductive effects, 2000 ppm, the highest concentration tested; and for neonatal toxicity, 1000 ppm (based on acute CNS depressive effects).

Organic solvents:

Logman et al, 2005:

Is a meta-analysis.

Period of exposure: Paternal exposure, adult human males exposed chronically to any organic solvent.

Neurodegeneration:
Overall random effects odds ratios and 95% confidence intervals (CI95%) were 1.30 (CI95%: 0.81-2.11, N=1,248) for spontaneous abortions (SA), 1.47 (CI95%: 1.18-1.83, N=384,762) for major malformations (MMs), 1.86 (CI95%: 1.40-2.46, N=180,242) for any neural tube defect, 2.18 (CI95%: 1.52-3.11, N=107,761) for anencephaly, and 1.59 (CI95%: 0.99-2.56, N=96,517; power=56.3%) for spina bifida.
Paternal exposure to organic solvents is associated with an increased risk for neural tube defects but not SAs.

Khattak et al, 1999:

DNT is not measured.

Hydrocarbon solvents:

Mckee et al, 2015:

Is a review.

Neurodegeneration:
Aromatic Solvents (C9–C12): In developmental toxicity tests, there was no evidence of…effects on the developing nervous system in studies in which offspring were examined at postnatal day 90.
Aliphatic/Aromatic Solvents (C9–C20): The only fetal effects in developmental toxicity studies are developmental delays at maternally toxic levels.
Light Aliphatic Solvents (C5-C9): The only fetal effects in developmental toxicity studies were developmental delays at maternally toxic levels.
Heavy Aliphatic Solvents (C9–C20): These solvents…do not produce developmental…effects.

Inhalant volatile solvent (abuse):

Kurtzman et al, 2001:

Period of exposure: During pregnancy.

When DNT effects measured: Infants, neonates.

Neurodegeneration:
Substance abuse during pregnancy poses particular danger in the adolescent population. Since most inhalants are highly lipophilic, they readily cross the placenta. Toluene abuse is consistently associated with infant malformation including oral clefts, micrognathia, microcephaly, growth deficiency, and developmental delay. Furthermore, craniofacial characteristics similar to those seen in fetal alcohol syndrome have also been associated with toluene exposure in utero. Cases of neonatal renal tubular acidosis (RTA) have been reported in the infants of toluene-sniffing mothers. Numerous other inhaled volatiles may cause symptoms of withdrawal in the neonate. Finally, abuse

of toluene and some halogenated hydrocarbons during pregnancy may increase the risk of spontaneous abortion and premature delivery.

Inhalant abuse:

Lorenc, 2003:

When DNT effects measured: Embryos, neonates.

Neurodegeneration:
Inhalants are highly lipophilic and, therefore, readily cross the placenta. Chronic toluene abuse, in particular, has been associated with an embryopathy similar to fetal alcohol syndrome. A neonatal withdrawal syndrome has been described with chronic inhalant abuse. Finally, abuse of toluene and some halogenated hydrocarbons during pregnancy can increase the risk of spontaneous abortion and premature delivery.

Organic solvents and pesticides:

Julvez and Grandjean, 2009:

See table 1 of this article, for a list of DNT studies.

Pesticides:

Costa et al, 2008:

Full-text not found.

Period of exposure: In utero, neonatally.

When DNT effects measured: Children, early in life, later in life.

Neurodegeneration and neurobehavioral deficits:

The possibility that developmental exposure to pesticides (both in utero and neonatally) may contribute to developmental disorders in children, such as attention deficit hyperactivity disorder, autism, or learning disabilities, needs to be further investigated. Finally, a most challenging endeavor would be that of ascertain whether developmental exposure to pesticides may result in "silent neurotoxicity", i.e. may cause nervous system damage that would be manifest as a clinical condition only later in life. For example, damage to nigrostriatal dopaminergic neurons early in life would be expected to result in clinical manifestations of Parkinson's disease as the individual ages, by adding the early insult to the normal age-related loss of neurons.

Methylmercury, lead, polybrominated diphenyl ether and organophosphate insecticides:

Giordano and Costa, 2012:

Is a review.

Neurobehavioral deficits:
Exposure to chemicals which may adversely affect the nervous system has been suggested to be associated with a number of developmental disabilities (learning disabilities, attention-deficit hyperactivity disorder, dyslexia, sensory deficits, mental retardation, and autism spectrum disorders) which are diagnosed in children at an alarming increasing rate.

Methylmercury (MeHg): period of exposure, when DNT effects measured, apoptosis, neurodegeneration, neurobehavioral deficits:
Notable differences were found in the distribution of pathological changes in the young, exposed in utero or as children, and in adults. In particular, while damage in adults is restricted to the cerebellum and the visual cortex, diffuse damage is seen in the developing brain. It was estimated that the nervous system during early development in utero has a five-fold greater vulnerability to MeHg. Signs and symptoms in MeHg-poisoned children included spastic paresis, mental retardation, movement disorders, seizures, primitive reflexes, and speech difficulty. The mechanisms of MeHg developmental neurotoxicity have been studied extensively, and a very complex picture has emerged. MeHg binds with high affinity to sulfhydryl groups, which are relevant for the proper functioning of a large number of proteins. As such, several key cellular processes are affected by MeHg. For example, MeHg has been reported to cause apoptotic cell death, to cause retraction of growth cones and extension, to impair the cytoskeleton, to affect cell's energetics metabolism, and to reduce cell proliferation and neuronal migration.
Three major longitudinal studies have examined the potential effects of low-level MeHg exposure in New Zealand, the Seychelles, and the Faroe Islands. In all locations, MeHg exposure is entirely due to diet, which consists mainly of marine animals. Two of these studies (in New Zealand and the Faroe Islands) reported a correlation between maternal levels of MeHg and subtle neurobehavioral deficits in the offspring. In particular, a small decrease in IQ points and deficits in memory attention and

visuospatial perception were noted in both studies.

Lead (Pb): period of exposure, when DNT effects measured, apoptosis, neurodegeneration, neurobehavioral deficits:
Epidemiological studies clearly showed an association between body burden of Pb in children and adverse neurobehavioral outcomes, namely, lower academic performance and shortened attention span.
Blood Pb levels as low as 2 ug/mL have been associated with declines in IQ and various adverse behavioral effects.
Animal studies in multiple species have confirmed that developmental Pb exposure causes similar cognitive dysfunctions, learning impairment, and distractibility.
Pb has been shown to exert neurotoxicity during differentiation and synaptogenesis; however, the greatest adverse effects are seen during the latest stages of brain development, suggesting that Pb may interfere with the apoptotic process and the trimming/pruning of synaptic connections. In vivo and in vitro studies have shown that Pb may disrupt the blood-brain barrier by injuring astrocytes, with a secondary damage to the endothelial microvasculature. Developmental Pb exposure has been shown to target the hippocampus, cerebral cortex, and cerebellum. At the molecular level, Pb is known to interfere with the regulatory action of calcium in cell functions. Pb is able to increase intracellular calcium concentrations and serves as a calcium substitute, and some calcium-binding proteins are capable of binding Pb. One important enzyme shown to be activated by low concentrations of Pb is protein kinase C (the classical isoforms), with ensuing perturbations of cellular homeostatic mechanisms including cell proliferation.

Polybrominated Diphenyl Ethers (PBDEs): period of exposure, when DNT effects measured, apoptosis, neurodegeneration, neurobehavioral deficits:
Animal studies have provided indications that exposure to different PBDEs (BDE-47 and BDE-99, in particular) during the prenatal and/or postnatal periods causes long-lasting behavioral abnormalities, particularly in the domains of motor activity and cognition.
In a study in Taiwan, elevated PBDE levels in breast milk were correlated with lower birth weight and length, lower head and chest circumference, and decreased Quetelet's (body mass) index.
In another study in the Netherlands, an association was found between blood PBDE levels in the mother at the 35th week of pregnancy and altered motor function, cognition, and behavior of the child up to age six. In an additional cohort in New York City, prenatal PBDE exposure (as indicated by cord blood PBDE levels) was associated with lower scores on tests of mental and physical development at the ages of 1–4 and 6 years. Two recent additional studies in Spain and in Taiwan reported of neurodevelopmental deficits (decreases in cognitive and motor scores, decreased attention) in infants and children exposed to PBDEs.
The mechanisms of PBDEs' developmental neurotoxicity are still elusive, though two general, and not mutually exclusive, modes of action are emerging: one indirect, related to effects of PBDEs on thyroid hormones, and the other involving possible direct effects of PBDEs on the developing brain. Some animal studies have reported alterations of thyroid hormones following developmental PBDE exposures, though developmental effects of PBDEs in animals have also been observed in the absence of thyroid hormone alterations. Various in vitro studies have investigated the ability of PBDEs to alter cell signaling, particularly protein kinase C and calcium homeostasis, to cause oxidative stress, and to induce apoptotic cell death.

Organophosphorus (OP) Insecticides: period of exposure, when DNT effects measured, apoptosis, neurotransmitters and receptors, neurodegeneration, neurobehavioral deficits:
The increased sensitivity to OPs of the young is not due to intrinsic differences in AChE, whose catalytic properties are not influenced by age, but rather to lower metabolic abilities of detoxication of young animals.
An increasing body of literature suggests that developmental exposure to OPs (though most work has been carried out with a single compound, chlorpyrifos), at dose levels causing little inhibition of AChE, results in biochemical and behavioral abnormalities. Experimental studies in rodents indicate that pre- or postnatal exposure to chlorpyrifos affects various cellular processes (e.g., DNA replication, neuronal survival, glial cell proliferation), noncholinergic biochemical pathways (e.g., serotoninergic synaptic functions, the adenylate cyclase system), and causes various behavioral abnormalities (e.g., locomotor skills, cognitive performance). In vitro studies have shown that some OPs inhibit astroglial cell proliferation and cause neuronal apoptotic death. These, and other, effects were seen, however, at relatively high OP concentrations, higher then those sufficient to inhibit AChE. In contrast, a few observations report of in vitro and in vivo effects of OPs at concentrations or doses below those required to inhibit brain AChE catalytic activity.
A series of studies in different cohorts in New York City and California have reported associations between developmental exposure to chlorpyrifos and other OPs and neurodevelopmental abnormalities in the domains of reflexes and cognitive performance.

Silent Neurotoxicity, Indirect Neurotoxicity, and Long-Term Effects:
Exposure to chemicals may cause direct damage or alter developmental programming, whose resulting functional deficits become apparent later in life. For example certain pesticides, such as the herbicide paraquat and the fungicide maneb, the organochlorine insecticide dieldrin.
In some occasions, early mild damage may worsen as the individual matures and ages. For example MeHg, in utero exposure to methylazoxymethanol, neonatal exposure of triethyltin.
In other situations, early mild damage may worsen as the individual matures and ages may not be the case, yet the neurotoxic effects of developmental exposure appear to be irreversible, and even if they do not worsen with age, they are certainly long-lasting. For example, for PCB-126, Pb.
The development of the nervous system can also be influenced by chemicals which may not interact directly with neuronal and glial cells but may act instead, or in addition, as endocrine disruptors. For example effects on thyroid hormones: PBDEs, propyl thiouracyl, PCBs.

Polycyclic Aromatic Hydrocarbons (PAHs):

Crépeaux et al, 2014:

Period of exposure: The same PAH mixture during gestation, or during gestation and lactation.

When DNT measured:
Neurobehavioral, see table 2 of this article. Short term.
Effects of early PAH exposures on brain regional cytochrome oxidase activity measured at PND10, PND21.
Concentration levels of monohydroxylated-PAHs in brain of pups, at PND0, PND21.

Neurodegeneration, neurobehavioral deficits:
No significant short-term modifications of behavioral development and of cerebral metabolism were observed following an early PAH exposure whatever the dose and the period of exposure. Surprisingly, the same brain levels of concentration of PAHs and metabolites were observed in control and exposed pups in both studies.

Polychlorinated biphenyls (PCB):

Pessah et al, 2010:

Is a review.

Apoptosis, receptors:
Each of the biological activities associated with non-coplanar PCBs have been shown to influence neuronal apoptosis and to contribute to the dynamic control of dendritic growth; however, to date, experimental evidence linking these activities to PCB-induced alterations in neuronal connectivity has been reported only for RyR-dependent mechanisms.
PCBs have been shown to induce caspase-dependent apoptosis in primary cultures of hippocampal neurons.
Data linking a direct molecular effect of PCBs (RyR activation) to disruption of specific neurodevelopmental events (neuronal apoptosis and dendritic growth and plasticity) provide the first evidence of a receptor-based mechanism for PCB developmental neurotoxicity.

Receptors, neurodegeneration:
Arguably the most pervasive biological effects of PCBs could be mediated by their ability to alter the spatial and temporal fidelity of Ca2+ signals through one or more receptor-mediated processes. This review will focus on our current knowledge of the structure and function of ryanodine receptors (RyRs) in muscle and nerve cells and how PCBs and related non-coplanar structures alter these functions. The molecular and cellular mechanisms by which non-coplanar PCBs and related structures alter local and global Ca2+ signaling properties and the possible short and long-term consequences of these perturbations on neurodevelopment and neurodegeneration are reviewed.
Explained in more detail in article.
NMDA receptor also involved.

Polybrominated diphenyl ethers (PBDEs):

Schreiber et al, 2009:

Period of exposure:
Normal human neural progenitor cells (hNPCs; Lonza Verviers SPRL, Verviers, Belgium) generated from gestational week 16.
For viability, migration, and differentiation analyses, neurospheres were preincubated for 1 week with PBDEs (0.1, 1, or 10 µM) in proliferation medium; afterward, differentiation was initiated and spheres differentiated with the same concentrations of PBDEs in differentiation
medium for 48 hr (migration measurements) or 7 days (differentiation analyses). This treatment scheme is supposed to imitate exposure of fetal cells during expansion and differentiation in vivo. For proliferation analyses, neurospheres were treated for 2 weeks with PBDEs (0.1, 1, or 10 µM) in proliferation medium. For cotreatment with T3 or NH-3, spheres were incubated for 48 hr (migration) or 7 days (differentiation) with the indicated concentrations after differentiation was initiated.

When DNT measured:
Proliferation analyses:
0 and 14 days after spheres were cultured in proliferation medium, sphere size was determined by software analyses.
Migration assay:
48 hr after initiation of differentiation.
Immunocytochemistry:
After differentiating for 7 days.
Calcium imaging:
We treated neurospheres for 1 week with 10 µM BDE-47 or BDE-99. Subsequently, differentiation was induced under ongoing PBDE exposure. After 24 hr, ratiometric calcium imaging was performed.
14C-BDE-47 accumulation:
After mitogen withdrawal neurospheres were allowed to attach to culture dish for 4 hr; afterward, cells were exposed to 1 µM 14C-BDE-47. The cells were incubated for 7 days at 37°C.

Apoptosis:
BDE□47 or BDE-99 did not cause cytotoxicity of primary fetal human neural progenitor cells (hNPC).

Receptors, neurodegeneration:
PBDEs do not disturb hNPC proliferation but decrease migration distance of hNPCs. Moreover, they cause a reduction of differentiation into neurons and oligodendrocytes. Simultaneous exposure with the TH receptor (THR) agonist triiodothyronine rescues these effects on migration and differentiation, whereas the THR antagonist NH-3 does not exert an additive effect.
PBDEs disturb development of hNPCs in vitro via endocrine disruption of cellular TH signaling at

concentrations that might be of relevance for human exposure.

Polybrominated diphenyl ethers (PBDEs) and their hydroxylated (OH-) or methoxylated forms:

Dingemans et al, 2011:

Is a review.

Apoptosis, GABA, neurotransmitters and receptors, neurodegeneration, neurobehavioral deficits:
Many rodent studies reported behavioral changes after developmental, neonatal, or adult exposure to PBDEs, and other studies documented subtle structural and functional alterations in brains of PBDE-exposed animals. Functional effects have been observed on synaptic plasticity and the glutamate-nitric oxide-cyclic guanosine monophosphate pathway. In the brain, changes have been observed in the expression of genes and proteins involved in synapse and axon formation, neuronal morphology, cell migration, synaptic plasticity, ion channels, and vesicular neurotransmitter release. Cellular and molecular mechanisms include effects on neuronal viability (via apoptosis and oxidative stress), neuronal differentiation and migration, neurotransmitter release/uptake, neurotransmitter receptors and ion channels, calcium (Ca^{2+}) homeostasis, and intracellular signaling pathways.
Bioactivation of PBDEs by hydroxylation has been observed for several endocrine end points. This has also been observed for mechanisms related to neurodevelopment, including binding to thyroid hormone receptors and transport proteins, disruption of Ca^{2+} homeostasis, and modulation of GABA and nicotinic acetylcholine receptor function.

Various compounds (Ethanol, PCB, PBDE, Chlorpyrifosoxone, Dieldrin, Manganese, Arsenic, Mercury, Lead, Valproic acid):

Kadereit et al, 2012:

Is a review.

See table 1 of this article for a short summary of DNT effects of the compounds mentioned above.

Aflatoxin B1:

Parmar et al, 2016:

Article not found in PubMed.

Anesthetics:

Reddy, 2012:

Is a review.

Period of exposure, apoptosis, GABA, receptors, neurodegeneration, neurobehavioral deficits:
Based on the work of Ikonomidou et al. and the work of others over the last few years, it is widely accepted that the commonly used general anesthetics potentiate inhibitory transmission through gamma-amino-butyric-acid type A ($GABA_A$) receptors and the excitatory transmission is reduced through N-methyl-D-aspartic acid (NMDA) glutamate receptors at the peak of synaptogenesis causes widespread apoptotic neurodegeneration. Furthermore, based on the studies by Jevtovic-Todorovic et al. it appeared that exposure to general anesthetics at the peak of synaptogenesis causes significant learning and memory deficiencies later on in life in comparison to control group, and progressively widening the gap in adulthood.
In the adult, $GABA_A$ receptor activation leads to an influx of chloride ions (Cl^-) into the cell. This results in hyperpolarization and can lead to neuroprotection in many models of hypoxia and ischemia. However, in the developing brain, especially during synaptogenesis, intracellular concentration of Cl^- is high; activation of $GABA_A$ receptor results in Cl^- efflux and depolarization of the neuron. Consequently, depolarization mediated rise in intracellular calcium concentration reaches levels that can be harmful to the cell, suggesting that this excitotoxic action of $GABA_A$ may contribute to neuronal injury. An imbalance between excitatory and inhibitory input in the central nervous system during synaptogenesis may trigger apoptosis and changes to the morphology of dendritic
spines.
Apoptosis takes place via different biochemical pathways resulting in activation of effector caspase as the final step. One pathway is the extrinsic pathway or receptor-dependent pathway.

Period of exposure, when DNT measured, neurodegeneration, neurobehavioral deficits:
Prior to the publication of animal data and after the publication of animal data, there are several human cohort studies that demonstrate the association of poor neurodevelopmental outcome in neonates, who underwent major surgery during their neonatal period.

Volatile anesthetic:

Chiao and Zuo, 2014:

Is a review.

Apoptosis, GABA, receptors, neurodegeneration:
See Figure 1 of this article.
Neuroprotection:
Inhibit cerebral metabolic rate.
Activate GABA receptors.
Inhibit glutamate receptors.
Activate protective signaling molecules, such as nitric oxidase synthase and Akt.
Neurotoxicity:
Inhibit glutamate receptors.
Activate GABA receptors.
Activate intrinsic pathway of apoptosis.
Imbalance of cell excitement and inhibition.

Period of exposure (surgery), when DNT measured (children), neurobehavioral deficits:
No studies specifically addressing neuroprotection induced by volatile anesthetics in pediatric populations have been published to date.
Clinical evidence for or against anesthetic induced neurotoxicity in humans is very weak. A few studies have shown that multiple (but not one) exposures to general anesthesia due to surgery carry an increased risk of development of learning disability. Multiple studies have produced results that demonstrate no association between early anesthetic exposure/surgery and the development of cognitive or behavioral disturbances. Since children in all of these studies had surgery, it is difficult to know whether any effects on cognition or behavior are due to surgery or an anesthetic. The studies have reached conflicting conclusions. It is still unclear whether there is association with early exposure to general anesthetics and later development of learning disability or behavioral disturbances. Nevertheless, the existing evidence suggests that anesthetic effect on learning and memory after an early exposure in humans is small, if there is any.

Anesthetics:

Olsen and Li, 2011:

Is a review.

Not about DNT.

Article is about on which protein domains of GABA(A)-R, anesthetics bind.

Dearomatized white spirit:

Hass et al, 2001:

Period of exposure: Rats (Mol:WIST) were exposed to 0 or 800 ppm dearomatized white spirit for 6 hr per day on gestation days 7-20.

When DNT measured: see table 1 of article.
Motor function (Rotarod) 16 weeks
Activity (Open field) 17 weeks
Learning and memory (Morris water maze):
- initial learning 1 months
- memory 2 months
- reversal learning 2 months
- memory 5 months
- transfer of learning 5 months

Neurobehavioral deficits:
No significant effects were recorded on motor function and the activity in Open Field. In the initial learning period (age 1 month), the performance in a Morris water maze was similar in exposed and control animals. When testing for memory at the age of 2 months, the exposed male offspring used more time to locate the hidden platform. After platform relocation, impaired cognitive function was revealed in the exposed females. At the age of 5 months, learning and memory deficits were observed in exposed offspring. The differences were not related to poorer swimming capabilities, because swim speeds were similar to control values. The results show that prenatal exposure to 800 ppm white spirit caused long-lasting learning and memory deficits in rats.

Anesthetics:

McGowan and Davis, 2008:

Is a review comment.

Apoptosis, GABA, receptors, neurotransmitters:
Most neurotransmission in the developing brain is primarily due to NMDA- and/or γ-aminobutyric acid-dependent mechanisms, as is the regulation of neuronal developmental and synaptogenesis. There are at least two possible general mechanisms of pro-apoptotic and/or neurotic injury. The first is that receptor blockade by anesthetic drugs decreases "trophic" stimulation at a critical point in time, leading to induction of cell death programs (analogous to the effects of growth factor deprivation in a variety of cell types). The second is that the transient receptor blockade caused by anesthetic drugs results in subsequent receptor upregulation (protein expression and/or receptor activity). When the blocking anesthetic is removed, the affected neuron is subsequently subjected to significantly increased NMDA activity, resulting in increased excitatory amino acid neurotoxicity. The mechanisms remain largely unsolved, although the available evidence weakly suggests the second.

Along with expected differences in pharmacokinetics and pharmacodynamics among species, interspecies variations make experimental differences in dose, duration of exposure, and time of exposure, both critical and problematic in terms of extrapolation to humans.

Increased research on mechanisms of anesthetic action are needed to discover drugs with greater specificity or mechanisms of action that do not involve γ-aminobutyric acid and NMDA receptors.

Stratmann, 2011:

Is a review.

For more details, see this article.

Neurobehavioral deficits, neurodegeneration:
In summary, the human literature is controversial as to whether anesthesia in infancy causes cognitive problems later in life. Furthermore, it is unclear what the period of vulnerability to anesthetic neurotoxicity is. We do not know whether there is a safe anesthetic technique or duration. The specific cognitive deficit caused by anesthesia, if any, that may underlie such outcomes as learning disabilities, has not been identified. None of the studies, alone or in combination, form a basis for informing clinical practice.

Neurobehavioral deficits, neurodegeneration:
If anesthesia caused cognitive dysfunction, the mechanism by which anesthesia caused cognitive dysfunction would be causally linked to both anesthesia and cognitive dysfunction. The following discussion suggests that the mechanism of anesthesia-induced cognitive dysfunction or decline, as the case may be, is much less clear than previously thought. Specifically, I discuss evidence for each of 3 cellular phenomena to qualify as a mediating mechanism of anesthesia-induced cognitive decline: neurodegeneration, synaptogenesis, and hippocampal neurogenesis.

GABA, neurotransmitters, receptors:
Developmental anesthetic neurotoxicity has largely been attributed to the combination of GABAergic and NMDA antagonist actions of anesthetic drugs.

Mellon et al, 2007:

Is a review.

Period of exposure, when DNT measured:
See the list of studies in table 3 and 4 of this article.

In this article, the terms neuroapoptosis and neurodegeneration, mean more or less the same. For example, table 3 and 4 of this article.

GABA, receptors, neurotransmitters, neurodegeneration, neurobehavioral deficits:
Numerous animal studies in rodents indicate that NMDA receptor antagonists, including ketamine, induce neurodegeneration in the developing brain. The effects of ketamine are dose dependent. The data suggest that limiting exposure limits the potential for neurodegeneration. There is also evidence that other general anesthetics, such as isoflurane, can induce neurodegeneration in rodent models, which may be exacerbated by concurrent administration of midazolam or nitrous oxide. There are very few studies that have examined the potential functional consequences of the neurodegeneration noted in the animal models. However, the studies that have been reported suggest subtle, but prolonged, behavioral changes in rodents. Although the doses and durations of ketamine exposure that resulted in neurodegeneration were slightly larger than those used in the clinical setting, those associated with isoflurane were not. There are insufficient human data to either support or refute the clinical applicability of these findings.
Animal studies suggest that neurodegeneration, with possible cognitive sequelae, is a potential long-term risk of anesthetics in neonatal and young pediatric patients. The existing nonclinical data implicate not only NMDA-receptor antagonists, but also drugs that potentiate gamma-aminobutyric acid signal transduction, as potentially neurotoxic to the developing brain. The potential for the combination of drugs that have activity at both receptor systems or that can induce more or less neurotoxicity is not clear; however, recent nonclinical data suggest that some combinations may be more neurotoxic than the individual components. The lack of information to date precludes the ability to designate any one anesthetic agent or regimen as safer than any other. Ongoing studies in juvenile animals should provide additional information regarding the risks.

GABA, receptors, neurotransmitters:
Anesthetic drugs are also capable of interacting with a variety of other neuronal systems, including GABAergic systems, or interact with a variety of other receptors, including glycine receptors, nicotinic-acetylcholine receptors, serotonergic receptors, and other glutamatergic receptors. The potential clinical significance of these interactions remains to be determined.
Opioid analgesics are μ-opioid agonists.

Weir, 2006:

Is a review.

DNT is not mentioned in this article.

General anaesthetics modulate the activity of the transmitter-gated ion channel superfamily to either enhance inhibitory, or inhibit excitatory neurotransmission. This superfamily includes γ-aminobutyric acid type A ($GABA_A$), strychnine-sensitive glycine, neuronal nicotinic acetylcholine (nAch) and 5-hydroxytryptamine (5-HT3) receptors.
At clinically relevant concentrations the majority of general anaesthetics augment the activity of the $GABA_A$ receptor.

$GABA_A$ receptor isoforms, which means variation within the subunits themselves of this receptor, determine the effects of anesthetics. Also which specific subunits are targeted by anesthetics, determine the effects of anesthetics.

DNT:

Hass U Methodologies to assess health outcomes in children 168-216-:

Article not found.

Hass, 2003:

Is a review.

This article does not fit in with the other articles of this assignment.

Important points of this article:

The regulatory authorities and toxicologists will be faced with the challenge that decisions have to be made concerning e.g. when testing should be requested, how testing should be performed, as well as evaluation of the results and the regulatory consequences.

In determining the necessity for developmental neurotoxicity testing, a weight-of-evidence approach should be used. Data from all available toxicity studies, as well as potential human exposure information should be considered. Developmental neurotoxicity testing should be conducted to further characterise neurological effects observed in other studies, and should be considered if the substance has been shown to cause neurotoxicity or structural abnormalities of the CNS in other studies, or suspected of interfering with neurotransmission or neuroendocrine pathways (thyroid, pituitary, or circulating sex hormones) at the CNS level. For example, neuroendocrine interference at the level of the hypothalamic-pituitary axis might be inferred from changes in the levels of circulating gonadotrophins or steroid sex hormones.

A developmental neurotoxicity study can be conducted as a separate study, or as an add-on study.
In the EU-TGD, it is recommended to perform developmental neurotoxicity testing as an add-on to a two-generation study using offspring that would otherwise be discarded at weaning since this will not involve the use of additional groups of animals. Consequently, the need for inclusion of a developmental neurotoxicity evaluation has to be considered at the planning stage of a two-generation study, i.e. normally before the prenatal developmental toxicity study has been performed. Therefore, the relevant data needed for triggering developmental neurotoxicity testing may not be available.

A standard test method is currently being developed, as the OECD draft TG 426 and this guideline is recommended for regulatory testing in the EU-TGD. Consequently, the choice of guideline will not be a great challenge for the regulators. The evaluation consists of observations to detect gross neurological and behavioural abnormalities; the assessment of physical development, including sexual maturation, reflex ontogeny, motor activity, motor and sensory function, and learning and memory; and the evaluation of brain weights and neuropathology during postnatal development and adulthood. The overall design of the study as well as the functional end points are relevant and should ideally allow the identification of potential developmental neurotoxicants.

The flexibility in the OECD guideline may become a disadvantage if methods with a low sensitivity are chosen.

Developmental neurotoxicity can be indicated by behavioural changes or morphological changes in the brain. The severity and nature of the effect should be considered.

Andersen et al, 2000:

Article is outdated. New data is available in for example **Grandjean and Landrigan, 2014**.

More epidemiological and experimental studies about DNT are available since this article was published. This article states:
Epidemiological evidence is very limited, but severe irreversible effects have been observed in humans following in utero exposures to a few known developmental neurotoxicants.
In experimental animals, exposure to neurotoxic chemicals during critical periods of brain development has induced permanent functional disturbances in the CNS.

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ANNEX 7

A7.1 Detailed information on grouping of selected narcotic organic solvents

The narcotic organic solvents presented in Annex 4 -and some more- are grouped in the table below.

Note: References are either completely presented in the table below, and/or are given under Annex 4 and 5.

A7.1.1 Abbreviations to Annex 7

ATE, acute toxicity estimate; ATSDR, Agency for Toxic Substances and Disease Registry; C, ceiling value; Ca, potential occupational carcinogen; CAS, Chemical Abstracts Service; CDC, Centers for Disease Control and Prevention; CID, compound identifier; CNS, central nervous system; CRC, CRC Press, a publishing group; DHHS, Department of Health and Human Services; EEG, electroencephalography; EPA, Environmental Protection Agency; ER, evaporation rate; FDA, Food and Drug Administration; FOB, functional observational battery; FP, flash point; GD, gestational day; GESTIS, Gefahrstoffinformationssystem; IDLH, immediately dangerous to life or health; IFA, Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung; IUPAC name, International Union of Pure and Applied Chemistry; LD_{50}, median lethal dose
LD_{Lo}, lowest lethal dose; LOAEL, lowest observed adverse effect level; Log Kow, log octanol-water partition coefficient; Log Pow, log octanol-water partition coefficient; MSDS, material safety data sheet; NIOSH, National Institute for Occupational Safety and Health; NOAEC, no observed adverse effect concentration; NOAEL, no observed adverse effect level; NS, nervous system; OECD TG, Organization for Economic Co-operation and Development test guideline; OSHA, Occupational Safety and Health Administration; OT, odor threshold; OTS, Office of Technology Solutions; PEL, permissible exposure limit; PNDT, prenatal developmental toxicity; QSAR, quantitative structure–activity relationship; RA, radioactive; REL, recommended exposure limit; ST, short term; STOT SE 3: H336, specific target organ toxicity, single exposure, category 3, may cause drowsiness or dizziness; TK, toxicokinetics; TLV, threshold limit value; TWA, time-weighted average; VP, vapor pressure.

A7.1.2 Table with detailed information on grouping of selected narcotic organic solvents

Annex 7 *(grouping attempt)* **Substances classified as STOT SE 3: H336 (see also Table 1 in main text of the document)***
Effects on (developing) nervous system, observed in acute, repeated dose and developmental neurotoxicity studies.**

** In this table, substances are categorized as proposed in Table 1 (See: Main text of the document).*
*** Cells are shaded (three most right columns) indicate: narcosis/CNS depression (blue), neurotoxicity (yellow), absence of effects (empty), no information available (grey); pink-shaded cells: substance is not included in Annex 4.*

CAS Number **Name** **Chemical formula** **IUPAC name** **Other name(s)**	**Substance information** From various sources	**Physical state** From various sources	**Acute toxicity studies** **Effects on nervous system**	**Repeated dose studies** **Effects on nervous system**	**Developmental neurotoxicity studies**
Pink-shaded cells: substance is not included in Table Annex 4. Substances are evaluated for the sake of comparison with substances in the group and narcotic/neurotoxic potency. Information collected from various sources.			**Blue-shaded cells**: narcosis/CNS depression **Yellow-shaded cells**: neurotoxicity **Grey cells**: no information available **Empty cells**: no effects observed on nervous system		

Aliphatic hydrocarbons (acyclic) Alkanes R-H (Most polar)	Straight or branched chains of carbon, saturated and unsaturated with hydrogen				
Aliphatic hydrocarbons (acyclic) **Saturated**	***n-pentane*** ***isopentane*** ***n-hexane*** ***n-heptane*** ***isoheptane*** ***n-octane*** ***2,2,4-trimethylpentane***				

203-692-4 n-Pentane C_5H_{12} IUPAC name: ***Pentane*** $CH_3(CH_2)_3CH_3$ Other name(s): n-pentane	**Pentane** is an alkane of five carbon atoms, with the chemical formula C_5H_{12}. Three structural isomers exist with this formula. The un-branched isomer is n-pentane (normal pentane: $CH_3(CH_2)_3CH_3$); the other two isomers are named isopentane (methylbutane) and neopentane (dimethylpropane). The term pentane may also refer to a mixture of the three isomers. Note: **Cyclopentane is not an isomer of pentane because it has only 10 hydrogen atoms where pentane has 12.** LD $_{50\ (median\ dose)}$: 3 g /kg (dermal, rabbit); 5 g /kg (oral, mouse)[1]. LD $_{50\ (median\ concentration)}$: 130,000 mg/m^3 (mouse, 30 min); 128,200 ppm (mouse, 37 min); 325,000 mg/m^3 (mouse, 2 hr)[1] PEL (Permissible): TWA 1000 ppm (2950 mg/m^3)[2] REL (Recommended): TWA 120 ppm (350mg/m^3)[2] C 610 ppm (1800mg/m^3) [15-minute][2] IDLH (Immediate danger): 1500 ppm[2] *(1) NIOSH, 'n-Pentane'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *(2) NIOSH. 'NIOSH Pocket Guide to Chemical Hazards #0486'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 579 hPa 20.0 °C Log Kow: 3.45 Water solubility: 38.5 mg/L at 20 °C Dielectric Constant: 1.84	Narcosis observed from acute inhalation studies. Mortalities at mid and high dose (complete anesthesia test, 4.5, and 4.9 mmol/L); LD_{50} was not calculated. --------- ***Acute toxicity, inhalation, n-pentane; male rats: 4 hours, 21000 ppm; female mice: 2 hours, 23500 ppm; hindlimb stretch test*** ***Acute toxicity, inhalation n-pentane, mice: exp. 1) 'light anesthesia' test: 3.0, 3.5, and 4.2 mmol; exp. 2) 'complete anaesthesia' test: 4.2, 4.5, and 4.9 mmol/L.***	No neurotoxicity effects were observed in the 90 day and 6 weeks inhalation studies. --------- ***Two 90 day inhalation studies each with a neurotoxicity screening component.*** ***6-Weeks inhalation study at 3000 ppm); testing conduction velocity peripheral nerve, and behavior.***	No literature was found on n-pentane showing effects in the developmental nervous system

201-142-8 Isopentane C_5H_{12} IUPAC name: ***2-Methylbutane*** Other name(s): Isopentane methylbutane	2-methylbutane (=isopentane; also called methylbutane) is a branched-chain alkane with five carbon atoms. 2-methylbutane is one of three structural isomers with the molecular formula C_5H_{12}, the others being pentane (n-pentane) and neopentane (dimethyl propane).	Physical state: Liquid VP: 1000 hPa 27.5 °C Water solubility: 0.049 g/L at 25 °C Log Pow: 4 Dielectric Constant: (68 °F) 1.8	Acute toxicity study results in depression in rats with cyclopentane (oral or inhalation) and with n-pentane, and complete anaesthesia in mice with 1-bromopropane.	Repeated dose toxicity studies with Naphtha (petroleum), light alkylate via inhalation in rats for 13 weeks results **no effects related to neurotoxicity.**	No information found from literature search on the effect of isopentane on neurodevelopment.

203-777-6 n-hexane C_6H_{14} IUPAC name: ***Hexane*** $CH_3(CH_2)_4CH_3$ Other name(s): n-hexane	**Hexane** is an alkane of six carbon atoms, with the chemical formula C_6H_{14}. Five structural isomers exist with this formula. The un-branched isomer is n-hexane (normal hexane: $CH_3(CH_2)_4CH_3$); the other four isomers are named as methylated derivatives of pentane and butane: 2-methylpentane (=isohexane: $(CH_3)2CH(CH_2)2CH_3$), 3-methylpentane ($CH_3CH_2CH(CH_3)CH_2CH_3$), 2,3-dimethylbutane ($CH_3CH(CH_3)CH(CH_3)CH_3$), 2,2-dimethylbutane (=neohexane: $CH_3C(CH_3)2CH_2CH_3$). LD $_{50\ (median\ dose)}$: 25 g /kg (oral, rat); 28710 mg/kg (rat, oral)[(1)]. LD $_{Lo\ (lowest\ published)}$: 56137 mg/kg (rat, oral) [(1)] PEL (Permissible): TWA 500 ppm (1800 mg/m^3)[2] REL (Recommended): TWA 50 ppm (180 mg/m^3)[2] IDLH (Immediate danger): 1100 ppm[2] *[(1)] NIOSH, 'n-Hexane'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *[(2)] NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0322'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 100 hPa 9.8 °C Water solubility: 0.01 g/L at 25 °C Log Pow: 4 Dielectric Constant: 1.89 TLV-TWA: 50 ppm OT: 65- 250 ppm FP: -7 F° ER: fast		Repeated dose toxicity studies with n-hexane via inhalation for 16 weeks results in effects on motor nerve conduction velocity and distal latency, and damage to neuronal tissues **(rats, n-hexane, 3000 ppm 12 hours/day, 7 days/week, 16 weeks).**	Information from literature showed that metabolite of n-hexane **(2,5-hexanedione) is a developmental neurotoxicant**.

205-563-8 n-heptane C_7H_{16} IUPAC name: ***Heptane*** $H_3C(CH_2)_5CH_3$ Other name(s): n-heptane	**Heptane** is an alkane of seven carbon atoms, with the chemical formula C_7H_{16} Nine structural isomers exist with this formula. The un-branched isomer is n-heptane (normal heptane: $H_3C–CH_2–CH_2–CH_2–CH_2–CH_2–CH_3$); the other eight isomers are named: 2-Methylhexane (=isoheptane: $H_3C–CH(CH_3)–CH_2–CH_2–CH_2–CH_3$), 3-Methylhexane ($H_3C–CH_2–C^*H(CH_3)–CH_2–CH_2–CH_3$ (chiral)), 2,2-Dimethylpentane (=neoheptane: $H_3C–C(CH_3)_2–CH_2–CH_2–CH_3$), 2,3-Dimethylpentane: $H_3C–CH(CH_3)–C^*H(CH_3)–CH_2–CH_3$ (chiral)), 2,4-Dimethylpentane ($H_3C–CH(CH_3)–CH_2–CH(CH_3)–CH_3$), 3,3-Dimethylpentane ($H_3C–CH_2–C(CH_3)_2–CH_2–CH_3$), 3-Ethylpentane ($H_3C–CH_2–CH(CH_2CH_3)–CH_2–CH_3$), 2,2,3-Trimethylbutane (also known as pentamethylethane and triptane [(1)] : ($H_3C–C(CH_3)_2–CH(CH_3)–CH_3$)). LD $_{50\ (median\ dose)}$: 17,986 ppm (mouse, 2 hr)[2] . LD $_{Lo\ (lowest\ published)}$: 16,000 ppm (human); 15,000 ppm (mouse, 30 min)[2] PEL (Permissible): TWA 500 ppm (2000 mg/m^3)[3] REL (Recommended): TWA 85 ppm (350 mg/m^3)[3] C 450 ppm (1800 mg/m^3) [15-minute][3] IDLH (Immediate danger): 15 ppm[3] *(1) Isomers. Members.optushome.com.au. Retrieved on 2012-03-04.* *(2) NIOSH, 'n-Heptane'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *(3) NIOSH. 'NIOSH Pocket Guide to Chemical Hazards #0312'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 60.9 hPa 25 °C Water solubility: "slightly soluble (0.1-100 mg/L)" Log Pow: 4.5 Dielectric Constant: 1.92	Acute inhalation study showed behavioral effects **(4h male rats and 2h female mice; 2740 ppm)**: 'Under the conditions of the test, Normal heptane was capable of blocking electrically evoked seizures at 2740 ppm (90% confidence interval = 730), underlining the effects on behavior'.	Repeated dose toxicity studies in rats with n-heptane via inhalation **(OECD TG 413 study: heptane, rats, inhalation, whole body, 398, 2970 ppm, 26 weeks. 'Auditory sensitivity study': n-heptane, male rats, inhalation, whole body, 28 days, 0, 3.3, 16.6 mg/L (re-calculated; corresponding to 0, 800, 4000 ppm)**, resulted in **acute CNS depression** in 13 weeks study, **loss of auditory sensitivity in 28 day study, transient neurochemical changes ('2 week inhalation neurochemical study': heptane, inhalation, 4.2, 21 and 62 µM, 2 weeks)**. But other effects like neuropathy in the 28 day study and nerve conduction velocity in the motor and sensory nerves or microscopic changes **(no guideline study: heptane, rats, inhalation, 1500 ppm)** in the peripheral nerves in the 16 weeks study **(n-heptane, male rats, 3000 ppm via inhalation 12 hours/day, 7 days/week, for 16 weeks)** were **not reported.**	No information found from literature search on the effect of n-heptane on neurodevelopment

250-610-8 Isohep.tane C_7H_{16} IUPAC name: ***2-methylhexane*** Other name(s): Isoheptane b-methylhexane	**2-methylhexane** (=isopentane (C_7H_{16})) is a saturated organic substance and an isomer of n-heptane.	Physical state: liquid VP: 89 hPa 25 °C log Kow: 3.7 Water Solubility: 2.5 mg/L	No studies (acute or repeated dose studies) to investigate the neurotoxic effect of n-heptane.	The registrant assigned STOT SE 3 (H336) **based on read-across** within a category approach. n-Heptane or Naphta (petroleum), light alkylate (CAS no: 64741-66-8) was not a neurotoxicant while n-hexane induced neuropathy in 16 weeks inhalation study at 3000 ppm. *n-Heptane: not neurotoxic;* *n-hexane: induced neuropathy (NOAEC: > 3000 ppm (12470 mg/m³); n-Pentane: no information.* **----------** ***Peripheral nerve toxicity of n-pentane, n-hexane and n-heptane, male rats, 0 or 3000 ppm, 12 hours/day, 7 days/week, 16 weeks, conduction velocity, behavior, microscopy nerves.***	No literature was found on isoheptane showing effects in the developmental nervous system.

203-892-1 n-octane C_8H_{18} IUPAC name: ***Octane*** $H_3C(CH_2)_6CH_3$ Other name(s): n-octane	**Octane** is an alkane of eight carbon atoms, with the chemical formula C_8H_{18} Octane has many structural isomers that differ by the amount and location of branching in the carbon chain. The un-branched isomer is n-octane (normal octane: $H_3C–CH_2–CH_2–CH_2–CH_2–CH_2–CH_2–CH_3$); others are: 2-Methylheptane; 3-Methylheptane (2 enantiomers); 4-Methylheptane; 3-Ethylhexane; 2,2-Dimethylhexane; 2,3-Dimethylhexane (2 enantiomers); 2,4-Dimethylhexane (2 enantiomers); 2,5-Dimethylhexane; 3,3-Dimethylhexane; 3,4-Dimethylhexane (2 enantiomers + 1 meso compound); 3-Ethyl-2-methylpentane; 3-Ethyl-3-methylpentane; 2,2,3-Trimethylpentane (2 enantiomers); 2,2,4-Trimethylpentane (=isooctane); 2,3,3-Trimethylpentane; 2,3,4-Trimethylpentane; 2,2,3,3-Tetramethylbutane. LD $_{50\ (median\ dose)}$: not given LD $_{Lo\ (lowest\ published)}$: 428 mg/kg (mouse, intravenous)[1] PEL (Permissible): TWA 500 ppm (2350mg/m^3)[2] REL (Recommended): TWA 75 ppm (350 mg/m^3)[2] C 385 ppm (1800 mg/m^3) [15-minute][2] IDLH (Immediate danger): 1000 ppm [2] *(1) NIOSH, 'n-Octane'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *(2) NIOSH. 'NIOSH Pocket Guide to Chemical Hazards #0470'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 18.6 hPa 25 °C Water solubility: "slightly soluble (0.1-100 mg/L)" Log Pow: 5.15 Dielectric Constant: 2.0	Acute toxicity study in rats via oral results in transient clinical effects like tremors and hyperactivity with n-octane **(~OECD TG 403, octane, rats, inhalation, 4h, 24.88 mg/L),** and, depression and salivation with read across substance 2,2,4-trimethylpentane **(~OECD TG 401, 2,2,4-trimethylpentane, rats, oral (gavage), 5000 mg/kg bw)**.	Repeated dose toxicity studies in rats with octane or read across substances via inhalation results in **no effects** on cognitive performance, functional observations and motor activity, in 3 days or 13 weeks studies. --------- ***Studies on 'Discrete-trial two-choice visual discrimination task' and on 'FOB and motor activities': octane, rats, inhalation, 8h, 300, 900, and 3000 ppm, 3 days.*** ***~ OECD TG 413, Naphtha (petroleum), light alkylate, rats, inhalation, whole body, 668, 2220, and 6646 ppm, FOB and motor activity.*** ***~OECD TG 413, male rats, inhalation, nonane, 1.9, 3.1, 8.4 mg/L.***	No information found from literature search on the effect of n-octane on neurodevelopment

208-759-1 2,2,4-trimethylpentane C_8H_{18} IUPAC name: ***2,2,4-trimethylpentane*** $(CH_3)_3CCH_2CH(CH_3)_2$ Other name(s): Isooctane Iso-octane	2,2,4-Trimethylpentane (=isooctane) is an isomer of octane C_8H_{18} $(CH_3)_3CCH_2CH(CH_3)_2$ Strictly speaking, if the standard meaning of 'iso' is followed, the name isooctane should be reserved for the isomer 2-methylheptane. However, 2,2,4-trimethylpentane is by far the most important isomer of octane and so, historically, it has ended up with this name [(1)]. *[(1)] Clayden, Jonathan (2005). Organic chemistry (Reprinted (with corrections). ed.). Oxford [u.a.]: Oxford Univ. Press. p. 315. ISBN 978-0-19-850346-0.*	Physical state: Liquid VP: 28 hPa 20 °C Water Solubility: 2.2 mg/L at 25 °C Log Pow: 4.08 Dielectric Constant: 1.94	Acute toxicity study with 2,2,4-trimethylpentane results in clinical effects like depression, and salivation via oral; and prostrate via inhalation. ---------- ***~OECD TG 401, 2,2,4-trimethylpentane, rats, oral (gavage), 5000 mg/kg bw.*** ***~OECD TG 403, 2,2,4-trimethylpentane, rats, inhalation, 4h, 33.52 mg/L.***	Repeated dose toxicity studies in rats with **read across substances** (Alkanes, C7-10-iso-, octane, Naphtha (petroleum), light alkylate, and nonane) in rats between 3 days to 13 weeks via inhalation results in **no neurotoxicity effects** related to cognitive performance, FOB, and motor activity. ---------- ***Alkanes, C7-10-iso-, male rats, inhalation (air), 8h, 3 days, 300, 900, 3000 ppm, discrete-trial two-choice visual discrimination task.*** ***Octane, rats, inhalation, 3 days, 300, 900, and 3000 ppm, FOB and motor activity examinations.*** ***~ OECD TG 413, Naphtha (petroleum), light alkylate, rats, inhalation, whole body, 668, 2220, and 6646 ppm, FOB and motor activity examinations.*** ***~OECD TG 413, nonane, male rats, inhalation, 1.9, 3.1, 8.4 mg/L.***	No information found from literature search on the effect of 2,2,4-trimethylpentane on neurodevelopment.

Aliphatic hydrocarbons (acyclic) **Unsaturated**	***ethylene*** ***2,4,4-trimethylpentene***				
200-815-3 Ethylene C_2H_4 IUPAC name: ***ethene*** $H_2C=CH_2$ Other name(s): *Ethylene*	Ethylene (IUPAC name: ethene) is a hydrocarbon with formula C_2H_4 or $H_2C=CH_2$	Physical state: Gas VP: 2124 hPa 90 °C Water Solubility: 131 mg/L at 25 °C Log Pow: 1.13	Acute toxicity study in rats with ethylene via inhalation results **no effects related to neurotoxicity.** ---------- ***No guideline, male rats, ethylene, inhalation, whole body, 5h, 10000 ppm.***	Repeated dose toxicity studies in rats with ethylene via inhalation for 13 weeks results **no effects related to neurotoxicity.** ---------- ***OECD TG 413, rats, inhalation, whole body, 300, 1000, 3000, 10000 ppm), 13 weeks, neurobehavior***.	No information found from literature search on the effect of ethylene on neurodevelopment.
246-690-9 2,4,4-trimethylpentene C_8H_{16} IUPAC name: ***2,4,4-Trimethylpentene*** Other name(s): *Pentene, 2,4,4-Trimethyl Diisobutylene, 2,4,4-Trimethylpentene, Diisobutylene, Diisobutene.*		Physical state: Liquid VP: 28 hPa 20 °C Water Solubility: 2.2 mg/L at 25 °C Log Pow: 4.08 Dielectric Constant: 2.1	Acute toxicity study in rats with 2,4,4-trimethylpentene via inhalation results **no effects related to neurotoxicity**. >>> transient reduction in motor activity (no guideline study). ---------- **No guideline, rats, oral gavage, 2,4,4-trimethylpentene, 250, 500, 1 000 or 2 500 mg/kg bw.** *At 1000 and 2 500 mg/kg bw, transient reduction in motor activity.*	Repeated dose toxicity studies in rats with 2,4,4-trimethylpentene via inhalation for 28 days results **no effects related to neurotoxicity**. ---------- **OECD TG 407, 2,4,4-trimethylpentene, rats, oral gavage, 100, 300, 1000 mg/kg/day, 28 days.** *'Considered no effect on nervous system function up to 1000 mg/kg/day'.*	No information found from literature search on the effect of 2,4,4-trimethylpentene on neurodevelopment.

Cyclic hydrocarbons *(cycloparaffins, naphthenes).* (not aromatic)	Ring structure saturated and unsaturated with hydrogen ***cyclo-hexane*** ***methylcyclohexane***				
203-806-2 cyclohexane C_6H_{12} IUPAC name: (preferred) ***Cyclohexane*** Other name(s): *Hexanaphthene*	CNS impairment. Chronic effects unknown. **Cyclohexane** is a cycloalkane with the molecular formula C_6H_{12} (abbreviated to Cy). Cyclohexane –being a non-polar, hydrophobic hydrocarbon– is rather unreactive. LD $_{50\ (median\ dose)}$: 12705 mg/kg (rat, oral), 813 mg/kg (mouse, oral)[(1)] LD $_{50\ (median\ concentration)}$: 10172 ppm (mouse, 2 hr), 10,000-12,500 ppm (mouse, 2 hr), 15227 ppm (rabbit, 1 hr)[(2)] PEL (Permissible): TWA 300 ppm (1050 mg/m^3)[(2)] REL: 300 ppm (1050 mg/m^3 IDLH (Immediate danger): 1300 ppm[(2)] *[(1)] NIOSH, 'Cyclohexane'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *[(2)] NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0163'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 127 hPa 20 °C Water Solubility: 52 mg/L at 23.5 °C Log Pow: 3.44 TLV-TWA: 100 ppm OT: 780 ppm FP: 1.4 F° ER: fast Dielectric Constant: 2.02	Acute toxicity study with cyclohexane in rats via inhalation results clinical **effects like gait** and a statistically significant **reduction in psychomotor speed at 28000 mg/m3**.	Repeated dose toxicity studies in rats with cyclohexane between 3 days to 13 weeks via inhalation results **no effects** like neuropathy or histopathological changes in the nerve tissue in 30 weeks study; and **no neuro-behavioral effects** in a 13-14 week study.	No information found from literature search on the effect of cyclohexane on neurodevelopment.

203-624-3 methylcyclohexane C_7H_{14}. IUPAC name: ***methylcyclohexane*** $CH_3C_6H_{11}$ Other name(s): *Hexahydrotoluene* *Cyclohexylmethane* *Toluene hexahydride*	**Methylcyclohexane** is saturated hydrocarbon with the molecular formula is $CH_3C_6H_{11}$. Methylcyclohexane is a monosubstituted cyclohexane, i.e. one branching via the attachment of one methyl group on one carbon of the cyclohexane ring. Note that, while methylcyclohexane is a substructure of 4-methylcyclohexanemethanol (MCHM), it is distinct in its physical, chemical, and biological (ecologic, metabolic, and toxicologic properties (CDC, 2014) [(3)]. Rather, its properties are similar to related saturated hydrocarbons such as heptane. LD $_{50\ (median\ dose)}$: 2250 mg/kg (mouse, oral))[(1)] LD $_{50\ (median\ concentration)}$: 10172 ppm (mouse, 2 hr), 10,000-12,500 ppm (mouse, 2 hr), 15227 ppm (rabbit, 1 hr)[(1)] PEL (Permissible): TWA 500 ppm (2000 mg/m^3)[(2)] REL: TWA 400 ppm (1600 mg/m^3)[(2)] IDLH (Immediate danger): 1200 ppm[(2)] *[(1)] NIOSH, 'Methylcyclohexane'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *[(2)] NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0406'. National Institute for Occupational Safety and Health.* *[(3)] CDC, 2014, 'Methylcyclohexane', NIOSH Pocket Guide to Chemical Hazards, see [2], accessed 27 May 2014.*	Physical state: Liquid VP: 61.8 hPa 25 °C Water solubility: 14 mg/L at 25 °C Log Pow: 3.88	Acute toxicity study with methylcyclohexane via inhalation results clinical effects like **lethargic without exhibiting true narcosis** in rats; **no CNS effects in dogs**; and **hyperactivity,** slight **loss of coordination** and prostration in mice and rat	Repeated dose toxicity studies with methylcyclohexane via inhalation results **no effects in neurobehavior in rats** exposed for 28 days. **No effects related to neurotoxicity** were reported in dogs, mice, and rats exposed for 12 months (however examination like neuro-behavioral examination were not included).	No information found from literature search on the effect of methylcyclohexane on neurodevelopment.

Aromatic hydrocarbons Aromatics Ar-H	Contain a 6 carbon ring structure with one hydrogen per carbon bound by energy from several resonant forms ***benzene*** ***toluene*** ***styrene*** ***xylenes*** ***ethyl benzene*** ***2,6-diiso-propylphenol (propofol)***				

CAS registry 71-43-2 Benzene C_6H_6 IUPAC name (preferred): ***Benzene*** Other names: Benzol Phene Phenyl hydride	**Benzene** –chemical formula C_6H_6 – is a hydrocarbon as it only contains carbon and hydrogen atoms; it has a sweet smell (the smell around petrol stations). The benzene molecule is composed of 6 carbon atoms joined in a ring with 1 hydrogen atom attached to each. Benzene is classed as an aromatic hydrocarbon because of the cyclic continuous pi bond between the carbon atoms. Many important chemical compounds are derived from benzene by replacing one or more of its hydrogen atoms with another functional group. Examples of simple benzene derivatives are phenol, toluene, and aniline (abbreviated PhOH, PhMe, and PhNH2, respectively). LD $_{50\ (median\ dose)}$: 930 mg/kg (rat, oral) LD $_{Lo\ (lowest\ published)}$: 44,000 ppm (rabbit, 30 min), 44,923 ppm (dog), 52,308 ppm (cat), 20,000 ppm (human, 5 min))[(1)] PEL (Permissible): TWA 1 ppm, ST 5 ppm[(2)] REL (recommended): Ca TWA 0.1 ppm ST 1 ppm [(2)] IDLH (Immediate danger): 500 ppm[(2)] *[(1)] NIOSH, 'Benzene'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *[(2)] NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0049'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 127 hPa 25 °C Water solubility: 1.79 g/L (15 °C); 1.84 g/L (30 °C) Log Kow: 103-245 Dielectric Constant: 2.4 TLV-TWA: 0.5 ppm OT: 34-119 ppm FP: 12 F° ER: med	Acute effects of **benzene inhalation** in both humans and animals include narcosis, anesthesia, CNS depression, respiratory arrest, unconsciousness, and death. Oral exposure results in symptoms similar to inhalation exposure. [(1)] **Note 1.** In its acute stages, benzene toxicity appears to be due primarily to the direct effects of benzene on the central nervous system, whereas the peripheral nervous system appears to be the target following chronic exposure. [(1)] *(1) ATSDR (2007) Toxicological profile for benzene. US Department of Health and Human Services. Public Health Service. Agency for Toxic Substances and Disease Registry. August 2007*	Chronic inhalation exposure to benzene in humans has been associated with distal neuropathy, difficulty in sleeping, and memory loss. Animal studies (acute and intermediate inhalation exposures) also reported adverse neurological effects reduced hind-limb grip strength and evoked electrical activity in the brain, and behavioral disturbances. Oral exposure results in symptoms similar to inhalation exposure. No neurological effects have been reported after dermal exposure to liquid benzene in either humans or animals. [(1)] **Note 2.** Low-level exposures: because benzene may induce an increase in brain catecholamines, it may also have a secondary effect on the immune system via the hypothalamus-pituitary-adrenal axis. Increased metabolism of catecholamines can result in increased adrenal corticosteroid levels, which are immunosuppressive. [(1)] *(1) ATSDR (2007) Toxicological profile for benzene. US Department of Health and Human Services. Public Health Service. Agency for Toxic Substances and Disease Registry. August 2007*	Effects of benzene on learning were investigated in male hooded rats (Sprague-Dawley), 550 mg/kg benzene in corn oil intraperitoneally, on days 9, 11, and 13 postpartum: significantly impaired learning ability in closed-field, maze-learning task. In another study, 47-day-old **juvenile** cotton rats, maintained on one of two isocaloric diets (with 4 or 16% crude protein) for a 26-days; intraperitoneally 0, 100, 500, or 1,000 mg/kg benzene in corn oil for 3 consecutive days (days 15– 17 of experiment-tation); animal termination on experimentation day 27. Results: severe loss of coordination in some rats on low protein diet immediately after exposure to benzene, but this subsided. [(1)] *(1) ATSDR (2007) Toxicological profile for benzene. US Department of Health and Human Services. Public Health Service. Agency for Toxic Substances and Disease Registry. August 2007*

203-625-9 toluene C_7H_8 IUPAC name (preferred): ***Toluene*** (systematic): ***Methylbenzene*** $C_6H_5CH_3$ Other names: Phenylmethane Toluol Anisen	**Methylbenzene (=toluene; toluol)** is a mono- substituted benzene derivative, consisting of a CH_3 group attached to a phenyl group; it is an aromatic hydrocarbon. Like styrene, toluene can severely damage the auditory function in adult rats, but the ototoxic potency of styrene is higher compared to that of the toluene. [1] LD 50 (median dose): >26700 ppm (rat, 1 hr); 400 ppm (mouse, 24 hr)[2] LD Lo (lowest published): 55,000 ppm (rabbit, 40 min)[2] PEL (Permissible): TWA 200 ppm C 300 ppm 500 ppm (10-minute maximum peak)[3] REL (Recommended): TWA 100 ppm (375 mg/m³) ST 150 ppm (560 mg/m³)[3] IDLH (Immediate danger): 500 ppm[3] *[1] Loqueta G, Campo P, Lataye R. 199. Comparison of Toluene-Induced and Styrene-Induced Hearing Losses. Neurotoxicology and Teratology 21 (6): November–December 1999, 689–697.* *[2] NIOSH, 'Toluene'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *[3] NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0619'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 30.9 hPa 21.1°C; 41.3 hPa 26.6°C. Water solubility: 573-587 mg at 25°C Log Kow: 2.73 Dielectric Constant: 2.3 TLV-TWA: 2.9 ppm OT: 40 ppm FP: -7 F° ER: med	Acute exposure to toluene in human volunteers show that dizziness and sleepiness are experienced at **air levels < 20 mg/L for 4h** and **rocking gait and narcosis** were observed in rats at this same concentration). --------- ***Acute Oral: EU B1; rats; toluene, 4000, 4560, 5200, 5930, 6760 mg/kg****. Highest dose NS effects; LD50 calculated 5580 mg/kg.* ***Acute Oral: Similar*** *LD50 values; about 5600 mg/kg and 6400 mg/kg for younger and older adult rats, respectively.* ***Acute inhalation: males/ female rats, 6.08, 20.00, 23.98, 38.87, 61.80 mg/L, 4 hour:*** *LC50 > 20 mg/L (28.1 mg/L in males and females; 25.7 mg/L in males and 30 mg/L in females).* ***Inhalation, Toluene, mice****: conclusion: anxiolytic behavior.* ***~OECD 43, toluene, rats, inhalation, whole body, 4h. Nominal: 7, 31.6, 52.2, 78.3, 104.4 mg/L; Analysed: 6.08, 20.00, 23.98, 38.87, 61.80 mg/L):*** *toluene 6.08 mg/L: no adverse clinical signs; effects up to 3 hours post exposure* ***Acute dermal:*** ***Single acute dermal toxicity toluene in the rabbit:*** *LD50 value 14.1 mL/kg = 12267 mg/kg (using density 0.87). No information on clinical signs or mortality.*	After repeated dose exposure toluene case adverse effects including **impairment of auditory function and morphological evidence of cell loss** in the rat cochlea, neuron loss in the central nervous system of animals and in humans neuropsychological effects, auditory dysfunction and effects on color vision have been reported. ---------- ***Non-human exposure:*** ***Two studies, rats and mice, respectively; 312, 625, 1250, 2500, 5000 mg toluene/kg in corn oil, oral gavage, 13 weeks:*** *Deaths at 5000 mg/kg (in all rats and mice), 2500 mg/kg (8 male and 1 female rat; and 4/sex mice), and 1250 mg/kg (1 mouse).* *NS signs (rats or mice): at 5000 and 2500 mg/kg.* *In rats at 1250 and 2500 mg/kg neuropathology in brain (dentate gyrus, hippocampus, cerebellum.* *In male mice, relative brain increased at 5000 mg/kg. NOAEL for repeat dose oral toxicity 625 mg/kg in rats and mice.* ***Inhalation toluene, 15 week, rats, inhalation, 100, 625, 1250, 2500, 3000 ppm, 6.5 hours/day, 5 days/week):*** *Death at 3000 ppm (males, week 2). Lower body weights, adverse clinical signs and organ weights at 3000, 2500 and 1250 ppm. Plasma cholinesterase activity decreased with increase of concentration. NOAEC = 625 ppm.* ***Other two repeated dose toxicity studies in rats and one in mice****: No effects neurobehavior, or other NS effects.*	Toluene resulted neurobehavioral effects in the rats and hamster offspring and the effect becomes more stronger in the hamster offspring. ---------- ***Toluene vapor (800 mg/m3), 6 h daily, gestation days 14 to 20 (rats) and 6 to 11 (hamsters):*** *effects on exploratory neurobehavior; effects in hamster > rat.*

			Human information: *Effects on acute neurobehavioral performance at 75 ppm;* ***Human Exposure, constant level of 50 ppm toluene****: At 50 ppm no behavioral effects but on smell and throat. A NOAEC of 50 ppm (188 mg/m3) can be determined for acute neurobehavioural effects in humans.*	***Special studies addressing neurotoxicity and ototoxicity:*** *toluene is ototoxic in rat and can produce neurochemical and pathological changes. Data insufficient to determine NOAEC values.* ***Human information:*** ***- Toluene repeated dose inhalation humans****: number of adverse effects, i.e. neuropsychological effects, auditory dysfunction, disturbances color vision.* ***- Long-term exposure to toluene, 26 ppm, 21 years:*** *no evidence of effects on cognitive function.* ***- Occupational exposure, toluene, below 50 ppm (mean exposure 26 ppm / 98 mg/m3) in longitudinal study over 5 years:*** *no evidence of ototoxicity* ***- Toluene occupational exposures, follow up over 4 years, three repeated examinations****: no effect on color vision.* ***Together****, studies show 26 ppm (98 mg/m3) is a NOAEC for human adverse effects (Value taken in risk characterization).*	

CAS registry 100-42-5 Styrene C_8H_8 IUPAC name (preferred): ***Ethenylbenzene*** $C_6H_5CH=CH_2$ C6H5CHCH2 or C6H5CH=CH2 Other names: Styrene Vinylbenzene Phenylethene Phenylethylene Cinnamene Styrol Diarex HF 77 Styrolene Styropol	**Styrene (= ethenylbenzene**, **vinylbenzene**, **phenylethene)**, is a derivative of benzene; chemical formula $C_6H_5CH=CH_2$. Colorless oily liquid, evaporates easily, sweet smell. Styrene is an aromatic solvent widely used as a precursor for polystyrene plastics. Central nervous system is the critical target of toxicity for styrene. Both short- and long-term exposures to styrene can result in neurological effects. Like toluene, styrene can severely damage the auditory function in adult rats; its ototoxic potency is higher than that of toluene [(1)]. LD $_{50\ (median\ concentration)}$: 2194 ppm (mouse, 4 hr), 5543 ppm (rat, 4 hr)[2] LD $_{Lo\ (lowest\ published)}$: 10,000 ppm (human, 30 min) 2771 ppm (rat, 4 hr)[2] PEL (Permissible): TWA 100 ppm C 200 ppm 600 ppm (5-minute maximum peak in any 3 hours)[3] REL TWA 50 ppm (215 mg/m^3) ST 100 ppm (425 mg/m^3)[3] IDLH (Immediate danger): 700 ppm[3] *[(1)] ATSDR. 2010. Toxicological Profile for Styrene, Draft for Public Comment was released in October 2007. Agency for Toxic Substances and Disease Registry, Division of Toxicology and Environmental Medicine, Applied Toxicology Branch. November 2010.* *[(1)] NIOSH, 'Styrene'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *[(3)] NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0571'. National Institute for Occupational Safety and Health.*	Physical state: Liquid Physical state: Liquid VP: 6.7 hPa 20 °C Water solubility: 300 mg/L at 25 °C log Kow : 2.95 Dielectric Constant: 2.4 TLV-TWA: 20 ppm OT: 0.02-0.47 ppm FP: 90 F° ER: slow	The nervous system is most sensitive target of styrene toxicity in humans following **acute-duration inhalation exposure**. Inhalation symptoms are: dizziness, drowsiness, headache, nausea, vomiting, weakness, unconsciousness [(1)]. **Acute exposure data (humans)** are limited to the finding of impaired performance on tests of vestibular function in test subjects exposed to 87–376 ppm styrene for 1–3 hours and studies finding no alterations in performance of neurobehavioral tests (reaction time, color discrimination, and tests of memory or attention) in subjects exposed to 20 or 49 ppm **[(2)]**. Lowest LOAEL for a relevant end point in humans is 87 ppm for vestibular impairment in subjects exposed to styrene for 1 hour (Ödkvist et al. 1982) **[(2)]**. *[(1)] Compound Summary for CID 7501* *[(2)] ATSDR. 2010. Toxicological Profile for Styrene, Draft for Public Comment was released in October 2007. Agency for Toxic Substances and Disease Registry, Division of Toxicology and Environmental Medicine, Applied Toxicology Branch. November 2010.* References therein.	**Chronic duration exposure** studies provide strong evidence that the nervous system is the most sensitive target of styrene toxicity. It is likely that this would also be the most sensitive effect following **intermediate-duration exposure** (human neurotoxicity data absent) [(1)]. **Chronic exposure** to styrene in humans results in effects on the CNS, with symptoms such as headache, fatigue, weakness, depression, CNS dysfunction (reaction time, memory, visuomotor speed and accuracy, intellectual function), and hearing loss, peripheral neuropathy [(1)]. There are currently no biomarkers specific for the effects of styrene that are not also typical of other central nervous system depressants. Additional studies in mammalian animal models are needed to determine if styrene causes chronic damage to the central and/or peripheral nervous systems and to determine the associated mechanism of toxicity. Also, information is needed to determine if neurotoxicity is a sensitive end point from exposure to styrene via the oral route [(1)]. A variety of neurological effects have been observed in **chronically exposed styrene workers**: symptoms of neurotoxicity, particularly "feeling drunk" and tiredness, decreased color discrimination, slowed reaction time (≥21 ppm styrene), impaired performance on other neurobehavioral tests, permanent hearing threshold shifts, vestibular effects (at ≥18 ppm), EEG alterations, altered nerve conduction velocity, and increases in subjective symptoms (≥18 ppm). Hearing loss	The nervous system is the most sensitive target of styrene toxicity in adults, but no consistent information is obtained from children and young animals. *[(1)]* No adverse styrene-related effects were observed in neuro-behavioral function tests in rats exposed to styrene during gestation and lactation [(2)]; however, neurological effects have been observed in another inhalation study of rats exposed during gestation [(3)] and in an oral gestation and lactation study [(4)]. No human or animal data were located on the toxicokinetic properties of styrene in children or immature animals or possible age-related differences in the toxicokinetics of styrene[(1)]. No studies identified examining toxicity of styrene in children. No consistency observed in developmental effects reported in occupational exposure studies **(Ahlborg et al. 1987; Härkönen et al. 1984; Lemasters et al. 1989)** or in animal studies

				and alterations in nerve conduction velocity findings not consistent across studies [(1)]. Other neurological effects include alterations in astroglial cells in rats continuously exposed to 320 ppm for 3 months (Rosengren and Haglid 1989) and decreased sensory nerve conduction velocity in rats exposed to 2,000 ppm 8 hours/day, 5 days/week for 32 weeks (Yamamoto et al. 1997) [(1)]. The existing data are inadequate to determine whether chronic styrene exposure results in permanent damage. Mixed results have been found in studies examining workers before and after an extended period without styrene exposure [(1)]. Neurotoxicity studies in animals have primarily focused on effects on hearing and damage to the organ of Corti. Styrene disrupts the auditory system in both humans and animals[(2), (3)]. --------- **Campo et al., 2001** [(2)]: A time course experiment was carried out to study sequence of events which could explain the cochlear impairments. Male 6-month-old Long-Evans rats, 1000 ppm styrene, 6 h/day, 5 days/week, for either 1, 2, 3, or 4 consecutive weeks. Testing: auditory function by recording the near field evoked potentials from the inferior colliculus; histological analyses of the cochleae with light and transmission electron microscopy. Results: electrophysiology supports a toxic mid-frequency process, which keeps worsening even after the end of the exposure. Histology: supporting cells are the	**(Cruzan et al. 2005b; Daston et al. 1991; Kankaanpää et al. 1980; Murray et al. 1978)[(1)]**. Additional studies are needed to examine the potential effects on the nervous systems of developing organisms. No human or animal data were located on the toxicokinetic properties of styrene in children or immature animals or possible age-related differences in the toxicokinetics of styrene[(1)]. --------- **Cruzan et al. 2005** [(2)]: Exposure-related developmental and neuromotor changes identified in F2 pups from dams exposed to 500 ppm occurred in endpoints known to be both age- and weight-sensitive parameters, and were observed in the absence of any other remarkable indicators of neurobehavioral toxicity. Thus, exposure level of 50 ppm was considered to be the NOAEL for growth of F2 offspring; an exposure level of 500 ppm was

				first targets of the solvent. Then, the outer hair cells of the third row (OHC3) are disrupted, followed successively by OHC2 and OHC1 from the basal (20 kHz) to the upper turn (4 kHz) of the cochlea. **Basically, the disorganization of the membranous structures could be the starting point for the cochlear injury induced by styrene**. This paper presents a hypothesis that the accumulation of K+ in the spaces of Nuel underlies the toxic effects of styrene. **Loqueta et al., 1999 [3]:** Styrene and toluene *both* can severely damage the auditory function in adult rats. Study in adult rats, 500 to 1500 ppm styrene, or, 1000 to 2000 ppm toluene; same schedule of exposure: 6 hours/day, 5 days/week, 4 weeks. Testing: auditory function by recording evoked potentials from the inferior colliculus over frequency range from 2-32 kHz; pathology by conventional histologic techniques. Results: **permanent threshold shifts (PTS) were obtained with a styrene dose 2.4 times lower than that of the toluene.** The slope of the regression line (PTS/doses) was 2.1 steeper with styrene than with toluene. The sequence of histopathological events along the organ of Corti, especially the orderliness and the location of the traumas, was similar for paired concentrations of styrene and toluene (respectively 650 ppm, 1500 ppm for the first match, and 850 ppm, 1750 ppm for the second one). **Both electrophysiological and histological findings point out the higher ototoxic potency of the styrene compared to that of the toluene.**	considered to be the NOAEL for F2 developmental neurotoxicity. [1] **Katakura et al. 2001 [3]**: Another inhalation study found impaired righting reflex in the offspring of rats exposed to 300 ppm during gestation. **Zaidi et al., 1985 [4]:** Similarly, impaired amphetamine-induced locomotor activity and apomorphine-induced stereotypy were observed in the offspring of rats orally administered 200 mg/kg/day styrene during gestation and lactation. --- [1] ***ATSDR. 2010.*** *Toxicological Profile for Styrene, Draft for Public Comment was released in October 2007. Agency for Toxic Substances and Disease Registry, Division of Toxicology and Environmental Medicine, Applied Toxicology Branch. November 2010.* See references therein. [2] ***Cruzan G , Faber WD, Johnson KA, Roberts LS,***

				[1] *ATSDR. 2010. Toxicological Profile for Styrene, Draft for Public Comment was released in October 2007. Agency for Toxic Substances and Disease Registry, Division of Toxicology and Environmental Medicine, Applied Toxicology Branch. November 2010.* References therein. [2] *Campo P, Lataye R, Loquet G, Bonnet P. 2001. Styrene-induced hearing loss: a membrane insult. Hearing Research 154: 170-180.* [3] *Loqueta G, Campo P, Lataye R. 199. Comparison of Toluene-Induced and Styrene-Induced Hearing Losses. Neurotoxicology and Teratology 21 (6): , November–December 1999, 689–697.*	***Hellwig J, Maurissen J, Beck MJ, Radovsky A, Stump DG.** (2005) Developmental neurotoxicity study of styrene by inhalation in Crl-CD rats. Birth Defects Res B Dev Reprod Toxicol. 2005 Jun;74(3):221-32.* [3] ***Katakura Y, Kishi R, Ikeda T, et al.** 2001. Effects of prenatal styrene exposure on postnatal development and brain serotonin and catecholamine levels in rats. Environ Res 85 (1): 41-47.* [4] ***Zaidi NF, Agrawal AK, Srivastava SP,** et al. 1985. Effect of gestational and neonatal styrene exposure on dopamine receptors. Neurobehav Toxicol Teratol 7:23-28.*

CAS registry 1330-20-7 (technical) 95-47-6 (o-) 108-38-3 (m-) 106-42-3 (p-) Xylenes C_8H_{10} IUPAC name (systematic): ***Dimethylbenzene*** $(CH_3)_2C_6H_4$ Other names: **Xylene** **Xylol**	**Xylene (=xylol or dimethylbenzene)** is any one of three isomers of dimethylbenzene, or a combination thereof. With the formula $(CH_3)_2C_6H_4$, each of the three compounds has a central benzene ring with two methyl groups attached at substituents. The mixture is referred to as both xylene and, more precisely, xylenes. Xylene exists in three isomeric forms, distinguished by the designations ortho- (o-), meta- (m-), and para- (p-), which specify to which carbon atoms (of the benzene ring) the two methyl groups are attached. The primary effects of xylene exposure involve the nervous system by all routes of exposure, the respiratory tract by inhalation exposure, and, at higher oral exposure levels, hepatic, renal, and body weight effects. No adverse health effects have been associated with the background levels of xylene to which the general population is typically exposed. Isomers of xylene have similar toxicokinetic properties and elicit similar toxicological effects, with no single isomer consistently exhibiting the greatest potency, depending on the end point. The chemical and physical properties of xylene differ according to the respective isomers. The melting point ranges from −47.87 °C (−54.17 °F) (m-xylene) to 13.26 °C (55.87 °F) (p-xylene). The boiling point for each isomer is around 140 °C (284 °F). The density of each isomer is around 0.87 g/mL (7.26 lb/U.S. gallon or 8.72 lb/imp gallon) and thus is less dense than water. Xylene in air can be smelled at concentrations as low as 0.08 to 3.7 ppm (parts of xylene per million parts of air) and can be tasted in water at 0.53 to 1.8 ppm.	Physical state: Liquid VP: 11.05 hPa m-xylene 8.81 hPa o-xylene 11.79 hPa p-xylene 25 °C Water solubility: 161, 178, 162 mg/L (25°C), m, o-, p-xylene, respectively Log Kow: 3.2, 3.12, 3.15, m, o, p-xylene, respectively Dielectric Constant: 2.3 TLV-TWA: 100 ppm OT: 0.08-0.40 ppm FP: 20 F° ER: slow	**Acute Toxicity to Humans** The principal systemic effects of acute xylene exposure are on the Central Nervous System (CNS) –although it is also a respiratory and eye irritant) – as was shown by numerous studies with healthy human volunteers [(1)]. At **acute-duration** inhalation concentrations as low as 50 ppm, xylenes produce mild central nervous system effects, including headache and dizziness [(1)] Increases in subjective reports of neurological effects (anxiety, forgetfulness, inability to concentrate, and a sensation of intoxication) were noted following **chronic-duration** occupational exposure at 14 ppm [(1)]. With increasing airborne xylene concentrations of 100– 400 ppm, other neurological effects reported in **acutely exposed human subjects** include retardation of response times and impairments in memory and body balance [(1)]. **Acute exposure** to an estimated 10,000 ppm xylenes elicited tremors, mental confusion, and depressant effects (narcosis) on the central nervous system that caused at least one fatality due to respiratory failure [(1)]. *[(1)] 'Xylene: Material Safety Data Sheet'.*	The neurotoxicity of xylenes has been examined in **short- and long-term inhalation studies in humans and animals** and **acute-duration oral studies in animals** and appears to be related to the interference of un-metabolized xylene with neuronal membranes. [(1)] The effects included narcosis, prostration, incoordination, tremors, muscular spasms, and labored respiration. All three xylene isomers elicited biphasic response rates in operant behavior studies in mice exposed for 30 minutes: increased responses at ≥1,400 ppm and half-maximal responses at 5,179–6,176 ppm. Motor coordination was impaired at concentrations above 2,000 ppm for the para isomer and above 3,000 ppm for the meta and ortho isomers. [(1)] Animal studies have shown that **oral exposure to xylenes at high concentrations** (single doses of ≥4,000 mg/kg or **repeated dosing** at 2,000 mg/kg/day for 2 weeks) may result in nervous system effects such as tremors, respiratory depression, weakness, lethargy, **unsteadiness**, and hyperactivity. **Hyperactivity** was also observed in all mice after oral dosing with 1,000 mg/kg mixed xylene during weeks 4–103. Rats dosed with p-xylene, but not m-or o-xylene at 900 mg/kg/day, 5 days/week for 2 weeks, experienced significant **loss of cochlear hair cells associated with hearing at medium frequencies** (10–25 kHz). [(1)] *[(1)] 'Xylene: Material Safety Data Sheet'. West Liberty University. Retrieved 16 December*	Animal studies on effects of xylenes on neurodevelopment showed transient alteration in learning and memory abilities [(1)]. --------- **Hass et al., 1995 a [(1)]; Hass *et al.*, 1997 [(2)]:** Persistence of neuro-behavioral effects in offspring of female rats (Mol:WIST), 500 ppm technical xylene, 6 hours/day, on days 7-20 of prenatal development. Result. Dose not maternally toxic and not decreasing viability of offspring. Learning and memory abilities with spatial navigation on a water maze were impaired at 16, 28 and 55 weeks of age, but no more at 55 weeks. Authors suggested results were compatible with two different conclusions: 1) effect was partly reversible over a long time period, or 2) practice at solving the problem led to compensation over unresolved neurotoxic effects. *[(1)] Hass U, Lund SP,*

	LD_{50} ranges from 200 to 5000 mg/kg for animals. Oral LD_{50} for rats is 4300 mg/kg.[1] *(1) 'Xylene: Material Safety Data Sheet'. West Liberty University. Retrieved 16 December 2013.*		*West Liberty University. Retrieved 16 December 2013.*	*2013.* References therein. --- **Mirkova E, Zaikov C, Antov G, Mikhailova A, Khinkova L. Benchev I. 1983.** (2) Prenatal inhalation toxicity of xylene (industrial mixture of isomers) studied in white Wistar rats, exposed daily (6 hr/day, 5 days/week) to 10, 50 (MAC for xylene in the air of work environment in Bulgaria) and 500 mg.m-3 throughout the period of gestation from first to 21st day. Both routine teratological indices and biochemical and physiological methods of observation used to evaluate the integrity of the individual organs - liver, brain, lungs and myocardium of the generation in the postnatal period of development. Concentrations of 50 and 500 mg.m-3 xylene, exhibit pronounced embryotoxic and teratogenic effects, among which are (but not limited to): delayed physical development; incidence of induced hydrocephalus enhanced, the processes of ossification of skull are impaired. In concentrations of 50 and 500 mg.m-3, xylene causes disturbances in postnatal development of F1 generation. The concentration of 50 mg.m-3 is the threshold of the embryotropic effect of the solvent. *(2) Mirkova E, Zaikov C, Antov G, Mikhailova A, Khinkova L. Benchev I. 1983. Prenatal toxicity of xylene. J Hyg Epidemiol Microbiol Immunol. 983: 27(3): 337-343.*	*Simonsen L. Long-lasting neurobehavioral effects of prenatal exposure to xylene in rats. 1995a. Neurotoxicology 1995; 16 (4): 761.* *(2) Hass U, Lund SP, Simonsen L. Long-lasting neurobehavioral effects of prenatal exposure to xylene in rats. Neurotoxicology 1997; 18(2): 547-551.* **Hass et al., 1995b** (3) **:** Effects of prenatal exposure on postnatal development and behavior in rats were studied. Pregnant rats (Mol:WIST), exposure 500 ppm technical xylene (dimethylbenzene, CAS-no 1330-20-7), for 6 h/day, on gestation days 7 to 20. Dose level selected not to induce maternal toxicity or decrease of viability of offspring. Results. In exposed offspring, delay in ontogeny of air righting reflex, lower absolute brain weight, and impaired performance in behavioral tests for neuromotor abilities and learning and memory; most marked in female

					offspring. *Hass U, Lund SP, Simonsen L, Fries AS. 1995b. Effects of prenatal exposure to xylene on postnatal development and behavior in rats. Neurotoxicol Teratol. 17 (3): 341-349.* **Hass and Jakobsen (1993) [4]:** Technical xylene (cas. nr. 1330-20-7) was investigated for development toxicity in a teratology and in a postnatal study. Rats (Mol: WIST), exposed to 500 ppm, 6 hr /day, on days 4 to 20 of gestation. Result. No signs of maternal toxicity. In teratology study, no exposure-related differences except delayed ossification of os maxillare. In postnatal study, xylene-exposed pups had a higher body weight and impaired performance on a motor ability test (Rotarod). Due to the possibility of direct toxic effects of xylene on the developing central nervous system, further studies are needed to investigate dose-effect relationship for this type

					of effects. [4] *Hass U. and Jakobsen B. M. 1993. Prenatal toxicity of xylene inhalation in the rat: a teratogenicity and postnatal study. Pharmacol Toxicol. 73 (1): 20-23.* **Edelfors *et al.*, 1996:** Rats of the same strain (Mol: WIST) exposed prenatally to the same regimen did not show any differences from control rats in synaptosomal cytosolic calcium concentration.

100-41-4 Ethyl Benzene C_8H_{10} IUPAC name ***Ethylbenzene*** $C_6H_5CH_2CH_3$ Other names: Ethyl benzol Phenylethane: *alpha*-Methyltoluene EB	**Ethylbenzene** is a monocyclic aromatic hydrocarbon with the formula $C_6H_5CH_2CH_3$. Highly flammable, colorless liquid; odor like gasoline. It is an intermediate in the production of styrene, the precursor to polystyrene, a common plastic material. Once inside the body, ethylbenzene biodegrades to 1-phenylethanol, acetophenone, phenylglyoxylic acid, mandelic acid, benzoic acid and hippuric acid. [1] Ethylbenzene exposure can be determined by testing for the breakdown products in urine. *(1) Welch VA, Fallon KJ, Gelbke H-P. 2005. "Ethylbenzene" Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim, 2005. doi:10.1002/14356007.a10_035.pub2* LD $_{50\ (median\ dose)}$: 5460 mg/kg LD $_{Lo\ (lowest\ published)}$: 4000 ppm (rat, 4 hr)(2) PEL (Permissible): TWA 100 ppm (435 mg/m^3) (3) REL (Recommended): TWA 100 ppm (435 mg/m^3) ST 125 ppm (545 mg/m^3) (3) IDLH (Immediate danger): 800 ppm(3) *(2) NIOSH, 'Ethylbenzene'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *(3) NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0264'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 12.80 hPa 25 °C Water solubility: 0.015 g/100 mL (20 °C) Log Kow: 3.15 Dielectric Constant: (68 °F) 2.5 TLV-TWA: 100 ppm OT: 0.0-6 ppm FP: 29 °F ER: slow	Exposure to high levels of ethylbenzene in air for short periods can cause eye and throat irritation. Exposure to higher levels can result in dizziness. [1] *(1) ATSDR. "Ethylbenzene" (PDF). Agency for Toxic Substances and Disease Registry. Retrieved 12 February 2013*	The potential for neurotoxicological effects of **ethylbenzene** was studied in young adult Crl:CD(SD) rats following 90-day oral (neurotoxicity) exposures. Neurotoxicity study (90-day oral, gavage), twice daily, 0, 25, 125, or 250 mg/kg ethylbenzene per dose (total daily dosages of 0, 50, 250, or 500 mg/kg bw/day [mg/kg bw/day]) for 13 weeks; functional observational battery (FOB), automated tests for motor activity and neuropathological examination conducted. Results: No-observed-adverse effect level (NOAEL) for adult neurotoxicity was the highest dose tested 500 mg/kg bw/day. (1) *(1) Li AA , Maurissen JP, Barnett JF Jr, Foss J, Freshwater L, Garman RH, Peachee VL, Hong SJ, Stump DG, Bus JS. (2010). Oral gavage subchronic neurotoxicity and inhalation subchronic immunotoxicity studies of ethylbenzene in the rat. Neurotoxicology. 2010 Jun; 31(3):247-58. doi: 10.1016/j.neuro.2010.02.001.*	**"**There are no studies evaluating the effects of ethylbenzene exposure on children or immature animals. It is likely that children would have the same health effects as adults. We do not know whether children would be more sensitive than adults to the effects of ethylbenzene. " (1) "We do not know if ethylbenzene will cause birth defects in humans. Minor birth defects and low birth weight have occurred in newborn animals whose mothers were exposed to ethylbenzene in air during pregnancy." (1) https://www.atsdr.cdc.gov/toxfaqs/tfacts110.pdf(1) *Agency for Toxic Substances and Disease Registry (ATSDR). 2010. Toxicological Profile for Ethylbenzene. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.* **Faber et al., 2007 (2):** No developmental neurotoxicity in 2-generation offspring: Evaluation of potential adverse effects of whole

					body inhalation exposure of F0 and F1 parental animals from a 2-generation reproduction study of **ethylbenzene** on nervous system functional and/or morphologic end points in the F2 offspring from four groups of male and female Crl:CD (SD)IGS BR rats; 0, 25, 100, and 500 ppm ethylbenzene, 6H/day, at least 70 consecutive days prior to mating for the F0 and F1 generations. Results: In current developmental neurotoxicity component, parental ethylbenzene exposure did not adversely affect offspring survival, clinical condition, body weight parameters, or acquisition of developmental landmarks of the F2-generation treatment derived offspring. **There were no alterations in FOB parameters, motor activity counts, acoustic startle endpoints, or Biel water maze performance in offspring attributed to parental ethylbenzene exposure.** A few isolated instances of statistically significant differences

					obtained in the treatment-derived groups occurred sporadically, and were attributed to unusual patterns of development and/or behavior in the concurrent control group. There were **no exposure-related differences in any neuropathology parameters in the F2-generation treatment derived offspring.** The no observed adverse effect level (NOAEL) for maternal reproductive toxicity, developmental toxicity, and developmental neurotoxicity in this study was considered to be 500 ppm/342 mg/kg/day ethylbenzene, the highest exposure level tested in the study. [1] [2] *Faber WD , Roberts LS, Stump DG, Beck M, Kirkpatrick D, Regan KS, Tort M, Moran E, Banton M. 2007. Inhalation developmental neurotoxicity study of ethylbenzene in Crl-CD rats. Birth Defects Res B Dev Reprod Toxicol. 80 (1): 34-48.*

2078-54-8 Propofol 2,6-diiso-propylphenol $C_{12}H_{18}O$	Propofol (=2,6-diiso-propylphenol) is an organic compound in the aromatic hydrocarbon class with the formula ($C_6H_{14}O$). Propofol is the most commonly used intravenous agent in current anesthesia practice. **It is noted that the US FDA has set propofol to 'pregnancy category: B': 'Animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women'.** Thus, in *humans* propofol is considered safe to mature and developing nervous system. Recent *animal* studies in neonates and fetuses, however, has raised some concern for the developing nervous system. Propofol-induced apoptosis of neurons and oligodendrocytes was observed during brain development, in rats, mice (in infant mice at one fourth the dose required for surgical anesthesia) and rhesus macaques (after propofol anesthesia for 5 hours). Acute Toxicity: Oral LD50: Category 4. ATE = 300 - 2000 mg/kg. Dermal LD50: on ATE data, the classification criteria are not met. ATE > 20 mg/L Vapor LD50: Based on ATE data, the classification criteria are not met. ATE > 2000 mg/kg. [1] *[1] Fisher Scientific. 2,6-diiso-propylphenol. Safety Data Sheet. Revision number 1. Revision Date 10-Feb-2015* *https://www.fishersci.com/shop/msdsproxy?storeId=10652&productName=AC115251000&productDescription=2*	Physical state: Liquid VP: 7.47 hPa 100 °C Water solubility: Insoluble in water; oil-in-water emulsion with a pKa of 11 Kow: 6761 at a pH of 6-8.5. Dielectric Constant: No data available	**Human**: generally considered safe in a mature brain. [1] Currently most commonly used general anesthetic. *[1] FDA. 'Propofol'. FDA prescribing information, side effects and uses. Revised: 06/2016. Hospira, Inc. (141588017)* *https://www.drugs.com/pro/propofol.html*	**Human**: generally considered safe in a mature brain. [1] Currently most commonly used general anesthetic. *[1] FDA. 'Propofol'. FDA prescribing information, side effects and uses. Revised: 06/2016. Hospira, Inc. (141588017)* *https://www.drugs.com/pro/propofol.html*	**Studies in humans**: There is no direct evidence in *humans* for propofol induced neurotoxicity to the *developing* nervous system (however, not that well studied). Otherwise, in the early days of its use, some conflicting literature has appeared as well, about the safety and serious adverse effects of propofol [1] **Studies in animals:** Animal studies (within past ten years) showed that propofol exposure induces similar neurotoxicity in **neonatal animals** of various species including rhesus monkey. Observed neuro-degeneration is associated with significant long-term behavioral deficits. Recent animal studies demonstrated that **prenatal exposure** of propofol in animals may trigger similar or more pronounced neuro-degeneration than that in neonatal animals. [2]

					[(1)] *Chidambaran V, Costandi A, D'Mello A (2015). Propofol: a review of its role in pediatric anesthesia and sedation. CNS Drugs; 29(7):543-563. doi:10.1007/s40263-015-0259-6.* References therein. [(2)] *Xiong M, Zhang L, Li J, Eloy J, Ye JH, Bekker A (2016). Propofol-Induced Neurotoxicity in the Fetal Animal Brain and Developments in Modifying These Effects—An Updated Review of Propofol Fetal Exposure in Laboratory Animal Studies. Brain Sci.; 6: 11. doi:10.3390/brainsci6020011* References therein.

Halogenated hydrocarbons	A halogen atom has replaced one or more hydrogen atoms on the hydrocarbon ***Trichloroethylene*** ***1-bromopropane***				
201-167-4 Trichloroethylene C_2HCl_3 IUPAC name: ***trichloroethene*** Other name(s): *1,1,2-Trichloroethene, 1,1-Dichloro-2-Chloroethylene, 1-Chloro-2,2-Dichloroethylene, Acetylene Trichloride, TCE, Trethylene, Triclene, Tri, Trimar, Trilene, HCC-1120*	**Trichloroethylene (=trichloroethene** (C_2HCl_3)) is a halocarbon (not to be confused with the similar 1,1,1-trichloroethane, known as chlorothene). The IUPAC name is trichloroethene. Industrial abbreviations include TCE, trichlor, Trike, Tricky and tri. Under the trade names Trimar and Trilene, trichloroethylene was used as a volatile anesthetic and as an inhaled obstetrical analgesic in millions of patients. LD $_{50\ (median\ dose)}$: 8450 ppm (mouse, 4 hr), 26300 (rat, 1 hr)[1] LD $_{Lo\ (lowest\ published)}$: 2900 ppm (human), 37,200 ppm (guinea pig, 40 min), 5952 ppm (cat, 2 hr), 8000 ppm (rat, 4 hr) 11,000 (rabbit)[1] PEL (Permissible): TWA 100 ppm C 200 ppm 300 ppm (5-minute maximum peak in any 2 hours)[2] REL: Ca[2] IDLH (Immediate danger): Ca [1000 ppm][2] *(1) NIOSH, 'Trichloroethylene'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *(2) NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0629'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 99 hPa 25 °C Water solubility: 1.1 g/L at 20 °C Log Kow: 2.53 Dielectric Constant: 3.2 TLV-TWA: 10 ppm OT: 82 ppm ER: med	No detailed information provided on the effect of the substance after acute exposure.	**No neurotoxicity effects** observed after long term repeated exposure to trichloroethylene.	Information from literature search showed **conflicting results**. One study concluded that trichloroethylene is developmental neurotoxicant. Similarly another prenatal study showed inflammatory and oxidative stress related effects which associated with neurotoxicity and also indicated that plasma level biomarkers can be used to predict trichloroethylene mediated neurotoxicity effects. While another PNDT study via oral gavage from GD 6 to GD 10 doses up to 750 mg/kg bw/day did not result neurobehavioural and neuropahological effects in the offspring.

203-445-0 1-bromopropane C_3H_7Br IUPAC name: ***1-Bromopropane*** $CH_3CH_2CH_2Br$ Other name(s): *Bromopropane* *1-propyl bromide* *1-bromopropane* *n-propyl bromide*	**1-Bromopropane (=n-propylbromide (nPB))** is an organobromine compound with the chemical formula $CH_3CH_2CH_2Br$. $LD_{50\ (median\ dose)}$: 2.950 mg kg^{-1} (intraperitoneal, rat) Reported symptoms of overexposure affect the nervous system and include confusion, slurred speech, dizziness, paresthesias, and difficulty walking, unusual fatigue and headaches, development of arthralgias, visual disturbances (difficulty focusing), and muscle twitching. Symptoms may persist over one year [8] Other symptoms include irritation of mucous membranes, eyes, upper respiratory tract, and skin, as well as transient loss of consciousness.[7] Loss of feeling in the feet, an example of paresthesia, is colloquially called "dead foot" by workers who suffer from it.[5] Of nationwide "more than 140 cushion workers nationwide, mostly from plants in Utah, Mississippi and North Carolina,[...] that had been exposed to dangerous levels of the chemical, many of them sickened and [are] unable to walk".[5] One worker's long-term exposure resulting in neurological damage was covered in the NY Times.[5] Air sampling for the level of 1-bromopropane and monitoring workers' urine for metabolites are both effective at measuring workers' exposure.[7] *- 5. Ian Urbina (March 30, 2013). "As OSHA Emphasizes Safety, Long-Term Health Risks Fester". The New York Times. Retrieved March 31, 2013.* *- 6. "Hazard Alert: 1-Bromopropane" (PDF). DHHS (NIOSH) Publication Number 2013-150. National Institute for Occupational Safety and Health. July 2013. Retrieved 17 January 2015.* *- 7. Trout, Doug; Hudson, Naomi; Dotson, Scott; Hanley, Kevin (1 August 2013). "1-Bromopropane". National Institute for Occupational Safety and Health. Retrieved 16*	Physical state: Liquid VP: 182.65 hPa 25 °C Water solubility: 2 500 mg/L Log Kow: 2.16 Dielectric Constant: 7.2	Acute toxicity study in rats with 1-bromopropane via inhalation results **sedation**, lateral decubitus, dyspnea and piloerection, and via oral results effects related to **CNS depression**.	Repeated dose toxicity studies in rats with 1-bromopropane for 12 weeks via inhalation; results: **neurobehavioral effects.**	Information from literature showed that 1-bromopropane **affects the neurobehavioral responses** during juvenile period.

	January 2015. *- 8. "Neurologic Illness Associated with Occupational Exposure to the Solvent 1-Bromopropane --- New Jersey and Pennsylvania, 2007--2008". Centers for Disease Control. December 5, 2008. Retrieved March 31, 2013.*				

Ethers Ethers R-O-R'	Contain a C-O-C binding ***diethyl ether*** ***diisopropyl ether*** ***2-methoxy-2-methylbutane***				
200-467-2 Diethyl ether ether $C_6H_{14}O$ IUPAC name ***Ethoxyethane*** Other names: Diethyl ether Ether Ethyl ether Ethyl oxide 3-Oxapentane Ethoxyethane Diethyl oxide Solvent ether Sulfuric ether	**Diethyl ether** or simply **ether**, is an organic compound in the ether class with the formula ($C_6H_{14}O$). Formerly used as a general anesthetic. **Metabolism**: A cytochrome P450 enzyme is proposed to metabolize diethyl ether. Diethyl ether inhibits alcohol dehydrogenase, and thus slows the metabolism of ethanol. It also inhibits metabolism of other drugs requiring oxidative metabolism. LD $_{50\ (median\ concentration)}$: 73,000 ppm (rat, 2 hr); 6500 ppm (mouse, 1.65 hr)[1] LD $_{Lo\ (lowest\ published)}$: 106,000 ppm (rabbit) 76,000 ppm (dog)[1)] PEL (Permissible): TWA 400 ppm (1200 mg/m^3)[2] REL (Recommended): No established REL[2] IDLH (Immediate danger): 1900 ppm[2] *[(1)] NIOSH, 'Ethyl ether'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *[(2)] NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0277'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 716 hPa 25 °C Water solubility: 64.9 g/L at 20 °C Log Pow: 0.83 Dielectric Constant: 4.0	**Convulsions** from acute inhalation study up to 120 minutes. **Acute inhalation study in adults and neonates for 170 minutes showed neonates are less sensitive than adults to diethyl ether.** --------- ***Inhalation, mice, 120 min, 2.7, 2.9, 3.2, 3.5, 3.8, 4.6 vol% (males) and 2.7, 2.9, 3.2, 3.5, 3.8, 4.2, 4.6 vol% (females):*** *convulsions at induction of anesthesia.* ***Inhalation, adult females and neonates (males and females), 170 min, 15vol% and 20vol%:*** *Higher blood concentration of diethyl ether in neonates than adults, reflecting longer exposure period. Compared to adults, neonates have decreased sensitivity to CNS than adults.*	Repeated dose toxicity studies do not report effects related to neurotoxicity**. Light anesthesia in 13wk rat study.** --------- ***Oral gavage, rat, 500, 2000, 3500 mg/kg bw, 13 weeks:*** *Light anesthesia at 2000 and 3500 mg/kg bw.* ***OECD TG 413, diisopropyl ether (EC no: 203-560-6), inhalation, test concentration (480 ppm, 3300ppm, 7100ppm):*** *no changes clinical signs.* ***Other repeated dose inhalation toxicity studies with RA substances*** *no neurotoxicity effects.* ***TK study****: showed distribution of diethyl ether in brain.*	No information found from literature search on the effect of diethyl ether on neurodevelopmental effect.

203-560-6 Diisopropyl ether $C_6H_{14}O$ IUPAC name ***2-[(Propan-2-yl)oxy]propane*** Other names: 2-Isopropoxypropane Diisopropyl oxide	**2-[(Propan-2-yl)oxy]propane (**=Diisopropyl ether; 'DIPE') is a secondary ether with formula $C_6H_{14}O$. LD $_{50\ (median\ dose)}$: 8470 mg/kg (rat, oral)[1] LD $_{Lo\ (lowest\ published)}$: 5000-6500 mg/kg (rabbit, oral)(1) LD $_{50\ (median\ concentration)}$: 38,138 ppm (rat); 30,840 ppm (rabbit); 28,486 ppm (rabbit)(1) PEL (Permissible): TWA 500 ppm (2100 mg/m^3)2) REL TWA 500 ppm (2100 mg/m^3)[2] IDLH (Immediate danger): 1400 ppm[2] *(1) NIOSH, 'Isopropyl ether'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *(2) NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0362'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 198.6 hPa 25 °C Water solubility: 3.11 g/L at 20.2 °C Log Pow: 2.4 Dielectric Constant: no information found	Narcosis reported from acute inhalation studies. --------- ***Acute oral toxicity, rabbits, gavage, 1.62, 3.3, 5.2, 6.0, 7.2, 8.2 g/kg bw/day:*** *Lack of coordination and unsteadiness from 5 to 20 min, followed by intoxication and light narcosis from 14 to 40 minutes. > 7.2 to 8.2 g isopropyl ether/kg bw, more rapid onset of symptoms, deeper narcosis.* ***Inhalation toxicity in monkeys, rabbits, and guinea pigs at 0.1, 0.3, 1.0, 3.0, or 6.0% for 180 minutes:*** *anesthesia signs in all species at 1.0 and 3.0% isopropyl ether (= 10000 and 30000 ppm); death at 6.0%.*	No report on effects on the NS from sub-chronic inhalation exposure to 3300 ppm diisopropyl ether. --------- ***~ OECD TG 413, rats, 480, 3300 and 7100ppm):*** *No effects on NS.*	No information found from literature search on the effect of diethyl ether on neurodevelopment.
213-611-4 2-methoxy-2-methylbutane $C_6H_{14}O$ IUPAC name: ***2-methoxy-2-methylbutane*** $(CH_3)_3CCH_2CH(CH_3)_2$ Other name(s): *tert*-Amyl-methylether, TAME	**2-methoxy-2-methylbutane** ($C_6H_{14}O$) is an **ether** produced by reaction of methanol with 2-methyl-2-butene and/of 2-methyl-1-butene.	Physical state: Liquid VP: 91 hPa 25 °C Water solubility: 10.4 g/L at 20 °C Log Pow: 1.55	Acute toxicity study in rats with 2-methoxy-2-methylbutane via inhalation or oral routes results in **CNS depression**. ***~OECD TG 401, 2-methoxy-2-methylbutane, rats, oral, gavage, 2000, 2500 and 3000 mg/kg.*** ***~OECD TG 403, 2-methoxy-2-methylbutane, rats, inhalation, whole body, 4h, 5400 mg/m³.***	Repeated dose toxicity studies in rats with 2-methoxy-2-methylbutane via inhalation for 13 weeks resulted **clinical effects related to CNS depression.** ***~OECD TG 407, 2-methoxy-2-methylbutane, rats, oral, gavage, 0.125, 0.5, 1.0 g/kg bw/day), 29 days.*** ***EPA OTS 798.2450 (90-Day Inhalation Toxicity), 2-methoxy-2-methylbutane, Fischer 344 rats, inhalation, whole body, 13 weeks, 250, 1500, 3500 ppm.***	No information found from literature search on the effect of 2-methoxy-2-methylbutane on neurodevelopment.

Esters Esters R-COOR'	Formed by interaction of an organic acid with an alcohol ***methyl acetate*** ***ethyl acetate*** ***propyl acetate*** ***isopropyl acetate*** ***n-butyl acetate*** ***2-ethoxy-1-methylethyl acetate***				
CAS nr 201-185-2 methyl acetate $C_3H_6O_2$ IUPAC name: (preferred) ***Methyl acetate*** (systematic) ***Methyl ethanoate*** CH_3COOCH_3 Other name(s): Methyl ester of acetic acid	**Methyl acetate (=MeOAc; acetic acid methyl ester;** or **methyl ethanoate)** is a carboxylate ester with the formula CH_3COOCH_3. LD $_{50\ (median\ dose)}$: 3700 mg/kg (oral, rabbit)[1] LD $_{Lo\ (lowest\ published)}$: 11,039 ppm (mouse, 4 hr), 21,753 ppm (cat, 1 hr), 32,000 ppm (rat, 4 hr))[1] PEL (Permissible): TWA 200 ppm (610 mg/m^3)(1) REL TWA 200 ppm (610 mg/m^3) ST 250 ppm (760 mg/m^3)[3] IDLH (Immediate danger): 3100 ppm[3] *(1) NIOSH, 'Methyl acetate'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *(2) NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0391'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 28 hPa 20 °C Water solubility: 243.5 g/L at 20 °C Log Pow: 0.18 Dielectric Constant: 8.0 TLV-TWA: 200 ppm OT: 4.6 ppm FP: 14 F° ER: fast	Acute toxicity studies in rats with methyl acetate via oral reported **no effects related to neurotoxicity.**	Repeated dose toxicity study in rats with methyl acetate via inhalation results **no effects related to neurotoxicity.**	No information found from literature search on the effect of methyl acetate neurodevelopment.

205-500-4 ethyl acetate $C_4H_8O_2$ IUPAC name: (preferred) ***Ethyl acetate*** (systematic) ***Ethyl ethanoate*** CH_3-COO-CH_2-CH_3 Other name(s): Acetic ester Acetic ether Ethyl ester of acetic acid	**Ethyl acetate** (abbreviated EtOAc or EA) is the organic compound with formula CH_3-COO-CH_2-CH_3, simplified to $C_4H_8O_2$. Ethyl acetate is the ester of ethanol and acetic acid.	Physical state: Liquid VP: 98.3 hPa 20 °C Water solubility: 83.1g at 20 °C Log Pow: 0.68 Dielectric Constant: 6.0 TLV-TWA: 400 ppm OT: 3.9 ppm FP: 24 F° ER: fast	Acute toxicity inhalation studies in mice and rabbits treated with ethyl acetate; results: **narcosis.**	Repeated dose inhalation toxicity study in rats with ethyl acetate; results: effects reported as **acute sedative effect**.	No information found from literature search on the effect of ethyl acetate neurodevelopment.

203-686-1 Propyl acetate $C_5H_{10}O_2$ IUPAC name: (preferred) ***Propyl acetate*** (systematic) ***Propyl ethanoate*** $C_5H_{10}O_2$ Other names *Acetic acid propyl ester* *n-Propyl ethanoate* *n-Propyl acetate* *n-Propyl ester of acetic acid*	**Propyl acetate** (=propyl ethanoate) is an ester, formed by the esterification of acetic acid and 1-propanol. LD $_{50\ (median\ dose)}$: 9370 mg/kg (oral, rat), 8300 mg/kg (oral, mouse), 6640 mg/kg (oral, rabbit), 8700 mg/kg (oral, rat)[1],17800 mg/kg (dermal, rabbit)[2] LD $_{Lo\ (lowest\ published)}$: 8941 ppm (cat, 5 hr)[1] PEL (Permissible): TWA 200 ppm (840mg/m^3)[3] REL TWA 200 ppm (840 mg/m^3) ST 250 ppm (1050 mg/m^3)[3] IDLH (Immediate danger): 1700 ppm[3] *(1) NIOSH, 'n-Propyl acetate'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *(2) Record in the GESTIS Substance Database of the IFA* *(3) NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0532'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 47.9 hPa 25 °C Solubility: Log Pow: 1.4	Acute toxicity study in mice treated with propyl acetate via inhalation; results: **effect on tonic extension of the hindlimbs**.	Repeated dose toxicity studies in rats with propan-2-ol via inhalation for up to 13 weeks results **effects related to CNS depression**. **No effects on neurobehavior** reported.	No information found from literature search on the effect of propan-2-ol on neurodevelopment.

203-561-1 Isopropyl acetate $C_5H_{10}O_2$ IUPAC name: (preferred) ***Propan-2-yl acetate*** Other names Isopropyl acetate 2-Acetoxypropane 2-Propyl acetate 2-Propyl ethanoate Propan-2-yl ethanoate Propan-2-yl acetate	**Isopropyl acetate** is an ester, an organic compound which is the product of esterification of acetic acid and isopropanol. LD $_{50\ (\text{median concentration})}$: 11,918 ppm (rat, 8 hr)[1] PEL (Permissible): TWA 250 ppm (950mg/m^3)[1] REL None established[1] IDLH (Immediate danger): 1800 ppm[3] *[1] NIOSH, 'Isopropyl acetate'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *[2] NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0358'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 60.3 hPa 20 °C Water solubility: "very soluble (> 10000 mg/L)" Log Kow: 1.03	Acute toxicity study in mice treated with isopropyl acetate via inhalation; results: **immobility.**	Repeated dose toxicity studies in rats with propan-2-ol via inhalation for 13 weeks; results :**CNS depression**.	No information found from literature search on the effect of isopropyl acetate on neurodevelopment.

204-658-1 n-butyl acetate $C_6H_{12}O_2$ IUPAC name: (preferred) ***Butyl acetate*** (systematic) ***Butyl ethanoate*** Other name(s): *n-Butyl acetate* *Acetic acid n-butyl ester* *Butilen*	Butyl acetate (=n-butyl acetate; butyl ethanoate) is an ester with formula $C_6H_{12}O_2$. The other three isomers of butyl acetate are: isobutyl acetate, tert-butyl acetate, and sec-butyl acetate. LD $_{50\ (median\ dose)}$: 10768 mg/kg (rats, oral)[1] LD $_{Lo\ (lowest\ published)}$: 14,079 ppm (cat, 72 min), 13,872 ppm (guinea pig, 4 hr)[2] LD $_{50\ (median\ concentration)}$: 160 ppm (rat, 4 hr), 2000 ppm (rat, 4 hr), 391 ppm (rat, 4 hr), 1242 ppm (mouse, 2 hr)[2] PEL (Permissible): TWA 150 ppm (710 mg/m^3)[1] REL TWA 150 ppm (710 mg/m^3) ST 200 ppm (950 mg/m^3)[3] IDLH (Immediate danger): 1400 ppm[3] *[1] 'MSDS of n-Butyl acetate'. https://www.fishersci.ca. Fisher Scientific. Retrieved 2014-06-28.* *[2] NIOSH, 'n-Butyl acetate'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *[3] NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0072'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 11.2 hPa 20 °C Water Solubility: 5.3 g/L at 20°C Log Pow: 2.3 Dielectric Constant: 5.01	**CNS depression** reported from acute inhalation study.	13 weeks, oral, study showed **ataxia and hypoactivity at 500 mg/kg bw**. 28 day inhalation study showed transient reduced activity and reduced response at **3000ppm. No effect in neurobehaviour**.	No information found from literature search on the effect of n-butyl acetate on neurodevelopment

259-370-9 2-ethoxy-1-methylethyl acetate $C_7H_{14}O_3$ IUPAC name: ***1-ethoxypropan-2-yl acetate*** Other names: *1-ethoxypropan-2-yl acetate, 1-ethoxypropan-2-yl ethanoate, (2-ethoxy-1-methyl-ethyl) acetate, 2-Ethoxy-1-methylethyl acetate, 54839-24-6, acetic acid (2-ethoxy-1-methyl-ethyl) ester, C7H14O3, CID171378, EINECS 259-370-9, ZINC02575428*		Physical state: Liquid VP: 2.03 hPa 25 °C Water solubility: 69.6 g/L at 18 °C Log Pow: 0.76	Acute toxicity study in mice treated with 2-ethoxy-1-methylethyl acetate via oral clinical effects like **hunched posture, abnormal gait (waddling), lethargy, pallor of the extremities and increased salivation**.	Repeated dose toxicity studies in rats with 2-ethoxy-1-methylethyl acetate results **slight loss of coordination** in 28 day study via oral, transient reduced response to external stimuli in 28 day study via inhalation, and **reduced 'startle response'** in 13 weeks study.	No information found from literature search on the effect of 2-ethoxy-1-methylethyl acetate neurodevelopment.

Ketones Ketones R-CO-R'	Contain the double bonded carbonyl group C=O with two hydrocarbon groups on the carbon ***acetone*** ***butanone***				
200-662-2 Acetone C_3H_6O IUPAC name: (preferred) ***Propan-2-one*** (systematic) ***2-propanone*** $(CH_3)_2CO$ Other name(s): *Acetone* *Dimethyl ketone* *Dimethyl carbonyl* *β-Ketopropane* *Propanone* *2-Propanone* *Dimethyl formaldehyde* *Pyroacetic spirit (archaic)* *Ketone propane*	**Acetone** (= **2-propanone)** is the simplest ketone with formula $(CH_3)_2CO$. LD $_{50\ (median\ dose)}$: 5800 mg/kg (rat, oral), 3000 mg/kg (mouse, oral), 5340 mg/kg (rabbit, oral)[1] LD $_{Lo\ (lowest\ published)}$: 45,455 ppm (mouse, 1 hr)[1] LD $_{50\ (median\ concentration)}$: 20,702 ppm (rat, 8 hr)[1] PEL (Permissible): TWA 1000 ppm (2400 mg/m³)[2] REL TWA 250 ppm (590 mg/m³)[2] IDLH (Immediate danger): 2500 ppm[2] *[1] NIOSH, 'Aceton'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *[2] NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0260'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 306 hPa 25 °C Solubility: 240 hPa at 20° Log Pow: -0.23 Dielectric Constant: 21.5 TLV-TWA: 500 ppm OT: 62 ppm FP: -4 F° ER: fast	**CNS depression** reported from acute inhalation study.	**No effect reported in the nervous system** after repeated exposure by inhalation or oral route.	No information found from literature search on the effect of acetone on neurodevelopment

201-159-0 Butanone C_4H_8O IUPAC name: (preferred) ***Butan-2-one*** $CH_3C(O)CH_2CH_3$ Other name(s): *Ethyl methyl ketone* *Methyl ethyl ketone* *MEK* *2-Butanone* *Methylpropanone* *Ethylmethylketone* *Methylacetone*	**Butanone (=methyl ethyl ketone (MEK))** is a liquid ketone with the formula $CH_3C(O)CH_2CH_3$. LD $_{50\ (median\ dose)}$: 2737 mg/kg (oral, rat), 4050 mg/kg, (oral, mouse)[1] LD $_{50\ (median\ concentration)}$: 12667 ppm (mammal), 13333 ppm (mouse, 2 hr), 7833 ppm (rat, 8 hr)[1] PEL (Permissible): TWA 200 ppm (590 mg/m^3)[2] REL TWA 250 ppm (590 mg/m^3) ST 300 ppm (885 mg/m^3)[2] IDLH (Immediate danger): 3000 ppm[2] *[1] NIOSH, '2-Butanone'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *[2] NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0069'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 126 hPa 25 °C Water solubility: "very soluble (> 10000 mg/L)" Log Pow: 0.3 TLV-TWA: 200 ppm OT: 5.4 ppm FP: 16 F° ER: fast	Acute toxicity studies in rats with butanone via oral results clinical effects like **gait and/or posture abnormalities and became comatose at the highest dose**.	Repeated dose toxicity study in rats with butanone via inhalation reported **no effects related to neurotoxicity.**	No information found from literature search on the effect of butanone neurodevelopment.

Alcohols Contain a single OH group Alcohols R-OH	***n-propanol*** ***isopropanol*** ***n-butanol*** ***2-methylpropan-1-ol*** ***Butan-2-ol*** ***1-methoxy-2-propanol*** ***1-ethoxy-2-propanol***				
200-746-9 n-propanol C_3H_7OH IUPAC name: ***Propan-1-ol*** $CH_3CH_2CH_2OH$ Other names *n-Propyl alcohol* *n-Propanol* *n-PrOH* *Ethylcarbinol* *1-Hydroxypropane* *Propionic alcohol* *Propionyl alcohol* *Propionylol* *Propyl alcohol* *Propylic alcohol* *Propylol*	**n-Propanol (=1-Propanol; propan-1-ol; 1-propyl alcohol; n-propyl alcohol)** is a primary alcohol with the formula $CH_3CH_2CH_2OH$ (sometimes represented as PrOH or n-PrOH). It is an isomer of isopropanol (2-propanol, isopropyl alcohol). It is formed naturally in small amounts during many fermentation processes. LD $_{50\ (median\ dose)}$: 2800 mg/kg (rabbit, oral), 6800 mg/kg (mouse, oral), 1870 mg/kg (rat, oral)[1] PEL (Permissible): TWA 200 ppm (500 mg/m^3)[2] REL TWA 200 ppm (500 mg/m^3) ST 250 ppm (625 mg/m^3) [skin][2] IDLH (Immediate danger): 800 ppm[2] *[1] Perkin, W. H.; Kipping, F. S (1922). Organic Chemistry. London: W. & R. Chambers. ISBN 0-08-022354-0.* *[2] Lide, David R., ed. (2006-06-26). CRC Handbook of Chemistry and Physics, 87th Edition (87 ed.). TF-CRC. ISBN 0-8493-0487-3.*	Physical state: Liquid VP: 28.2 hPa 25 °C Water solubility: "substance completely miscible in water at 25 °C" log Pow: 0.2 at 25 °C Dielectric Constant: 14.4 TLV-TWA: 100 ppm OT: 5.3 ppm FP: 59 F° ER: med	**Narcosis** effect reported from the acute oral or inhalation studies.	**No effects in the nervous system including clinical signs** were reported **in the 28 day or 90 day inhalation studies.**	**In vitro effects** of five short chain aliphatic alcohols (ethanol, n-propanol and t-butanol) on muscarinic receptor-stimulated phosphoinositide metabolism in cerebral cortical slices from 7 day-old rats. "These results suggest that muscarinic receptor-coupled phosphoinositide metabolism might be a common neurochemical target for the developmental neurotoxicity of short chain aliphatic alcohols."

200-661-7 Isopropanol C_3H_8O or C_3H_7OH or $CH_3CHOHCH_3$ IUPAC name: (systematic) ***Propan-2-ol*** Other names *2-Propanol* *Isopropanol (incorrect)* *Rubbing alcohol* *sec-Propyl alcohol* *s-Propanol* *iPrOH* *Dimethyl carbinol* *IPA*	**Isopropyl alcohol (=isopropanol; dimethyl carbinol)** is a primary alcohol with the chemical formula C_3H_8O or C_3H_7OH or $CH_3CHOHCH_3$ (sometimes represented as i-PrOH) with a propyl group linked to a hydroxyl group. It is the simplest example of a secondary alcohol, where the alcohol carbon atom is attached to two other carbon atoms, sometimes shown as $(CH_3)_2CHOH$. It is a structural isomer of 1-propanol (=n-Propanol, propan-1-ol; 1-propyl alcohol; n-propyl alcohol). LD $_{50\ (median\ dose)}$: 12800 mg/kg (dermal, rabbit), 3600 mg/kg (oral, mouse), 5045 mg/kg (oral, rat), 6410 mg/kg (oral, rabbit)[1] LD $_{Lo\ (lowest\ published)}$: 16,000 ppm (rat, 4 hr), 12,800 ppm (mouse, 3 hr)[1] LD $_{50\ (median\ concentration)}$: 53000 mg/m^3 (inhalation, mouse), 12,000 ppm (rat, 8 hr)[1] PEL (Permissible): TWA 400 ppm (980 mg/m^3)[2] REL TWA 400 ppm (980 mg/m^3) ST 500 ppm (1225 mg/m^3)[2] IDLH (Immediate danger): 2000 ppm[2] *[1] NIOSH, 'Isopropyl alcohol'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *[2] NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0359'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 44 hPa 20 °C Water Solubility: "Compound is soluble, however, unable to determine degree of solubility from the word "miscible"". Log Pow: 0.05 Dielectric Constant: 18.0 TLV-TWA: 200 ppm OT: 22 ppm FP: 53 F° ER: med	Narcosis and **increase in motor activity** was observed from acute inhalation study.	In **Chronic inhalation study** showed clinical signs of toxicity in the nervous system at 5000 ppm and at 2500 ppm (hypoactivity and lack of startle reflex). **A 13 weeks inhalation study** showed clinical signs of toxicity in the nervous system including narcosis in rats (**narcosis reversed** after 2 weeks of the study) and mice at 5000 ppm and in some mice and rats (only hypoactivity) at 1500ppm. **Increase motor activity** only in female rats at 5000 ppm. **No effects in functional observation battery, and no treatment related neuropathological effects**.	**PNDT study** via oral **gavage from GD 6 to GD 10 at doses up to 750 mg/kg bw/day did not result in neuro-behavioral and neuro-pathological effects in the offspring.**

200-751-6 n-butanol C_4H_9OH IUPAC name: (systematic) ***Butan-1-ol*** Other name(s): *Butalcohol* *Butanol* *1-Butanol* *Butyl alcohol* *Butyl hydrate* *Butylic alcohol* *Butyralcohol* *Butyric alcohol* *Butyryl alcohol* *n-Butyl alcohol* *1-Hydroxybutane* *n-Propylcarbinol*	***n*-Butanol** (=n-butyl alcohol or normal butanol) is a primary alcohol with a 4-carbon structure and chemical formula C_4H_9OH. Its isomers are isobutanol, 2-butanol, and tert-butanol. LD $_{50\ (median\ dose)}$: 790 mg/kg (rat, oral) LD $_{Lo\ (lowest\ published)}$: 3484 mg/kg (rabbit, oral), 790 mg/kg (rat, oral), 1700 mg/kg (dog, oral)[(1)] LD $_{50\ (median\ concentration)}$: 9221 ppm (mammal), 8000 ppm (rat, 4 hr)[(1)] PEL (Permissible): TWA 100 ppm (300 mg/m^3)[(2)] REL: C 50 ppm (150 mg/m^3) [skin][(2)] IDLH (Immediate danger): 1400 ppm[(2)] *(1) NIOSH, 'N-Butyl alcohol'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *(2) NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0076'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 9.31 hPa 25 °C Water solubility: 66 g/L at 20 °C Log Pow: 1 at 25 °C Dielectric Constant: 17.51 (25°C) TLV-TWA: 20 ppm OT: 1.2 ppm FP: 95 F° ER: slow	Acute oral study showed **narcosis effect**.	**90 day inhalation study** resulted reduced activity related to CNS depression at **3000 ppm (ca. 14.1 mg/L). Transient effect in motor activity** was also reported.	**PNDT study** up to **5 g/kg/day for 8 weeks resulted effects in the CNS** (hydrocephalus, dilation of subarachnoid space, cerebral ventricles).

201-148-0 2-methylpropan-1-ol $C_4H_{10}O$ IUPAC name: ***2-methylpropan-1-ol*** $(CH_3)_2CHCH_2OH$ Other names *Isobutanol* *Isobutyl alcohol, IBA,* *2-methyl-1-propanol,* *2-methylpropyl alcohol,* *Isopropylcarbinol*	**2-Methylpropan-1-ol (=isobutanol)** is a primary alcohol with the formula $(CH_3)_2CHCH_2OH$ (sometimes represented as i-BuOH). Its isomers, the other butanols, include n-butanol, 2-butanol, and tert-butanol. 2-Methylpropan-1-ol or isobutanol is produced by the carbonylation of propylene. LD $_{Lo\ (lowest\ published)}$: 3750 mg/kg (rabbit, oral) 2460 mg/kg (rat, oral)[1] PEL (Permissible): TWA 100 ppm (300 mg/m^3)[2] REL TWA 50 ppm (150 mg/m^3)[2] IDLH (Immediate danger): 1600 ppm[2] *[1] NIOSH, 'Isobutyl alcohol'. Immediately Dangerous to Life and Health. National Institute for Occupational Safety and Health.* *[2] NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0352'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: < 16 hPa 20 °C Water solubility: 70 g/L at 20 °C Log Pow: 1 Dielectric Constant: 16.68	Acute toxicity study in rats with 2-methylpropan-1-ol via inhalation or oral routes; results: **CNS depression.**	Repeated dose toxicity studies in rats with 2-methylpropan-1-ol via inhalation for up to 13 weeks; results; **clinical effects related to CNS depression.**	No information found from literature search on the effect of 2-methylpropan-1-ol on neurodevelopment.

201-158-5 Butan-2-ol $C_4H_{10}O$ IUPAC name: ***Butan-2-ol*** $CH_3CH(OOH)CH_2CH_3$ Other names *sec-Butanol* *sec-Butyl alcohol* *2-Butanol* *2-Butyl alcohol*	**Butan-2-ol (=sec-butanol; 2 butanol)** is an isomer of butanol and is a secondary alcohol with chemical formula $CH_3CH(OOH)CH_2CH_3$. 2-Butanol is chiral (non-superposable on its mirror image) and thus can be obtained as either of two stereoisomers designated as (R)-(−)-2-butanol and (S)-(+)-2-butanol, i.e. enantiomers or optical isomers. It is normally found as an equal mixture of the two stereoisomers — a racemic mixture. $LD_{Lo\ (lowest\ published)}$: 16,000 ppm (rat, 4 hr), 10,670 ppm (mouse, 3.75 hr), 16,000 ppm (mouse, 2.67 hr)[(1)] PEL (Permissible): TWA 150 ppm (450 mg/m^3)[(1)] REL TWA 100 ppm (305 mg/m^3) ST 150 ppm (455 mg/m^3)[(1)] IDLH (Immediate danger): 2000 ppm[1] *(1) NIOSH, 'NIOSH Pocket Guide to Chemical Hazards #0077'. National Institute for Occupational Safety and Health.*	Physical state: Liquid VP: 16.67 hPa 20 °C Water solubility: > 1 000 000 mg/L at 20ºC Log Pow: < 1	Acute toxicity study in rats with butan-2-ol via oral route; results: **gait and/or posture abnormalities**. No effects reported after inhalation exposure.	Repeated dose toxicity studies in rats with isopropanol via inhalation for 13 weeks; results: clinical effects related to **CNS depression. No effects related to neurobehavior reported**.	No information found from literature search on the effect of butan-2-ol on neurodevelopment.

203-539-1 1-methoxy-2-propanol $C_4H_{10}O_2$ IUPAC name: ***1-methoxy-2-propanol*** $H_3CCHOHCH_2OCH_3$ $CH_3OCH_2CHOHCH_3$ Other names *1-Methoxy-2-propanol 1-Methoxypropan-2-ol 2-Propanol 1-methoxy-; Propylene glycol monomethyl ether PGME*	**1-Methoxy-2-propanol (=propylene glycol monomethyl ether (PGME))** with formula $C_4H_{10}O_2$ or $H_3CCHOHCH_2OCH_3$ or $CH_3OCH_2CHOHCH_3$	Physical state: Liquid VP: 15.60 hPa 25 °C Water solubility: > 1 000 000 mg/L at 20 °C Log Pow: < 1	Acute toxicity study in rats and with 1-methoxy-2-propanol via oral and inhalation routes; results: effects related to **CNS depression.**	Repeated dose toxicity studies in rats/ rabbits/guinea pigs/monkey with 1-methoxy-2-propanol via inhalation between 13 weeks to 2 year; results: clinical effects related to **CNS depression which was mostly recovered after the exposure period.**	No information found from literature search on the effect of 1-methoxy-2-propanol on neurodevelopment.

216-374-5 1-ethoxy-2-propanol $C_5H_{12}O_2$ IUPAC name: ***1-ethoxy-2-propanol*** Other names	Oral/Parenteral Toxicity: oral-rat LD50 4400 mg/kg Raw Material Data Handbook, Vol.1: Organic Solvents, 1974. Vol. 1, Pg. 104, 1974. Dermal Toxicity: skin-rabbit LD50 8100 mg/kg Raw Material Data Handbook, Vol.1: Organic Solvents, 1974. Vol. 1, Pg. 104, 1974. Inhalation Toxicity: inhalation-rat LC50 > 10000 ppm/4H Raw Material Data Handbook, Vol.1: Organic Solvents, 1974. Vol. 1, Pg. 104, 1974.	Physical state: Liquid VP: 9.65-14.48 hPa 25 °C Water solubility: 897 at 20 °C Log Pow: 0 (QSAR)	Acute toxicity study in rats and with 1-ethoxy-2-propanol via oral; results: **clinical effects such as inactivity, unsteady gait, partial ptosis, hunched posture and prostration**.	CNS depression	No information found from literature search on the effect of 1-ethoxy-2-propanol on neurodevelopment.

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ANNEX 8

A8. Organic solvents and ion channel receptors

Organic solvents were found to inhibit and potentiate excitatory and inhibitory ion channel receptors, respectively. In this **Annex 8**, some receptors are discussed in more detail. Thereby, attention is drawn to differences in action of receptors in the mature versus developing nervous system as this may change the vulnerability of the nervous system when exposed to (narcotic) organic solvents.

A8.1 Excitatory ion channel receptors

A8.1.1 NMDA-glutamate receptor

The N-methyl-D-aspartate receptor (NMDA receptor or NMDAR) is a glutamate receptor and ion channel protein found in nerve cells. It is activated when glutamate and glycine (or D-serine) bind to it; when activated it allows positively charged ions to flow through the cell membrane (Hiroyasu et al., 2005). NMDAR is very important for controlling synaptic plasticity and memory function (Li and Tsien, 2009). NMDAR is a specific type of ionotropic glutamate receptor (Moriyoshi et al., 1991). The agonist molecule N-methyl-D-aspartate (NMDA) binds selectively to it. Ca^{2+} flux through NMDARs is thought to be critical in synaptic plasticity. The opening and closing (gating) of the NMDA receptor is complex. While it is primarily a ligand-gated channel, it does display weaker voltage-dependence modulation of the ligand-dependent gating. The ligand gating requires co-activation by two ligands: glutamate and either D-serine or glycine (Kleckner and Dingledine, 1988). The activity of the NMDA receptor is affected by many psychoactive drugs, such as phencyclidine (PCP), alcohol (ethanol) and dextromethorphan (DXM). **Thus, organic solvents with narcotic properties may partly act via NMDA receptors. However, there is no further information on narcotic substances to support this assumption.**

As NMDARs are important for controlling synaptic plasticity and memory function (Li and Tsien, 2009) it seems likely that narcotic organic solvents acting via NMDA receptors may be mechanistically linked with learning and memory impairments in developing brain.

A8.2 Inhibitory ion channel receptors

A8.2.1 Gamma aminobutyric acid-A ($GABA_A$)

Also the GABA transmission pathway has been focus of studies on possible mechanisms involved in solvent-induced neurological alterations, including narcosis.

GABA receptors respond to the neurotransmitter gamma-aminobutyric acid (GABA), the main *inhibitory* compound in the mature vertebrate CNS. GABA receptors influence cognition by coordinating with glutamatergic processes (Farahmandfar et al., 2016). There are two classes of

GABA receptors: $GABA_A$ and $GABA_B$. $GABA_A$ receptors are ligand-gated ion channels, i.e. 'ionotropic receptors', whereas $GABA_B$ receptors are G protein-coupled receptors, i.e. 'metabotropic receptors'.

$GABA_A$ receptors are ligand–gated ion channels composed of various subunits (α1-6, β1-4, γ1-3, δ, ε and ρ1-3) forming a pentameric structure containing a central chloride channel (Sieghart, 1995; Barnard et al., 1998).

The *fast* response of neurons to GABA –which is blocked by bicuculline and picrotoxin– is caused by direct activation of the $GABA_A$ receptor (Takeuchi and Onodera, 1972). Fast-responding GABA receptors are all members of a family of Cys-loop ligand-gated ion channels (Barnard et al, 1998; Hevers and Lüddens, 1998; Sieghart and Sperk, 2002); they all possess a characteristic loop formed by a disulfide bond between two cysteine residues. $GABA_A$ receptors, glycine, nicotinic acetylcholine receptors, and 5-HT3 receptors all belong to this family.

A *slow* response to GABA is mediated by $GABA_B$ receptors (Bowery et al., 2002). In studies focusing on the control of neurotransmitter release, it was noted that a GABA receptor was responsible for modulating evoked release in a variety of isolated tissue preparations. The ability of GABA to inhibit neurotransmitter release from these preparations was not blocked by bicuculline, was not mimicked by isoguvacine, and was not dependent on Cl^-, all of which are characteristics of the $GABA_A$ receptor.

In ionotropic $GABA_A$ receptors, binding of GABA molecules to their binding sites in the extracellular part of the receptor, triggers opening of a chloride ion-selective pore. The increased chloride conductance drives the membrane potential towards the reversal potential of the Cl^- ion, which is about –65 mV in neurons, inhibiting the firing of new action potentials. This mechanism is responsible for the sedative effects of $GABA_A$ allosteric agonists such as e.g. benzodiazepines (McKernan et al., 2000; Sigel et al., 2012)

There are, however, also numerous reports of *excitatory* $GABA_A$ receptors. This phenomenon is due to increased intracellular concentration of Cl^- ions either during *development* of the nervous system (Ben-Ari et al., 1997; Taketo and Yoshioka, 2000) or in certain cell populations (Tomikoet al., 1983; Cherubini et al., 1991; Lamsa and Taira, 2003). Ben-Ari and co-workers (Ben-Ari et al., 1997) demonstrated that the main ionotropic receptors ($GABA_A$, NMDA and AMPA) display a sequential participation in neuronal excitation in the *neonatal* hippocampus. 1) GABA, the principal *inhibitory* transmitter in the *adult* CNS, acts as an *excitatory* transmitter in *early postnatal stage*. 2) Glutamatergic synaptic transmission is first purely NMDA-receptor based and lacks functional AMPA receptors. Therefore, initially glutamatergic synapses are 'silent' at resting membrane potential, NMDA channels being blocked by Mg^{2+}. When GABA and glutamatergic synapses, however, are co-activated during the physiological patterns of activity, $GABA_A$ receptors can facilitate the activation of NMDA receptors, playing the role conferred to AMPA receptors later on in development.

These subtle cascades of events on the one hand form a beautiful example of the architecting of an impressive fine-tuned network, taking place during nervous system development; otherwise, it also demonstrates how critical the timing may be when it comes to exposure windows and the vulnerability of the developing nervous system to the toxic potency of substances. For example, studies have found that **anesthetic agents** that bind to the GABA and NMDA receptors during the **period of brain growth spurt (BGSP),** may potentially trigger excess apoptosis and affect neuronal development (Gao et al., 1998 *(propofol)*; Ikonomidou et al., 1999; Irifune et al.,2003; Kargaran et al., 2014) and the rational for the increased vulnerability during this period of BGS is that exposure to anesthetics during this period leads to excitation, rather than inhibition of the $GABA_A$ receptors (Herlenius, 2004; Nguyen et al., 2001).

Also, Taketo and Yoshioka (2000) demonstrated that $GABA_A$ergic inhibitory postsynaptic currents in hippocampal CA3 pyramidal cells change developmentally and indicate that **different receptor isoforms are functionally expressed between neonates and adults.**

Glutamate and GABA display a wide range of trophic roles in the developing brain, and in the transition from neonatal to adult forms of plasticity. Clearly, such developmental changes in transmitter action and role of receptors are complicated and by far not completely understood. But also here, not knowing the 'window' (time-frame) in which processes occur increases the vulnerability of the developing nervous system when exposed for example to narcotic organic solvents. The period of brain growth spurt (BGS) appears to be a period of increased vulnerability, which is related to *excitation* of the $GABA_A$ receptor rather than inhibition (as is more likely the situation in the mature nervous system). Therefore, narcotic organic solvents acting via $GABA_A$ receptors are likely to be more potent to harm the developing nervous system, as compared to the adult.

A8.2.2 Glycine

The glycine receptor (GlyR) is known to co-localize with the $GABA_A$ receptor on some hippocampal neurons; Gephyrin appears critical for GlyR clustering at inhibitory synapses, but not for formation of functional GABAergic synapses in hippocampal neurons (Levi et al., 2004). GlyR is the receptor for the amino acid neurotransmitter glycine and is an ionotropic receptor producing its effects through chloride current; it is the most widely distributed inhibitory receptor in CNS; plays important roles in a variety of physiological processes, especially in mediating inhibitory neurotransmission in the spinal cord and brainstem (Lynch, 2004). GlyR can be activated by a range of simple amino acids including glycine, β-alanine and taurine and can be selectively blocked by high-affinity competitive antagonist strychnine (Rajendra et al., 1997); caffeine is a competitive antagonist of GlyR (Duan et al., 2009).

A8.2.3 Dopamine

Also the dopamine pathway has been evaluated for involvement in the mechanism of CNS **narcotic organic solvents of abuse** (inhalant effects).

Dopamine receptors are a class of G protein-coupled receptors; the neurotransmitter dopamine is their primary endogenous ligand. Dopamine receptors are implicated in many neurological processes, including motivation, pleasure, cognition, memory, learning, and fine motor control, as well as modulation of neuroendocrine signaling (Williams and Castner, 2006). They also control neural signaling that modulates many important behaviors, such as spatial working memory (Williams and Castner, 2006). Thus, dopamine receptors do not seem to be directly involved in the narcotic effects of organic solvents.

Dysfunction of dopaminergic neurotransmission in the CNS has been implicated in a variety of neuropsychiatric disorders (Girault and Greengard, 2004): Parkinson's disease (Fuxe et al., 2006), schizophrenia (Kienast and Heinz, 2006), neuroleptic malignant syndrome (Mihara et al., 2003), attention-deficit hyperactivity disorder (ADHD) (Faraone and Khan, 2006), and drug and alcohol dependence (Kienast and Heinz, 2006; Hummel and Unterwald, 2002). Some of these disorders are

also recognized in relation to narcotic organic solvent-induced neurotoxicity. For example, Goldman et al. (2012) tested the hypothesis that exposure to specific solvents is associated with Parkinson's disease (PD) risk using discordant twin pair design. These investigators found that exposure to trichloroethylene (TCE) was associated with significantly increased risk of PD, and exposure to perchloroethylene (PERC) and carbon tetrachloride (CCl_4) tended toward significance. Results were interpreted as that exposure to specific solvents may increase risk of PD (Goldman et al., 2012). Otherwise, these findings were based on small numbers, and dose–response gradients were not observed and, moreover, numerous epidemiologic studies investigating the potential links between solvents and PD yielded mostly null or weak associations (Lock et al., 2013 *(Systematic review)*). The association between chronic pesticide exposure and the prevalence of dementias, including Alzheiner's disease (AD) has not been as well studied as with other environmental risk factors, and results are often inconsistent. Some studies, however, found positive assosiations between pesticide exposure and AD (reviewed by Chin-Chan et al., 2015 *(references therein)*). The occurrence of solvent-related schizophrenia is rare but Stein et al. (2010) reported on a patient with schizophrenia diagnosis after a sustained period of 6 months of everyday exposure to neurotoxic solvents in an unprotected occupational setting.

Overall, there is a serious concern regarding a possible causal link between exposure to narcotic organic solvents and neurodegenerative diseases, however, findings are inconsistent and more research will be needed to come to a firm conclusion.

To conclude:

1. Organic solvents with narcotic properties may partly act via NMDA receptors. However, there is no further information on narcotic substances to support this assumption.
2. For their narcotic effects –most likely the effects observed in repeated dose– their action at $GABA_A$ receptor is probably crucial.
3. Studies have found that anesthetic agents that bind to the GABA and NMDA receptors during the period of brain growth spurt (BGSP), may potentially trigger excess apoptosis and affect neuronal development, and the rational for the increased vulnerability during the period of BGS is that exposure to *anesthetics* during this period leads to *excitation,* rather than inhibition of the $GABA_A$ receptors.
4. GlyR is the receptor for the amino acid neurotransmitter glycine and is an ionotropic receptor producing its effects through chloride current; it is the most widely distributed *inhibitory* receptor in CNS; plays important roles in a variety of physiological processes, especially in mediating inhibitory neurotransmission in the spinal cord and brainstem.
5. Dopamine receptors do not seem to be directly involved in the narcotic effects of organic solvents; there is a serious concern regarding a possible causal link between exposure to narcotic organic solvents and neurodegenerative diseases, however, findings are inconsistent and more research will be needed to come to a firm conclusion.
6. Glutamate and GABA display a wide range of trophic roles in the developing brain, and in the transition from neonatal to adult forms of plasticity. Clearly, such developmental changes in transmitter action and role of receptors are complicated and by far not completely understood. But also here, not knowing the 'window' (time-frame) in which processes occur increases the vulnerability of the developing nervous system when exposed for example to narcotic organic solvents. The period of brain growth spurt (BGS) appears to be a period of increased vulnerability that is related to *excitation* of the $GABA_A$ receptor rather than inhibition (as is more likely the

situation in the mature nervous system). Therefore, narcotic organic solvents acting via $GABA_A$ receptors are likely to be more potent to harm the developing nervous system, as compared to the adult.

ANNEX 9

A9.1 Pre-/postnatal exposure to general anesthetics

In the past, some organic solvents (e.g. trichloroethylene and chloroform) were used in medicine as anesthetics because of their narcotic properties (Viaene, 2002). Propofol (2,6-diisopropylphenol) is still widely used in this respect.
Since the key research question in the present review focuses on *the potency for developmental neurotoxicity of organic solvents with narcotic properties*, it seems relevant to look into the information available on general anesthetics used on fetal and neonate brain and their known mechanisms/modes of action relative to developmental neurotoxicity. Propofol is used as example.

A9.1.1 Anesthetics-induced developmental neurotoxic alterations in animal studies

Neonatal animal studies on developmental neurotoxicity induced by general anesthetics like e.g. **halothane** ($C_2HBrClF_3$), **enflurane** ($C_3H_2ClF_5O$; 2-chloro-1,1,2,-trifluoroethyl-difluoromethyl ether), **isoflurane** ($C_3H_2ClF_5O$; (*RS*)-2-Chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane), **sevoflurane** ($C_4H_3F_7O$; (1,1,1,3,3,3-hexafluoro-2-(fluoromethoxy)propane; synonym: fluoromethyl hexafluoroisopropyl ether), **ketamine** ($C_{13}H_{16}ClNO$) and **midazolam** ($C_{18}H_{13}ClFN_3$; 8-chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazol) were reported to cause adverse neurological effects in various animal species including non human primates (Brambrink et al., 2010; Chalon et al., 1981; Jevtovic-Todorovic et al., 2003; Lunardi et al., 2010; Mazoit et al., 2010; Satomoto et al., 2009; Slikker et al., 2007; Stratmann et al., 2009; Quimby et al., 1974, 1975; Uemura et al., 1985; Zhu et al., 2010). Wise-Faberowski et al. (2005) pointed at the potential risk of anesthetics for brain development using organotypic hippocampal slice cultures and isoflurane. Other animal studies showed that fetal exposure to volatile anesthetics causes neurodegeneration and long-term changes (Kong et al., 2011; Schubert et al., 2012; Wang et al., 2012; Zheng et al., 2013). Studies also showed functional cognitive impairment corroborating the neuronal degeneration in the developing brain after exposure to such anesthetics (Jevtovic-Todorovic et al., 2003; Kodama et al., 2011; Ma et al., 2007; Zhang et al., 2012).

A9.1.2 Anesthetics-induced increase in neuroapoptosis during the brain growth spurt period

The neurotoxic changes observed in the various animal species following intravenous or inhaled exposure to anesthetic agents included an increase in neuroapoptosis during postnatal brain development, more specifically during the so called brain growth spurt period (Brambrink et al., 2010; Creeley et al., 2007, 2013, 2014; Jevtovic-Todorovic et al., 2003; Ma et al., 2007; Pearn et al., 2012; Slikker et al., 2007; Tu et al., 2011; Yu et al., 2013; Zou et al., 2011). Neurons appear most

vulnerable during this period, which is typically characterized by synaptogenesis, i.e. during outgrowth of dendrites and the formation of contact zones between neurons (Semple et al., 2013). In humans the peak of this brain growth period occurs from the second trimester to three years of age (Bayer et al., 1993; Dobbing and Sands, 1979); in rats around postnatal day 7 (Jevtovic-Todorovic et al., 2003; Stratmann et al., 2009). It is especially this maximal vulnerability of the brain during the growth spurt period and the adverse effects, demonstrated for pre-/postnatal exposure to general anesthetics in neonatal animals, which increasingly raised concerns on the use of anesthetics in children and neonates; after all, their use coincides this vulnerable period of brain development.

Some anesthetics were shown to be more potent than others in causing neurotoxicity, like e.g. isoflurane being more potent than sevoflurane (Mazoit et al., 2010) or desflurane (Straiko et al., 2010); but conflicting results were reported as well (Ma et al., 2004; Pellegrini et al., 2014), although these might also be explained by differences in species and testing protocols (Dobbing and Sands, 1979; Ma et al., 2004; Mazoit et al., 2010; Sanders et al., 2010; Straiko et al., 2010; Pellegrini et al., 2014). Also, the chemical structure of the anesthetics differs, which obviously has consequences for their binding possibilities to e.g. cell membrane and receptors (see for example below the anesthetics mentioned above under section *A10.1.1):*

Halothane : halogenated hydrocarbon
Enflurane : halogenated hydrocarbon and ether
Isoflurane : halogenated hydrocarbon and ether
Sevoflurane : halogenated hydrocarbon and ether
Ketamine : halogenated hydrocarbon and aromatic hydrocarbon and cylic hydrocarbon and ketone (and amine; *not included in the grouping table for the organic solvents (Table 1)*)
Midozalam : halogenated hydrocarbon and aromatic hydrocarbon (and heterocylic aromatic hydrocarbon; *also not included in the grouping table for the organic solvents (Table 1)*)

ANNEX 10

A10.1 Propofol

Propofol is a relatively new and widely used general anesthetic. It has been evaluated for this review as an example of general anesthetics to learn about underlying mechanisms of anesthesia-like effects and narcosis.

A10.1.1 Propofol in anesthesia

Propofol (2,6-diisopropylphenol; formula: $C_{12}H_{18}O$) is a versatile drug, i.e. it can be given for short or prolonged sedation, as well as for general anesthesia. There are review articles covering the features of propofol (Fulton and Sorkin, 1995; Langley and Heel, 1988). The original formulation of Propofol –Diprivan®– received FDA approval in October 1989. Since then, also other formulations of propofol, like Propoven® and Lipuro®, became commercially available (Chidambaran et al., 2015 and references therein). Propofol is highly protein-bound *in vivo* and is metabolized by conjugation in the liver (Favetta et al., 2002). Its half-life of elimination is assessed between 2 and 24 hours, but clinical effect duration is much shorter as propofol rapidly distributes into peripheral tissues. Because of its rapid onset and recovery along with its amnestic effects it has been widely used for sedation and anesthesia since the 1990th (Veselis et al., 1997) and, to date, it is the most commonly used intravenous agent in current anesthesia practice and generally accepted to be safe in humans for the *mature* brain (Xiong et al., 2016).

The efficacy of propofol as a sedative for *children* has been established in several clinical trials and case studies since the 1990s (Borgeat et al., 1990; Reed and Blumer, 1996; Reed et al., 1996). Pediatric use of propofol includes induction and maintenance of general anesthesia as well as sedation during non-surgical intervention and intensive care (Angelini et al., 2001; Kulling et al., 2003). And because of its fast induction and recovery time, propofol is also widely used for sedation of infants and children undergoing MRI (Machata et al., 2008). A review on current use of propofol in pediatric anesthesia is published by Chidambaran et al. (2015).

Propofol is considered safe for use during pregnancy but propofol is not recommend for use during cesarean section; the rapid placental transference of propofol during caesarean section may require additional dosing, either by rapid intravenous (i.v.) injection or by maintaining the anesthesia by continuous i.v. infusion. In such a situation it is of prime importance to consider the time elapsing prior to removal of the fetus and the consequences of the anesthesia for the fetus, since the propofol plasma level in the newborn at the time of delivery depends on the level in maternal plasma (Sanchez-Alcaraz et al., 1998).

An early study of 20 children receiving propofol infusion did not show any negative neurological conditions (Macrae and James, 1992). Otherwise, in the early days of its use, some conflicting literature has appeared as well, about the safety and serious adverse effects of propofol (Chidambaran et al., 2015 (references therein)): prolonged sedation in a premature infant (Bacon and Razis, 1994); impaired motor function and blindness in a 23 month old individual (Lanigan et al.,

1992); muscle weakness and twitching which lasted 9-18 days as well as seizures (Trotter and Serpell, 1992); ataxia and hallucinations in a 6 year old individual after propofol infusions (Bendiksen and Larsen, 1998). Is has been disputed that increased vulnerability of the individual relative to the dosing scenario used might have been responsible for the latter results. It is noted that the US FDA has set propofol to 'pregnancy category: B': 'Animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women'. Thus, in humans propofol is considered safe to mature and developing nervous system. Recent animal studies in neonates and fetuses, however, has raised some concern for the developing nervous system. Propofol-induced apoptosis of neurons and oligodendrocytes was observed during brain development, in rats, mice (in infant mice at one fourth the dose required for surgical anesthesia) and rhesus macaques (after propofol anesthesia for 5 hours).

Not only it is recognized that in humans developmental neurotoxicity by propofol has not been studied that well, it has also been acknowledged to date that the developing brain differs from the adult in vulnerability. –'*Children are no adults'*– in that they have an immature blood-brain barrier, incomplete detoxification capabilities and different metabolic competencies. Moreover, as discussed in previous chapters of the present review, most important is the window of exposure relative to the targeted developmental processes/structures, and the dose, duration and frequency of exposure (James and Glen, 1980; Jevtovic-Todorovic et al., 2003; Uemura et al., 1985). Moreover, from early studies in rodents it had been known already for a long time that anesthetic exposure of the dam exposes the fetus since the highly lipid soluble propofol readily transfers the placenta (Gin et al., 1990; Sanchez-Alcaraz et al., 1998).

Together, this has led to an increase in the research on potential developmental neurotoxicity of propofol and other general anesthetics, in particular studies with neonatal animals of various species as these agents are applied on human fetus and neonate brain. Nevertheless, there is still no conclusive answer on the risk for fetus and neonate and in ***humans*** propofol is still considered safe to mature and developing nervous system.

A10.1.2 Propofol and potential developmental neurotoxicity

Propofol exposure may induce comparable neurotoxicity in neonatal animals of several species (Al-Jahdari et al., 2006; Bercker et al., 2009; Briner et al., 2011; Cattano eta al., 2008; Fredriksson et al., 2007; Gao et al., 2014; Karen et al., 2013; Milanovic et all., 2014; Pearn et al., 2012; Pesic et al., 2009; Tang et al., 2012; Yang et al., 2014; Yu et al., 2013; Wang et al., 2013) including rhesus monkeys (Creeley et al., 2013). Significant long-term behavioral impairments and cognitive functioning appeared associated with the observed neurodegeneration (Bercker et al., 2009; Gao et al., 2014; Yu et al., 2013).

Similar to the findings on general anesthetics, also for propofol apoptosis of neurons and oligodendrocytes was observed during brain development. This was observed in rats (Li et al., 2014; Yu et al., 2013; Xiong et al., 2014), mice –in infant mice at one fourth the dose required for surgical anesthesia– (Cattano et al., 2008), and rhesus macaques –after propofol anesthesia for 5 hours– (Creeley et al., 2013).

The excessive apoptosis is believed to cause the neurodegeneration following early exposure to propofol and appears the main toxicity event (Milanovic et al., 2014; Pearn et al., 2012; Pesic et

al., 2009; Popic et al., 2012). It is known that various internal and external agents may initiate neuronal apoptosis. Apoptotic DNA fragmentation requires the activation of internal Ca2+ and Mg2+-dependent DNA endonuclease (Yu et al., 1999), but also excitatory and inhibitory amino acids may involve in the apoptosis of neurons. Increasing evidence has demonstrated that glutamate and GABA directly act in the neuroapoptosis of drug abuse, alcohol addiction, and for example in chronic stress-induced neuroapoptosis in particular via respective NMDA and $GABA_A$ receptors (Desfeux et al., 2010; Llorens-Martin and Trejo, 2011; Lussier et al., 2013).

Thus, in humans propofol is considered safe to mature and developing nervous system. Recent information from animal studies –rodents and non-human primates (NHP)) has raised some concerns with respect to possible neurotoxic changes in the developing brain and accompanying neurocognitive deficits following propofol exposure (Bercker et al., 2009; Chidambaran, et al., 2015; Xiong et al., 2016) and anesthetic drugs in neonates and young children in general (Ikonomidou et al. 1999).

A10.2 Mechanism of action

A10.2.1 Propofol and propofol-induced developmental neurotoxicity

The mechanism of action of propofol on the central nervous system involves interactions at various neurotransmitter receptors

A10.2.1.1 Gamma-aminobutyric acid A ($GABA_A$) receptor

- Activation of inhibitory $GABA_A$ receptors contributes to the hypnotic effects of propofol
- Propofol suppresses seizure activity via GABA-mediated inhibition of neuronal firing

The mechanism of action of propofol on the central nervous system involves interactions at various neurotransmitter receptors, especially gamma-aminobutyric acid A ($GABA_A$) receptor (Chidambaran et al., 2015). Activation of inhibitory $GABA_A$ receptors by propofol in the central nervous system is well known and putatively contributes to the narcotic effects of propofol (Collins, 1988; Concas et al., 1991; Sanne et al., 1995; Zhao et al., 2011).
Propofol is sharing this mechanism with general anesthetics (Zhao et al., 2011). $GABA_A$ receptors are ligand–gated ion channels composed of various subunits (α1-6, β1-4, γ1-3, δ, ε and ρ1-3) forming a pentameric structure containing a central chloride channel (Barnard et al., 1998; Sieghart, 1995). There is evidence that α-, β-, and γ-subunits all contribute to $GABA_A$ receptor sensitivity to propofol. Within the β-subunits, propofol, appeared less efficient at β1 containing receptors than at those containing β2 or β3 subunits (Sanna et al., 1997). Binding of the propofol molecule to the $GABA_A$ receptor leads to increased chloride ion influx and hyperpolarization of the neuron which, in turn, results in insensitivity to external stimuli. Also presynaptic mechanisms of GABAergic transmission such as GABA uptake and release are affected by propofol (Trapani et al., 2000).

Propofol –being a GABA agonist– suppresses seizure activity via GABA-mediated inhibition of neuronal firing, i.e. GABA mediated inhibition of acetylcholine release is enhanced. Propofol

selectively blocks release of acetylcholine in the baso-cortical and septo-hippocampal pathways, which are under tonic innervation by GABAergic input (Imperato et al., 1993).

Propofol is activiating other receptor systems as well –further discussed below– which, however, are not likely to be directly involved in the narcotic effects of propofol.

A10.2.1.2 Glycine, serotonin and NMDA receptors

- The proconvulsant properties of propofol may be due to GABAergic agonism, or, antagonism of intrinsic subcortical glycine
- The anti-emetic action of propofol may be explained by activation of serotonin receptors
- Propofol may modulate calcium influx through inhibition of NMDA receptor

A possible mechanism for the proconvulsant properties of propofol may also be due to GABAergic agonism, or, antagonism of intrinsic subcortical glycine (Albertson et al., 1991; Dolin et al., 1992; Hadipour-Jahromy and Daniels, 2003). Glycine receptors are widely distributed in the human CNS (Tao and Ye, 2002.). Propofol, in addition, leads to a concentration-dependent activation of inhibitory glycine receptors at spinal cord level (Hales and Lambert, 1991), serotonin (5-Hydroxy Trytophan) receptors –which might explain its **anti-emetic action**– (Machu and Harris, 1994), with mild activity at excitatory glutamate/N-methyl D-aspartate (NMDA) receptors (Yamakura et al., 1995).

Other mechanisms of action include inhibition of the NMDA receptor and the modulation of calcium influx through slow calcium ion channels (Kanai et al., 1999; Rossetti et al., 2005; Hayashi et al., 2007)

A10.2.1.3 TRPV1 and TRPA1 receptors

Propofol excites nociceptors by activating TRPV1 and TRPA1 receptors, most likely causing the pain upon intracutaneous injection of propofol

The intense **pain upon intracutaneous Injection of propofol** is *not* caused by GABA. Studies claimed that propofol excites nociceptors by activating TRPV1 and TRPA1 rather than GABA receptors (Fischer et al., 2010). TRPV1 –*transient receptor potential cation channel subfamily V member 1 (TrpV1), also known as the capsaicin receptor and the vanilloid receptor 1*– is involved in the transmission and modulation of pain (nociception), as well as the integration of diverse painful stimuli (Cui et al., 2006; Huang et al., 2002). TRPV1 is a nonselective cation channel that may be activated by a wide variety of exogenous and endogenous physical and chemical stimuli. The activation of TRPV1 leads to a painful, burning sensation. TRPA1 –*transient receptor potential cation channel, subfamily A, member 1*– is believed to function as a mechanical stress sensor and is known to be activated by compounds capable of forming covalent chemical bonds with the cysteins in the protein, like isothiocyanates and many others (Nillius et al., 2007).

A10.3 Mechanisms of propofol-induced neurotoxicity

The mechanisms underlying propofol-induced developmental neurotoxicity are not clearly understood so far. Quite a number of similarities with anesthetics-induced apoptosis, neurodegeneration and behavioural deficits were recognized after propofol-induced neurotoxicity in neonatal animals. As such, it seems very likely that –at least partly– common mechanisms are responsible. It has been suggested that anesthetics-induced neurodegeneration involves the activation of both mitochondrial (intrinsic) and death receptor-mediated (extrinsic) apoptotic pathways (Yon et al., 2005). They both converge on the activation of executioner caspase-3, which subsequently cleaves multiple downstream cellular targets (Regula and Kirshenbaum, 2005). So, apoptosis is likely to be the principle common mechanism for general anesthetics –including also propofol– underlying the neurotoxic effects.

A10.3.1 Caspase-3, neurotrophins and downstream enzymes relevant in the apoptotic cascade

The neuroapoptosis by propofol was in particular demonstrated by the presence in the neurons of increased levels of Caspase-3 –a protein playing a crucial role in apoptotic pathways– (Cattano et al., 2008; Fredriksson et al., 2007; Karen et al., 2013; Milanovic et al., 2014; Pearn et al., 2012; Pesic et al., 2009; Tang et al., 2012; Yang et al., 2014; Yu et al., 2012). The neurotoxicity of caspase-3 activation in fetal and neonate brains may represent mechanisms underlying learning and memory impairment in adult rats (Zheng et al., 2013; Shen et al., 2013; Sanders et al., 2009).

Others studied the role of neurotrophins and further specific enzymes involved in the apoptotic cascade (Li et al., 2014, 2016; Milanovic et al., 2014; Pearn et al., 2012; Pesic, et al., 2009; Pontén et al., 2011; Popic et al., 2012), brain microglia activation, and expression of tumor necrosis factor (Milanovic et al., 2014; Pesic et al., 2009; Popic et al., 2012). Neurotrophines and downstream enzymes may also play a role in the cascade of events leading to apoptosis, as well as the expression of tumor necrosis factor, and the activation of microglia in the brain. During brain development when apoptosis plays a fundamental role in regulating cell fate, the neurotrophins antagonize naturally occurring programmed cell death and thus promote neuronal survival in mammals (Kaplan and Miller, 2000).

Propofol anesthesia in pregnant rats (GD20) can induce acute neurotoxicity, such as increases in the amount of cleaved caspase-3 and IBA1 protein (marker of microglia activation) in the brain tissues of fetal rats. The same propofol infusion in pregnant rats also induced learning and memory impairment in the juvenile offspring rats (Li et al., 2016).

A10.3.2 GABA receptor, p75 neurotrophin receptor, inositol trisphosphate (InsP3) receptors

Mechanisms suggested to underlie propofol-induced developmental neurotoxicity based on similarities with general anesthetics-induced apoptosis leading to neurodegeneration and behavioral deficits were in particular: GABA receptor activation to trigger the apoptosis (Zhao et al., 2011), P75 neurotrophin receptor activation which also may lead to apoptosis (Head et al., 2009; Pearn et al., 2012), and intracellular calcium dysregulation by over activation of inositol trisphosphate (InsP3) receptors also linked to apoptosis (Peng et al., 2011; Wei et al., 2008; Zhao et al., 2010).

A10.3.2.1 GABA receptor

Propofol's primary mechanism of action on the nervous system is through activation of

$GABA_A$ receptors (see above). Alterations in these actions may result for example in excitotoxic injury of immature neurons, as was shown for isoflurane (Zhao et al., 2011).

A10.3.2.2 The p75 neurotrophin receptor

Researchers also proposed a mechanism of propofol neurotoxicity mediated by p75 Neurotrophin receptor activation (Pearn *et. al*., 2012; Head et al., 2009) and intracellular calcium dysregulation by over activation of inositol trisphosphate (InsP3) receptors (Peng et al., 2011; Wei et al., 2008; Zhao et al., 2010). The p75 neurotrophin receptor belongs to a family of transmembrane molecules, which also serve as receptors for the tumor necrosis factor family of cytokines (Chao, 1994). The p75 receptor is a low affinity neurotrophin receptor, to which all neurotrophins bind; p75 receptors activate signaling pathways which can also result in apoptosis.
https://www.nlm.nih.gov/cgi/mesh/2011/MB_cgi?mode=&term=Nerve+Growth+Factor+Receptor+p75

A10.3.2.3 Inositol trisphosphate (InsP3) receptors

A mechanism suggested for propofol-induced developmental neurotoxicity and shared with general anesthetics, is intracellular calcium dysregulation by over-activation of inositol trisphosphate (InsP3) receptors (Peng et al., 2011; Wei et al., 2008; Zhao et al., 2010). Inositol trisphosphate receptor (InsP3R) is a membrane glycoprotein complex acting as a Ca2+ channel activated by inositol trisphosphate (InsP3). InsP3R is very diverse among organisms, and is necessary for the control of cellular and physiological processes including cell division, cell proliferation, apoptosis, fertilization, development, behavior, learning and memory (Bosanac et al., 2002). Inositol triphosphate receptor represents a dominant second messenger leading to the release of Ca2+ from intracellular store sites. There is strong evidence suggesting that the InsP3R plays an important role in the conversion of external stimuli to intracellular Ca2+ signals, e.g. Ca2+ waves and oscillations (Yoshida and Imai S, 1997).

A10.3.3 Mitochondrial function

It has also been shown that mitochondrial function is depressed by propofol by inhibiting in the mitochondria Complex I, Complex IV, Cytochrome c and acylcarnitine transferase, and also by acting as an uncoupling agent in oxidative phosphorylation (Bains et al., 2009; Branca et al., 1991; Mehta et al., 1999; Rigoulet et al., 1996; Wallace et al., 1998).

Four membrane-bound complexes have been identified in mitochondria. Each is an extremely complex trans-membrane structure that is embedded in the inner membrane. Three of them are proton pumps. Lipid-soluble electron carriers and water-soluble electron carriers electrically connect the structures. Briefly, the overall electron transport chain is as follows: complex I (NADH coenzyme Q reductase) accepts electrons from the Krebs cycle electron carrier nicotinamide adenine dinucleotide (NADH), and passes them to coenzyme Q (ubiquinone), which also receives electrons from complex II (succinate dehydrogenase). Coenzyme Q passes electrons to complex III (cytochrome bc1 complex), which passes them to cytochrome c (cyt c). Cyt c passes electrons to Complex IV (cytochrome c oxidase), which uses the electrons and hydrogen ions to reduce molecular oxygen to water.

Oxidative phosphorylation is a vital part of metabolism; the enzymes carrying out this metabolic pathway are also target of many drugs and poisons that inhibit their activities. Oxidative

phosphorylation may produce reactive oxygen species such as superoxide and hydrogen peroxide leading to propagation of free radicals that, in turn, may damage the cells.

More recently, Vanlander et al. (2015) suggested a pathogenic mechanism involving interruption of the electron flow in the mitochondrial membrane at the site of Coenzyme Q (CoQ, or Q_{10}), which transfers electrons from Complex II to Complex III.
CoQ is a coenzyme resembling a vitamin and is abundantly present in the body of most animals, primarily in the mitochondria of most eukaryotic cells. This fat-soluble substance is a component of the electron transport chain and participates in aerobic cellular respiration, which generates energy in the form of ATP. Ninety-five percent of the human body's energy is generated this way (Karp, 2008; Murray et al., 2003).

A10.3.4 Other features contributing to propofol-induced developmental neurotoxicity

Researchers also investigated other features contributing to propofol-induced developmental neurotoxicity like stimulation of blood-brain barrier breakdown –as was demonstrated recently by Sharma et al. (2014) for the developing mouse brain–, suppression of neurogenesis (Al-Jahdari et al., 2006; Wang et al., 2013) and alterations of dendritic spinogenesis (Briner et al., 2011).

To summarize,

1. Propofol is widely used for anesthesia in humans (adults and children/neonates). It is generally accepted to be safe for the mature nervous system, and –in current human practice– considered safe for the developing nervous system as well. However, potential neurotoxic effects in children /neonates have not that well been studied.
2. Exposure of neonate animals to propofol, specifically during the period of brain growth spurt i.e. the period of synaptogenesis, leads to increased neuroapoptosis. The apoptosis appears responsible for the neurodegeneration and behavioral cognitive deficits. The developmental neurotoxic effects are determined by exposure window –i.e. the period of brain growth spurt / synaptogenesis– and the dose, duration and frequency of exposure. Effects are comparable among different species studied, although differences in vulnerability between species are known to exist.
3. The mechanism of action of propofol on the central nervous system involves interactions at various neurotransmitter receptors. Activation of inhibitory $GABA_A$ receptors by propofol in the central nervous system is well known and putatively contributes to the narcotic effects of propofol.
4. The processes and the cascade of events leading to observed neurotoxicity –in laboratory research– in neonatal animals by anesthetics –including also propofol- is poorly understood. A number of potential mechanisms have been –and still is– investigated like interference of receptor mediated and mitochondrial pathways, activation of caspase-3 and involvement of neurotrophins inducing neuroapoptotic damage.
5. Mechanisms suggested to underlie propofol-induced developmental neurotoxicity based on similarities with general anesthetics-induced apoptosis, neurodegeneration and behavioral

deficits are in particular: GABA receptor activation, P75 neurotrophin receptor activation, and intracellular calcium deregulation by overactivation of inositol trisphosphate (InsP3) receptors.

6. Recent evidence shows that imbalance in GABA and glutamate transmitters promotes neuronal apoptosis and resulting neurodegeneration. Glutamate and GABA are known to directly act in drug-induced neuroapoptosis and alcohol addiction via their respective receptors. Relevant in this respect is the finding that there are transmitters that play a different role during development versus maturity and GABA is an example. GABA, the principal *inhibitory* transmitter in the *mature* CNS, acts as an *excitatory* transmitter in *early postnatal stage*. Information from literature suggests that in particular during development of the nervous system, the excitatory $GABA_A$ receptor plays a critical role in the increase in neuroaptosis that leads to structural and functional neural impairment.