Canadian Environmental Protection Act, 1999

Follow-up Assessment Report

Aniline

November 2010

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LIST OF ACRONYMS AND ABBREVIATIONS

CAS	Chemical Abstracts Service
CEPA 1988	*Canadian Environmental Protection Act, 1988*
CEPA 1999	*Canadian Environmental Protection Act, 1999*
kg-bw	kilogram body weight
K_{ow}	octanol/water partition coefficient
LOAEL	Lowest-Observed-Adverse-Effect Level
LOEL	Lowest-Observed-Effect Level
NOAEL	No-Observed-Adverse-Effect Level
PSL	Priority Substances List
TD_{05}	Tumourigenic dose 05 - the dose associated with a 5% increase in tumour incidence above controls
TDI	Tolerable Daily Intake
TDL_{05}	95% lower confidence limit of the TD_{05}

SYNOPSIS

More than 28 tonnes of aniline and its salts were manufactured as a by-product of chemical manufacturing in Canada in 2007. Between 13 and 48 tonnes of aniline and aniline salts were imported into Canada in the period 2000 to 2007. Aniline may be released during the production and use of rubber products but specific monitoring data are not available.

Aniline was included on the first Priority Substances List (PSL) under the 1988 *Canadian Environmental Protection Act* (CEPA) for assessment of potential risks to the environment and human health. As outlined in the assessment report released in 1994, relevant data identified before June 1993 were considered insufficient to conclude whether aniline met the criterion relating to human health under paragraph 11(c) of CEPA.

Additional data relevant to characterization of exposure of the population of Canada have become available since 1994. The available monitoring data in environmental media and food are sufficient to serve as a basis for derivation of average and upper-bounding estimates of exposure for the general population. The predominant route of exposure is from dietary intake as aniline is present in some fruits and vegetables, including apples. In addition, information on the presence of aniline in consumer products (cooking utensils used in food preparation, some permanent markers) was sufficient to estimate exposure from the use of these products.

On the basis of consideration of a comparison of average and upper-bounding estimates of exposure of the general population to the Tolerable Daily Intake derived for aniline, it is proposed that aniline is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

This substance will be included in the upcoming Domestic Substances List inventory update initiative. In addition and where relevant, research and monitoring will be undertaken to confirm assumptions used during the screening assessment.

Based on the information available, it is proposed that aniline does not meet criterion defined in Paragraph 64(c) of the *Canadian Environmental Protection Act, 1999* (CEPA 1999).

1.0 INTRODUCTION

Aniline was one of the substances included on the first *Priority Substances List* (PSL). Under this process, in 1994, the Government of Canada concluded that aniline was not of concern for the environment but available information was considered insufficient to conclude whether aniline was harmful to human health as defined under Paragraph 11 of *Canadian Environmental Protection Act*, 1988.. A follow-up report was published in October 5, 2002, under CEPA 1999, for a 60-day public comment period. This report proposed that there was reason to suspect that exposure to aniline may be harmful to human health but invited stakeholders to submit relevant data to inform the conclusion as there was considerable uncertainty with respect to exposure characterization. Further information relevant to exposure characterization was subsequently identified and has been taken into consideration in current assessment. Additionally, the health effects information has been updated.

The strategy for the literature search to identify critical new data, including commercial activity in Canada, human exposure and effects is presented in Appendix A of this report. Only relevant toxicological data acquired prior to March 2009, and exposure related information developed up to June 2008, with two exceptions, SRI Consulting (2009), and ACMI (2009) were considered in this assessment.

2.0 SUMMARY OF THE HUMAN HEALTH RISK ASSESSMENT FOR ANILINE PUBLISHED IN 1994

At the time of release of the PSL assessment (Government of Canada, 1994), at least 10 tonnes of aniline and its salts were being manufactured, processed or otherwise used annually in Canada. Aniline and aniline hydrochloride were manufactured and imported for use primarily as intermediates in the production of chemicals for the synthesis of polyurethane and rubber. In the United States, aniline was being used in the rubber, plastics, agricultural, dye, photographic chemical and pharmaceutical industries, including in the manufacture of sulphonamide drugs, acetanilide, hydroquinone, sweetening agents, optical whitening agents, resins, marking inks, perfumes and shoe polishes. Derivatives of aniline were also used in the United States as herbicides, fungicides, insecticides, animal repellents and defoliants.

It was estimated that in 1994, 1.1 tonnes of aniline were being released into the Canadian environment each year from various stages of its commercial life cycle. Aniline was not expected to be persistent in the environment. Data on concentrations of aniline in the principal media of exposure (air, food and water) for the general population of Canada and on concentrations of aniline in breast milk and soil in Canada were not available. Therefore, deterministic estimates of the average daily intake were developed based on limited monitoring data that were identified for ambient air in the United States, indoor air in Canada, food in Germany and mainstream cigarette smoke, and on the detection limit for measurement of aniline in drinking water samples from the province of Quebec. These estimates differed by approximately seven orders of magnitude from ones based on concentrations of aniline in air, water and soil in southern Ontario predicted by Level III fugacity modelling. These modelled values were based, in part, on the amount of aniline imported into Canada in 1989. While such estimates are more relevant to the Canadian situation, they are considerably less reliable than ones based on monitoring data.

Available epidemiological studies were inadequate to serve as a basis for assessment of the carcinogenicity of aniline. In identified chronic studies, aniline induced splenic tumours in male (but not female) rats at high doses; no such tumours were observed in male or female mice. Results from the two most extensive carcinogenesis bioassays were consistent with a greater metabolism of aniline by detoxification pathways in mice than in rats, the saturation of this pathway at higher doses in rats, and sex-related differences in metabolism. The increased incidence of splenic tumours in male rats (at doses that may saturate the detoxification pathway) was associated with cellular damage. Hence, the induction of these tumours was considered potentially a consequence of enhanced cellular proliferation associated with cell damage resulting from the accumulation of damaged erythrocytes within this tissue. Data on the genotoxicity of aniline were mixed but consistent with this hypothesis, since aniline had not been genotoxic in the spleen; however, this observation was based on limited data.

A Tolerable Daily Intake (TDI) (i.e., the level of intake to which it is believed that a person may be exposed daily over a lifetime without deleterious effects) of 1.4 µg/kg-bw per day was derived, therefore, for non-neoplastic effects. This value was derived by dividing the lowest dose of aniline (Lowest-Observed-Adverse-Effect Level [LOAEL] = 7.2 mg/kg-bw per day) at which adverse effects (increased splenic haemosiderin, extramedullary haematopoiesis and congestion in male CD-F rats) were observed in the only available long-term animal study in which an adequate range of endpoints had been examined (CIIT, 1982) by an uncertainty factor of 5000 (×10 for intraspecies variation; ×10 for interspecies variation; ×10 for use of a LOAEL rather than a No-Observed-Adverse-Effect Level [NOAEL]; ×5 for limited evidence of carcinogenicity). This TDI value is similar to that which could be derived (4.2 µg/kg-bw per day) based on the results of a (limited) clinical study on formation of methaemoglobin in volunteers exposed by ingestion (Jenkins *et al*., 1972) which reported a no effect dose of 15 mg/man (NOEL of 0.21 mg/kg bw for a 70 kg person) and to which an uncertainty factor of 50 (which takes into account intraspecies variation and limitations of the study) was applied.. However, this clinical study was considered inadequate in itself to serve as a basis for development of a TDI since it was a short-term investigation of a limited range of biochemical effects in a very small number of subjects.

The highly uncertain estimates of exposure to aniline, which were based primarily on data from other countries, and the considerable difference between those estimates and the modelled values precluded meaningful comparison with the TDI in 1994.

Therefore, data were considered insufficient to conclude whether aniline was "toxic" as defined under Paragraph 11(c) of CEPA 1988.

3.0 AN UPDATE OF ANILINE ASSESSMENT

3.1 Production, importation, use and release

3.1.1 Production

In 2007, one facility reported manufacturing more than 28 tonnes of aniline and its salts as a by-product of chemical manufacturing. In the period 2000-2007, the quantity of aniline and its salts manufactured, processed or otherwise used in one year varied from more than 10 tonnes to more than 50 tonnes (Environment Canada, 2008). Precise figures are not available from the National Pollutant Release Inventory (NPRI), the source of this information.

3.1.2 Import

From 2000 to 2007, between 13 and 48 tonnes of aniline and its salts, between 4 and 44 tonnes of N,N- diethylaniline, as well as 3 to 8 tonnes of other aniline derivatives and their salts were imported annually (CIMT, 2008).

3.1.3 Use

Worldwide, 73% to 85% of all aniline is used to produce methylenebis(4-phenyl isocyanate), MDI, also known as methylene diisocyanate. Nitrobenzene, which is the most common chemical precursor to aniline, is typically produced in facilities in which aniline and MDI are also produced. MDI is reacted to make flexible and rigid polyurethane foam. MDI is not produced commercially in Canada. After MDI production, the next largest use of aniline is in the production of rubber processing chemicals. Aniline is a raw material for most of the major groups of rubber processing chemicals: antidegradants; accelerators, activators and vulcanizing agents; and miscellaneous rubber processing aids (Bizzari and Kishi, 2007; Amini and Lowenkron, 2003). Aniline-based rubber processing chemicals and agricultural chemicals are manufactured in Canada (SRI Consulting, 2009). Aniline is also used in petrochemical processing in Canada (Environment Canada, 2008).

3.1.4 Sources and Releases

Each year in the period 2000-2007, more than 2 tonnes of aniline and its salts were sent to off-site waste treatment facilities and the quantity of aniline and its salts reported as on-site fugitive releases varied from 1 to 440 kg. In 2007, slightly more than 28 tonnes of aniline and its salts were sent to off-site treatment prior to disposal from one facility in Ontario. No fugitive emissions were reported from this site in 2007 (Environment Canada, 2008). The criteria for reporting release of aniline to the NPRI are such that facilities manufacturing, processing or otherwise using fewer than 10 tonnes annually are not required to report and these amounts are therefore not represented in the NPRI database.

Rubber production and abrasion of automobile tires are potential sources of aniline in the environment. In the report "European Union Risk Assessment Report Aniline", it was estimated that aniline could be emitted in stack gases from a rubber curing plant at a concentration of about 60 ppm of rubber cured[1] (European Chemicals Bureau, 2004). Based on this and Canadian rubber production in 2004 of 1,464,900 tonnes (Lamb, 2005), the potential annual release of aniline before any emission controls, would be about 88 tonnes All rubber processing plants in Canada have emission controls. The sources of aniline emissions are thought to be sulfenamide and guanidine compounds used as vulcanizing agents as well as n-phenyl-p-phenylenediamine derivatives used as anti-ageing agents. The EU report also stated that aniline was present in rubber abraded from automobile tires and it was estimated that this source could result

[2] Concentrations of aniline, one of 221 detected organic compounds, in stack gases were up to 3,800 $\mu g/m^3$

in 6 tonnes of aniline per year being distributed throughout Germany (European Chemicals Bureau, 2004). Similarly, the use of automobile tires in Canada is expected to be a source of tonnes of aniline released to the Canadian environment each year.

In 2004, several reports were made of aniline migrating from cooking utensils. The source seemed to be particular batches of black polyamide. The aniline content of the utensils was determined to be 121 mg/kg and the migration levels in water simulant at 100°C were 11 to 39 $\mu g/dm^2$, higher than the migration rate permitted for primary aromatic amines in European Union Directive 2002/72/EC (Brede and Skjevrak, 2004). The results of analysis of polyamide cooking utensils by the cantonal laboratory for Basel, Switzerland in 2004, 2005, and 2006 showed between 10% and 18% of such utensils contained 4, 4' methylenediphenylamine and in 2006, 7% contained aniline that migrated from the utensils at a rate exceeding the permitted rate in Europe of 0.02 mg/l (Kantonales Laboratorium, 2006). Although no data are available, it can be assumed that similar polyamide cooking utensils may be sold and used in Canada.

The concentration of aniline in the ink of certain green and pink marker pens sold in Denmark in 2006 was measured and found to be 0.22 and 0.11 mg per gram of ink (0.022 – 0.011%), respectively (Hansen, 2008). Data on the aniline content of green marker pen inks sold in the United States were received from the Art and Creative Materials Institute (ACMI). Estimated concentrations of aniline combined with the concentration of azo dyes assuming full metabolism to aniline following ingestion, in 17 inks ranged from 1 x 10^{-5} to 1.2 % with an average concentration of 0.2%. Among the data was information on green marker pen ink that contains 1.2% aniline and C.I. acid black 2 combined. C.I. acid black 2 is an azine pigment converted to aniline following ingestion (ACMI, 2009). A study of pens and markers was conducted by Health Canada, to determine the level of aniline in commonly used writing instrument inks. The level of aniline was below the limit of quantification (67 mg/kg; equivalent to 0.067 mg/gm) in all markers intended for children, and overall 94% of pens and markers sampled had levels of aniline below the limit of quantification. Only 5 samples (two ball point pens and three permanent markers) out of 86 samples tested had aniline concentrations above the limit of quantification (Health Canada internal report, 2010). While some research suggests that black printer inks, for example, can contain up to 10% C.I. acid black 2 (Xandex, 2006), results from samples tested in Canada do not indicate these levels of aniline would typically be present in Canadian products. The extent to which inks containing aniline used in other products such as stamp inks, temporary tattoos. in Canada is unknown.

Aniline derivatives are used in numerous dyes and pigments and residual aniline may remain in the dye or pigment, as well as in the treated material (e.g., textiles, plastics). The Danish Technological Institute (1999) analysed samples of dyed textiles and found aromatic amines, including aniline, at concentrations from 0.4 to 160 mg per kg textile. However, it is unknown whether similar textiles are available in Canada.

Aniline was reported in samples of shoe dye in Spain at a concentration of 1-2% (European Chemicals Bureau, 2004); however it is unknown whether similar shoe dye is available in Canada. (European Chemicals Bureau, 2004).

Aniline has not been registered as an active ingredient or as a formulant in pest control products in Canada. Many agricultural chemicals contain the aniline substructure, and while these compounds are not all registered for use in Canada, some are in use and they represent a potential source of aniline in the environment through biotic and abiotic degradation processes.

3.2 Population exposure

The information presented below is limited to that which is recent and considered critical to quantitative characterization of exposure of the general population in Canada to aniline. Pertinent new Canadian data are limited and include measurements of aniline in ambient and indoor air, in the breast milk of Canadian women, in fruits and vegetables included in the Canadian Total Diet Study, and findings of no detectable levels in agricultural soils.

Monoaromatic amines, including aniline, were measured in samples of residential indoor air in two regions of eastern Ontario. The levels of aniline in the homes of smokers were significantly higher than those in the homes of non-smokers. The levels of aniline detected in the homes of non-smokers were not statistically different from those found in outdoor air. Aniline was detected in 26 of 69 homes. The maximum level detected in indoor air was 0.054 $\mu g/m^3$ in the home of a smoker and the mean level in the homes of smokers was 0.034 $\mu g/m^3$. Results of this study suggest that cigarette smoke can be a source of aniline in indoor air (Zhu and Aikawa, 2004). The analysis of a composite sample of indoor air taken from 757 Canadian residences reported by Otson *et al*., 1994 included in the earlier follow-up report is not considered reliable due to problems with low analytical recovery and sample handling.

Zhu and Aikawa (2004) reported that the blank-corrected mean levels of aniline in outdoor air in two regions of eastern Ontario were 0.012 $\mu g /m^3$ and 0.007$\mu g/m^3$, and the overall mean concentration of aniline in outdoor air was 0.011 $\mu g/m^3$. The authors did not indicate the frequency of detection of aniline in samples of ambient air. The much higher concentration of aniline in air in the United States reported by Shah and Heyerdahl in 1988 (170 $\mu g /m^3$) which was used for the 1994 assessment and the earlier follow-up report was deemed not to be representative of ambient air concentrations for residential areas.

Composite samples of 39 kinds of fruit and vegetables included in the Canadian Total Diet Study were analysed for aniline. In the analysis of composite samples of raw apples from different Canadian cities and different years, the concentration of aniline

ranged from not detected (limit of detection of 0.010 mg/kg) to 0.483 mg/kg. Aniline was detected in apple samples collected from the 2001, 2004 and 2005 studies (with mean concentrations of 0.468, 0.085 and 0.278 mg/kg respectively) but it was not detected in apple samples collected in the 2002, 2003, 2006 or 2007 studies. The average aniline concentration in samples with detectable levels was 0.277 mg/kg. In all other fruits and vegetables, aniline was not detected (Cao *et al.* 2009). The concentration of aniline in garlic of 19.25 µg/g (equivalent to 19.25 mg/kg) purchased in Taiwan (Yu and Wu, 1989) is much higher than the level of aniline measured in fruits and vegetables sampled in Canada. Data from the Neurath *et al*., (1977) study were used in the estimation of human exposure to aniline in the 1994 assessment report, but the recent data from the Canadian study cited above are used in the estimation of human exposure to aniline in this assessment. One other study reported aniline at the following levels: 0.19 to 12.6 ng/ml coloured soft drink and 0.66 to 9.15 ng/g hard candy (equivalent to 0.00066 to 0.00915 mg/kg) (Lancaster and Lawrence, 1992).

Samples of the fat-free fraction of breast milk collected from 31 healthy, lactating mothers attending hospitals in Hamilton and Guelph in south-central Ontario contained aniline at concentrations ranging from 0.05 to 5.2 ppb (ug/kg); concentrations in 30 of the samples were between 0.05 and 0.8 ppb (ug/kg). There was no statistically significant difference in the concentration of aniline in the milk of the mothers who smoked and those who did not (DeBruin *et al*., 1999). The source of the aniline found in these samples of breast milk was not identified.

Aniline was not detected (limit of detection 0.3 mg/kg dry weight) in agricultural soil collected from nine provinces across Canada, including those where there had been repeated heavy use of agricultural pesticides at intensively cropped farms (Webber and Wang, 1995).

Studies identified but not considered to contribute to quantitative estimates of population exposure are those on aniline in indoor and outdoor air (Palmiotto *et al*., 2001; Luceri *et al*., 1993), garlic (Yu and Wu, 1989), a cyclamate sweetener (Hernando *et al*., 1999) and in consumer products (European Chemicals Bureau, 2004; Brede and Skjevrak, 2004).

Methodology for exposure assessment has evolved since completion of the 1994 assessment. Deterministic estimates of average and upper-bounding total multi-media daily intake of aniline for six age groups of the general population of Canada, which incorporate these developments in methodology (Health Canada, 1998) and the more recent monitoring data described in this section are presented in Tables 1 and 1a. Estimates of average daily intake of aniline for the six age groups range from 0.045 µg/kg-bw per day for breast-fed infants to 0.73 µg/kg-bw per day for children aged six months to four years while upper-bounding estimates of total daily intakes of aniline for these age groups range from 0.068 µg/kg-bw per day for formula fed infants to 1.16

µg/kg-bw per day for children aged six months to four years. The assumptions on which these estimates are based are listed in footnotes to the table.

Exposure scenarios were developed for dermal and oral intake of marker pen ink by a child aged two to three years, assuming that the concentration of aniline in the ink was 0.22 mg/gm. The calculations are shown in Table 2. It was estimated that a young child using marker pens daily would have a chronic aniline intake of 0.047 µg/kg-bw per day from marker pen ink. An acute exposure arising from applying 50 cm^2 to the skin (equivalent to the central area of two palms) is estimated to result in an intake of 0.71 µg/kg-bw per event. Because of behaviour and body weight, it is assumed that children aged 2-3 years are more highly exposed to marker ink than people in other age groups, so exposure was modelled for that age group only. Available information on the concentration of aniline in marker ink was used to estimate exposure, however, since Health Canada (2010) reported markers intended for children did not contain aniline at levels above the limit of quantification (67mg/kg; equivalent to 0.067 mg/gm), exposure to aniline from these types of inks is expected to be much lower.

Polyamide cooking utensils from which measurable quantities of aniline migrate into a water simulant were found in Europe in 2004, 2005, and 2006. In Table 2 are the results of a conservative estimation of exposure, based on the assumption that a polyamide tool is used to stir soups and sauces and 0.03mg aniline per litre per hour migrates from the tool to the food and that the tool remains in the soup or sauce for one hour at 100 degrees Celsius. Based on this calculation, the estimated exposure to aniline ranges from 0.04 to 0.14 µg/kg-bw per day. This estimate is considered to be conservative as it is unlikely that all soups or sauces will be stirred continually for this length of time or at this temperature; also, information from Europe indicated that less than 10% of all polyamide cooking utensils tested contained aniline.

Exposure scenarios based on levels of aniline reported in samples of shoe dye in Spain, developed by the European Chemicals Bureau, resulted in an estimated dermal intake of 0.1 µg/kg-bw per day for adults and 0.043 µg/kg-bw per day for children (European Chemicals Bureau, 2004). However, it is unknown whether similar shoe dye is available in Canada.

A separate estimate was made of the aniline exposure arising from smoking based on the mean concentration of aniline (102 ng/cigarette) in mainstream smoke from cigarettes purchased in the United States (Patriankos and Hoffmann, 1979) and an estimated 20 cigarettes smoked per day (Health Canada, 1998). The estimated exposure from cigarette smoking for an adult weighing 70.9 kg is 0.03 µg aniline/kg-bw/day

3.3 Hazard characterization and dose–response analyses

3.3.1 Hazard characterization

Additional toxicological data on aniline or aniline hydrochloride identified in the period since the 1994 assessment was released include the results of *in vivo* genotoxicity studies in which DNA damage was observed in the organs of rodents exposed to a single oral high dose (Sekihashi *et al*., 2002; Sasaki *et al*., 2000), and mixed results were reported regarding the induction of micronuclei in rats or mice after high-dose short- or long-term exposure to aniline via oral or intraperitoneal (ip) routes (reviewed in Bomhard and Herbold 2005; Bomhard, 2003; Jones and Fox, 2003; Ress *et al*., 2002; Bayer AG, 2001a, 2001b, cited in European Chemicals Bureau 2004; Witt *et al*., 2000). *In vitro* study results were negative for induction of micronuclei or transformation in Syrian hamster embryo cells (Fritzenschaf *et al*., 1993), for mutagenicity in Escherichia coli (Martinez *et al*., 2000), the Ames assay (Aßmann *et al*., 1997; Chung *et al*., 1995, 1996; Brennan and Schiestl, 1997). However, results were positive for chromosomal aberrations in Chinese hamster ovary cells (Chung *et al*., 1995, 1996), for micronuclei in Chinese hamster lung cells (Matsushima *et al*.1999), and for intrachromosomal recombination in *S. cerivisiae* (Brennan and Schiestl, 1997). The updated genotoxicity information is presented in table 4.

Additional repeated-dose toxicity studies conducted in experimental species include one subchronic study in which male Sprague-Dawley rats were exposed to a single concentration of aniline hydrochloride in drinking water for 90 days (Khan *et al*., 1993). Although the subchronic study by Khan *et al.*, (1993) was considered inadequate for characterization of exposure–response, the results of this investigation are similar to those of other repeated-dose toxicity studies in which the blood and spleen have been identified as critical tissues for toxicological effects of aniline.

New studies regarding the short-term toxicity of aniline did not identify significant effects other than those already characterized (erythrotoxicity or splenotoxicity) in the 1994 assessment and were further investigating the possible mechanism of aniline toxicity (Khan, 2006; Khan, 2003a, 2003b, 2003c; Zwirner-Baier *et al*., 2003; BASF AG 2001, cited in European Chemicals Bureau, 2004). Acute exposure to a single dose of aniline (equivalent to 15 mg/kg), via inhalation or oral administration, resulted in methaemoglobinemia in dogs within a few hours of exposure, but the animals recovered fully the next day (Pauluhn, 2002; Bayer AG, 2000, cited in European Chemicals Bureau 2004). The symptoms of methaemoglobinemia are those typically associated with a lack of oxygen (cyanosis). It has been reported that up to 20% of methaemoglobinemia does not cause health related symptoms in a healthy population i.e those with normal hematocrit. However, higher levels of methaemoglobinemia ranging from 20-50% may cause shortness of breath (dyspnoea), headache, tachycardia (increased

heart rate) or dizziness, whereas levels greater than 60-70% may cause coma or death (De Gruchy 1970; Wintrobe 1970, cited in Harrison 1977).

An acute no-effect dose of 15 mg/man (0.21 mg/kg bw) has been identified for methaemoglobinemia formation in adult human volunteers (Jenkins et al., 1972; Government of Canada, 1994; European Chemicals Bureau, 2004). A analysis conducted by Health Canada of human data reported in Jenkins et al (1972) suggested that an acute oral exposure of 71 mg of aniline (1 mg/kg bw per day) may be required to cause an adverse increase in methaemoglobinemia formation (20%) in humans.

During the 1994 assessment, the available data were considered inadequate to meaningfully characterize exposure–response for the effects of aniline following inhalation. In a single identified long-term inhalation study, minimal effects (mild cyanosis, a slight [unspecified] reduction in body weight and a slight [statistical evaluation not presented] increase in methaemoglobin) were reported in male Wistar rats exposed (whole body) to a single concentration (19 mg/m^3) of aniline for 26 weeks (Oberst *et al*., 1956).

A recently conducted inhalation study reported development of methaemoglobinemia and associated erythrocytotoxicity in male Wistar rats exposed to 96.5 or 274.9 mg/m^3 aniline for 6 hr/day, 5 days/week, for 2 weeks which was followed by a 2 week post exposure period. The authors reported a no-observed-adverse-effect concentration (NOAEC) of 32.4 mg/m^3 for erythrocytotoxicity and associated sequestration of erythrocytes, iron accumulation, and lipid peroxidation and no effects were seen at 9.2 mg/m^3 of aniline exposure (Pauluhn, 2004).

Potential developmental effects of aniline have been investigated in rats since the 1994 assessment. Although the incidence of cleft palate and cardiovascular malformations were observed in fetuses of dams injected subcutaneously with aniline hydrochloride, the authors considered them to be indirect teratogenic effects of aniline due to maternal methaemoglobinemia hypoxia (Matsumoto *et al*., 2001a, 2001b). No evidence of developmental effects was reported by the authors in a previous study in which Fischer 344 rats were exposed via gavage to maternally toxic doses (10, 30 or 100 mg/kg/day) of aniline from GD 7 - 20 (Price *et al*., 1985).

Relevant human data were limited to the results of (limited) epidemiological studies, in which workers were exposed to aniline and other chemicals within the working environment; however, no clear relationship was established between exposure to aniline and incidence of cancer (Sorahan and Pope, 1993; Mikoczy *et al*., 1996; Alguacil *et al*., 2000; Sathiakumar and Deizell, 2000). In an update of the Sorahan and Pope (1993) study, additional data analyses indicated no association between duration of

employment in the aniline department and increased risk of bladder cancer in chemical product workers (Sorahan *et al* 2000).

3.3.2 Possible Mode(s) of Action of Aniline

The mode of action of potential carcinogenicity of aniline or aniline hydrochloride is not fully understood. As described above, the genotoxicity of aniline in various *in vitro* or *in vivo* assays is mixed. Studies have shown that long-term dietary exposure to high or toxic doses (more than 100 mg/kg-bw per day for 2 years) of aniline hydrochloride produced significant levels of splenic tumours only in male rats, but not in mice (CIIT, 1982; NCI, 1978).The relevance of mechanism of aniline-related toxicity in rats to humans is also not clear.

There is some information available on the potential mode of action of aniline or its metabolites (reviewed in Bomhard and Herbold, 2005; Bus and Popp, 1987). Exposure to aniline hydrochloride at a dose range of 10-30 mg/kg-bw per day caused haematological effects and repeated long-term high-dose exposure (100 mg/kg-bw per day or more) produced splenic tumours in rats subsequent to haematological effects (Mellert *et al*., 2004; CIIT 1982; NCI 1978). The primary toxicity of aniline is characterized by injury to erythrocytes and production of methaemoglobinemia in rats (CIIT, 1982) and humans (Kearney *et al*., 1984; Harrison, 1977; Jenkins *et al*., 1972). A possible mode of action for carcinogenicity is that repeated high-dose exposure to aniline causes injury to erythrocytes and scavenging of these chemically damaged erythrocytes by the spleen produces an iron overload or oxidative damage to macromolecules, which may result in a carcinogenic response in the spleen (Ma *et al*., 2008; reviewed in Bomhard and Herbold, 2005; Wu *et al*., 2005; Khan, 2000; 1999). Alternatively, it has been proposed that the oxidized metabolites of aniline, including phenylhydroxylamine (PHA) or nitrosobenzene (NB), may cause damage to erythrocytes and contribute to splenic toxicity by causing oxidative damage which may initiate the events leading to the development of splenic tumours (reviewed in Bomhard and Herbold, 2005; Khan *et al*., 2000; Bus and Popp, 1987; Goodman *et al*., 1984). A recent study provided evidence that short-term repeated-dose exposure to aniline in rats caused initiation of an oxidative-stress signalling pathway, by activation of nuclear factor κB (NF-κB) and activator protein-1 (AP-1), in the rat spleenocytes. This leads to the phosphorylation of critical cell signalling proteins which may result in the upregulation of pathologic precursors (pro-inflammatory and pro-fibrogenic cytokines) of tumourigenesis. The authors concluded that these early molecular events could ultimately lead to splenic fibrosis and/or fibrosarcomas following continuous exposure to aniline (Wang *et al*., 2008).

There is some evidence from *in vivo* studies to indicate that aniline may be genotoxic and that a genotoxic mode of action may exist for the carcinogenicity of aniline. However, there is no evidence to directly support that the underlying mechanism of aniline-related splenic carcinogenicity is based on genotoxic activity (reviewed in

Bomhard and Herbold 2005; European Chemicals Bureau, 2004). In addition, it has been proposed that the methodological differences in genotoxicity assays (e.g., dose selection and route of exposure) and tumourigenic response confined only to high-dose (100 mg/kg bw per day) rats support a non-genotoxic mode of action which may be associated with a threshold (Bus and Popp, 1987; CIIT 1982; Bomhard, 2003; Mellert, *et al*., 2004; reviewed in Bomhard and Herbold, 2005). There is also some indication in *in vivo* studies that aniline, when administered at high dose, may interact directly with DNA in the spleen of predosed rats (but not mice), although DNA binding in the spleen is low compared with that in other tissues (McCarthy *et al*., 1985).

Elucidation of the mode of action of aniline has not become more defined since the release of the 1994 report. There is insufficient information to determine whether the tumourigenic response is mediated by direct interaction of aniline or its metabolites with splenic macromolecules (proteins, DNA or lipids) or if other possible cytotoxic responses of the spleen are involved. Possible involvement of a genotoxic or other multiple mode(s) of action needs further investigation.

3.3.3 Dose–response analyses

In view of the absence of critical recent toxicological data, the dose–response analyses presented here reflect primarily those developed in the 1994 assessment released under CEPA 1988.

3.3.3.1 Oral exposure

In the assessment of aniline for 1994, non-neoplastic histopathological lesions in the spleen of rats (the most sensitive rodent species) were considered to be the critical endpoint for characterization of dose–response. Since the cytotoxicity of aniline may be the crucial determinant in the carcinogenicity of this compound in the spleen of rats (but not mice) at high doses, measures of dose–response for non-neoplastic effects may be protective for tumours, although this conclusion is uncertain. In view of uncertainty concerning the mode of induction of tumours, therefore, measures of cancer potency are also presented here and compared with those for non-cancer effects (described in section 2.0).

Estimates of carcinogenic potency, tumourigenic dose 05 (TD_{05}) associated with a 5% increase in tumour incidence above controls, for aniline have been derived based on the incidence of splenic tumours (stromal sarcoma, haemangiosarcoma, fibrosarcoma, osteogenic sarcoma and capsular sarcoma) in control and three dose groups of CD-F rats exposed in the diet to 10–100 mg aniline hydrochloride/kg-bw per day (7.2–71.9 mg aniline/kg-bw per day) for up to 104 weeks (CIIT, 1982). This investigation was considered the most appropriate for quantitative assessment of the TD_{05}, since it was the only identified long-term study in which an adequate range of endpoints was examined in

the most sensitive rodent species. In addition, compared with the NCI (1978) bioassay, there were more dose groups (three dose groups and controls vs. two dose groups) in this study, as well as larger numbers of animals per group (n = 130 per sex vs. n = 50 males) and more extensive histopathological examination.

Measures of tumourigenic potency have been developed, based on multistage modelling of incidence using GLOBAL 82 (Howe and Crump, 1982). The incidences of tumours on which the estimates of potency are based, degrees of freedom, parameter estimates and nature of any adjustments for mortality or period of exposure are presented in Table 3 and Figure 1. The lowest calculated TD_{05} is 46 mg/kg-bw per day, based on stromal sarcoma in the spleen of male rats; the lower 95% confidence limit (TDL_{05}) for this value is 35 mg/kg-bw per day. The most conservative estimate of carcinogenic potency (i.e., the TDL_{05} of 35 mg/kg-bw per day) is one order of magnitude greater than the LOAEL (7.2 mg/kg-bw per day) that formed the basis of the TDI.

3.4 Human health risk characterization

The 1994 assessment for aniline (Government of Canada, 1994) concluded that there was inadequate information from epidemiological studies to assess the carcinogenicity of aniline in humans, and the limited evidence of carcinogenicity of aniline in laboratory animals exposed to high doses. Therefore, a tolerable daily intake (TDI) was derived on the basis of a Lowest-Observed-(Adverse)-Effect-Level [LO(A)EL], divided by an uncertainty factor, taking into account the limited evidence of carcinogenicity (as described in section 2.0 above). Since the publication of the 1994 assessment, no additional carcinogenicity studies, or epidemiological studies of aniline have been published. The most conservative estimate of carcinogenic potency (i.e., the TDL_{05} of 35 mg/kg-bw per day) is one order of magnitude greater than the LOAEL (7.2 mg/kg-bw per day) that formed the basis of the calculated TDI estimate for non-cancer effects (1.4 µg/kg-bw per day).

In the current analysis of multi-media exposure, fruits and vegetables consumed as food is the predominant source of exposure to aniline. Estimates of average daily intake of aniline range up to 0.73 µg/kg-bw per day for children aged six months to four years while the upper-bounding estimates of total daily intakes of aniline for this age group is 1.16 µg/kg-bw per day. Intake from food is based primarily on analysis of fruits and vegetables from Canadian Total Diet Studies for the years 2001-2007 (Cao *et al.* 2009). The concentration of aniline in composite samples of raw apples purchased in different Canadian cities and different years, ranged from not detected to 483 µg/kg, with the highest concentration in the 2001 samples of apples purchased in Newfoundland for the Canadian Total Diet Study (Cao *et al.* 2009). Data indicating that Canadians are exposed to aniline was demonstrated by the detection at parts per billion levels of this substance in each of 31 breast milk samples collected by DeBruin *et al*. (1999).

Incidental ingestion of inks containing concentrations of aniline limited to 0.022%, either directly of indirectly via prior dermal exposure and mouthing behaviour has been conservatively estimated to result in a chronic exposure of 0.094 µg/kg-bw per day for children aged 6 months to 4 years. A separate acute exposure scenario results in estimated exposure of 0.71 µg/kg-bw per event. These estimates are considered to be an overestimate as Health Canada did not identify aniline in markers intended for children at levels above the limit of detection (Health Canada internal report, 2010). The quantity of aniline migrating from cooking utensils during food preparation was conservatively estimated to result in an exposure of an additional 0.14 µg/kg-bw per day for this same age group.

The average and upper-bounding estimates of total daily intake of aniline from all media (up to 0.73 and 1.16 µg/kg-bw per day respectively), for the most highly exposed age groups are below the TDI of 1.4 µg/kg-bw per day.

Potential exposure for children aged 6 months to 4 years from the use of marker pens were conservatively estimated to range from 0.71 µg/kg-bw/event to 0.047 µg/kg-bw/day and from cooking utensils to be 0.04 to 0.14 µg/kg-bw/day. These exposures are lower than the TDI of 1.4 µg/kg-bw per day.

In terms of acute exposures, comparison of estimated acute exposure from inks (0.71 µg/kg bw per event) with the acute no-effect dose of 0.21 mg/kg bw or the value of 1.0 mg/kg bw derived by Health Canada as a level which may be required to cause an adverse increase in methaemoglobinemia formation in humans results in margins of exposure of 300 and 1400, respectively. These MOEs are considered adequate to address uncertainties in the health effects and exposure databases.

Given the conservative nature of the product exposure estimates as well as the upper-bounding multimedia intake combined with the conservative uncertainty factor applied to obtain the TDI (factor of 5000(×10 for intraspecies variation; ×10 for interspecies variation; ×10 for use of a LOAEL rather than a No-Observed-Adverse-Effect Level [NOAEL]; ×5 for limited evidence of carcinogenicity), Government of Canada. 1994) and accounting for that fact that estimated exposures would decrease with age it is proposed that aniline be considered a substance that is not entering the environment in a quantity and or concentration or under conditions that constitute or may constitute danger in Canada to human life or health.

3.5 Uncertainties and degree of confidence in human health risk characterization

Confidence in a single study that reported levels of aniline in indoor and ambient air measured in locations in eastern Ontario is moderate. The level of aniline measured in field blanks in this study exceeded the method detection limit for field blank-corrected

values for many samples. No samples were taken in heavily industrialised areas of Canada or in the vicinity of any point source of aniline emission, reducing confidence that the estimates of exposure via ambient air are upper-bounding.

The current assessment indicates that food, specifically fruits and vegetables (Canadian total diet study), is the predominant source of exposure to aniline and this is consistent with the prediction in the European Union Risk Assessment Report Aniline (European Chemicals Bureau, 2004). Exposure to aniline from foods other than fruits and vegetables was not accounted for, thus exposure may be underestimated. The source of aniline found in raw apples purchased in Canada is unknown, nor is it known whether these apples were grown in Canada or imported. Confidence in the reported levels of aniline in breast milk of Canadian women is high.

There is a relatively high degree of certainty that consumption of drinking water and ingestion of soil do not contribute significantly to the intake of aniline by Canadians, based on sensitive measurements of drinking water and of agricultural soil collected from several sources in Canada, in which aniline was consistently not detected.

The availability in Canada of consumer products that may result in exposure to aniline is not known. Although aniline is not an intentional ingredient in consumer products, it may be present in consumer products as a residual as aniline derivatives have various uses, including as dyes and pigments. Aniline may also be formed endogenously following ingestion of certain aniline derivatives. Confidence in the results of modelling of exposure of children to inks from marker pens is moderate since the Health Canada Survey of 86 samples of markers and pens (Health Canada, 2010) did not find aniline above the level of quantification in those markers intended for use by children.

The degree of confidence in the database on toxicity that serves as the basis for the development of the TDI is moderate, although there is a relatively high degree of certainty that the critical effects following ingestion are those that occur in the spleen. Available data on effects of aniline following inhalation are also inadequate to characterize exposure–response.

3.6 Proposed Conclusion

Based on the available information on its potential to cause harm to human health, it is proposed that aniline is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is therefore proposed that aniline does not meet the criterion in paragraph 64(c) of CEPA 1999.

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Figure 1: Cancer potency estimates (TD_{05}s) based on splenic tumours in rats

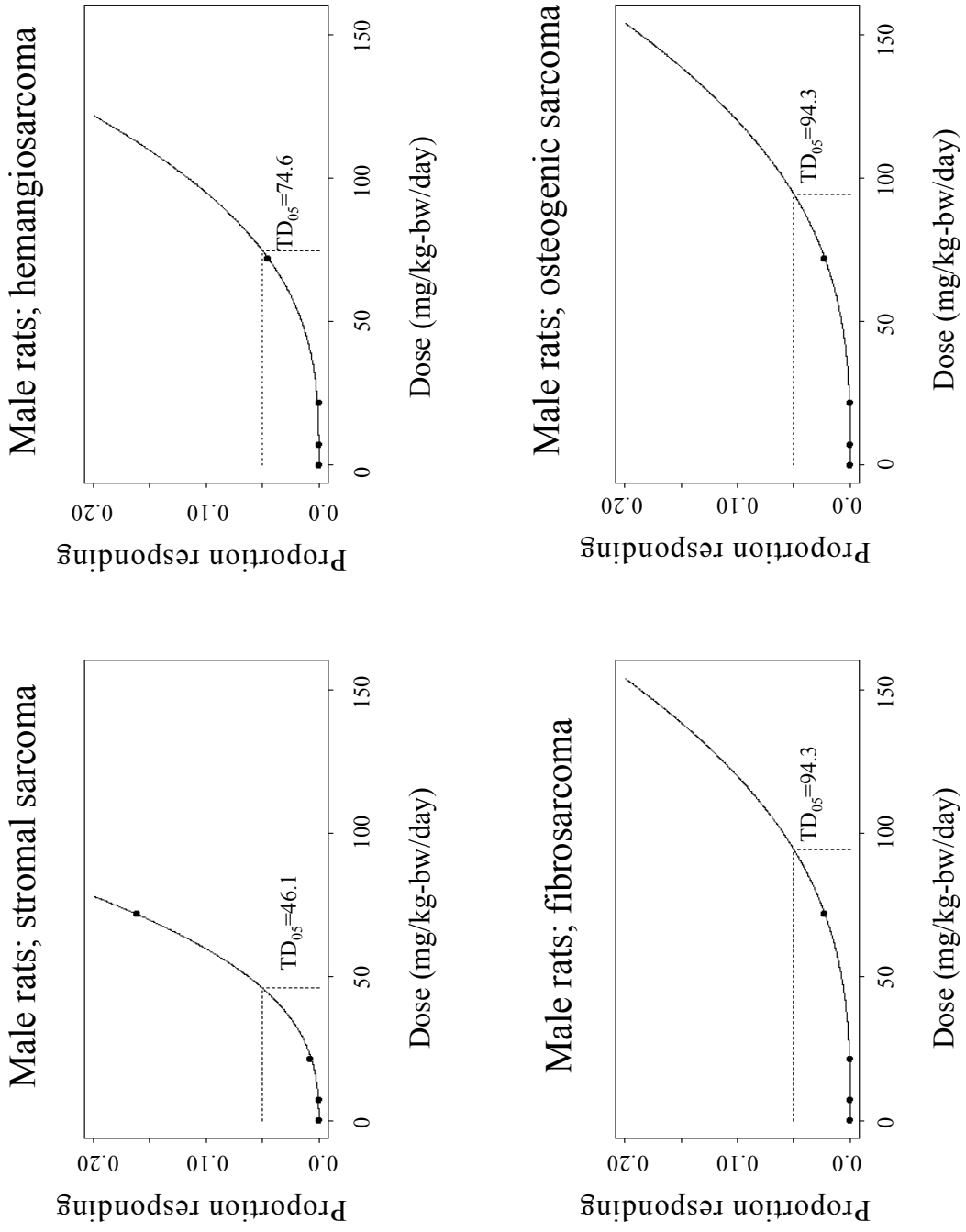
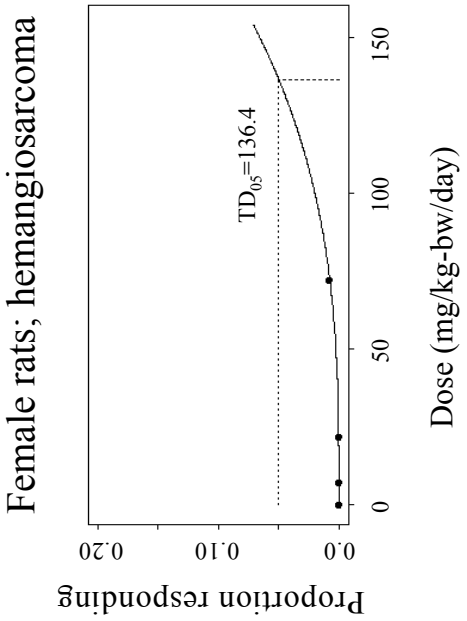


Figure 1 (continued)



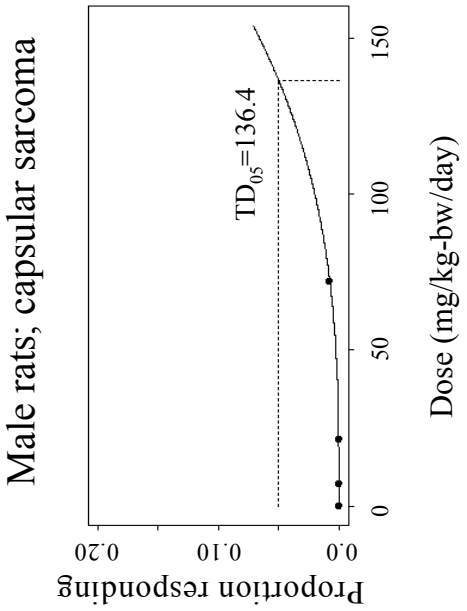


Table 1: Upper-bounding estimates of daily intake of aniline by the general population in Canada

	Estimated intake (µg/kg-bw per day) of Aniline by various age groups							
Age Group:	**0 - 0.5 yr[1]**			**0.5 - 4 yr[5]**	**5 - 11 yr[6]**	**12 - 19 yr[7]**	**20 - 59 yr[8]**	**60 + yr[9]**
Route of Exposure	**Breast Milk Fed[2]**	**Formula Fed[3]**	**Fed Solid Food[4]**					
Ambient Air[10]	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**
Indoor Air[11]	**0.013**	**0.013**	**0.013**	**0.028**	**0.022**	**0.013**	**0.011**	**0.009**
Drinking Water[12]	**na**	**0.053**	**0.050**	**0.023**	**0.018**	**0.010**	**0.011**	**0.011**
Food and Beverages[13]	**0.520**	**nd**	**0.667**	**1.11**	**0.924**	**0.384**	**0.252**	**0.200**
Soil[14]	**0.001**	**0.001**	**0.001**	**0.002**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Total Intake	**0.535**	**0.068**	**0.732**	**1.16**	**0.965**	**0.407**	**0.274**	**0.221**
Maximum Total Intake From All Routes of Exposure - Upper-Bounding								**1.16**

Abbreviations: na = not applicable nd = no data

1 Assumed to weigh 7.5 kg, to breathe 2.1 m^3 of air per day, to drink 0.8 L of water per day (formula fed) or 0.3 L/day (fed solid food) or 0.0 L/day (breast fed) and to ingest 30 mg of soil per day (Health Canada, 1998).

2 Based on the highest measured concentration of aniline, 5.2 ppb in 31 samples of breast milk from women residing in Ontario (DeBruin *et al*., 1999), a daily consumption of 750 g breast milk per day for infants and the assumption that infants in Canada are exclusively breast-fed (Health Canada, 1998).

3 For exclusively formula-fed infants, intake of water is only that required to reconstitute formula. Based on the limit of detection of 0.5 µg/L reported in a survey of drinking water conducted in 1991 in 17 municipalities in Quebec, in which aniline was not detected (St Martin, 1992).

4 The dietary intake is based on consumption of 0.3 litres of water and up to 1.18 kg of food daily. This intake pattern is presented as a hypothetical extreme case and does not reflect recommended infant feeding practice.

5 Assumed to weigh 15.5 kg, to breathe 9.3 m^3 of air per day, to drink 0.7 L of water per day and to ingest 100 mg of soil per day (Health Canada, 1998).

6 Assumed to weigh 31.0 kg, to breathe 14.5 m^3 of air per day, to drink 1.1 L of water per day and to ingest 65 mg of soil per day (Health Canada, 1998).

7 Assumed to weigh 59.4 kg, to breathe 15.8 m^3 of air per day, to drink 1.2 L of water per day and to ingest 30 mg of soil per day (Health Canada, 1998).

8 Assumed to weigh 70.9 kg, to breathe 16.2 m^3 of air per day, to drink 1.5 L of water per day and to ingest 30 mg of soil per day (Health Canada, 1998).

9 Assumed to weigh 72.0 kg, to breathe 14.3 m^3 of air per day, to drink 1.6 L of water per day and to ingest 30 mg of soil per day (Health Canada, 1998).

[10] Based on the higher mean level of aniline (0.012ug/m^3) in ambient air reported by Zhu and Aikawa 2004 in two regions of eastern Ontario and an estimated 3 hours per day spent outdoors (Health Canada, 1998)

[11] Based on the highest reported concentration of aniline (0.054ug/m^3) in the indoor air of 69 homes in two different locations in eastern Ontario (Zhu and Aikawa, 2004) and an estimated 21 hours per day spent indoors (Health Canada, 1998).

[12] Based on the limit of detection of 0.5 µg/L reported in a survey of drinking water conducted in 1991 in 17 municipalities in Quebec, in which aniline was not detected (St Martin, 1992).

[13] Based on the maximum measured concentrations of aniline in raw apples (0.483 mg/kg), and the detection limit of 0.010 mg/kg for other fruits and vegetables tested from Canada (Cao *et al.* 2009). For canned apple products, a concentration of 0.483 mg/kg was used and for apple pie, a concentration of 0.160 mg/kg was used. The highest concentrations of aniline measured in soft drinks and the concentration of aniline in hard candies were used (Lancaster and Lawrence, 1992). The data set considered includes reports by Neurath *et al*., 1977, by Yu and Wu 1989, and by Hernando *et al*., 1999. For breast milk-fed babies, see footnote 2. Amounts of foods consumed on a daily basis by each age group are described by Health Canada (Health Canada, 1998).

[14] Based on the limit of detection (0.3 mg/kg) of aniline in a survey of agricultural soil from nine provinces in Canada in which aniline was not detected (Webber and Wang, 1995).

Table 1a: Average estimates of daily intake of aniline by the general population in Canada

	Estimated intake (µg/kg-bw per day) of Aniline by various age groups							
Age Group:	**0 - 0.5 yr[1]**			**0.5 - 4 yr[5]**	**5 - 11 yr[6]**	**12 - 19 yr[7]**	**20 - 59 yr[8]**	**60 + yr[9]**
Route of Exposure	**Breast Milk Fed[2]**	**Formula Fed[3]**	**Fed Solid Food[4]**					
Ambient Air[10]	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**
Indoor Air[11]	**0.003**	**0.003**	**0.003**	**0.006**	**0.005**	**0.003**	**0.002**	**0.002**
Drinking Water[12]	**na**	**0.053**	**0.050**	**0.023**	**0.018**	**0.010**	**0.011**	**0.011**
Food and Beverages[13]	**0.036**	**nd**	**0.501**	**0.690**	**0.566**	**0.241**	**0.169**	**0.139**
Soil[14]	**0.001**	**0.001**	**0.001**	**0.002**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Total Intake	**0.040**	**0.058**	**0.556**	**0.722**	**0.590**	**0.254**	**0.182**	**0.152**
Maximum Total Intake From All Routes of Exposure – Central Tendency								**0.722**

Abbreviations: na = not applicable nd = no data

[1] Assumed to weigh 7.5 kg, to breathe 2.1 m^3 of air per day, to drink 0.8 L of water per day (formula fed) or 0.3 L/day (fed solid food) or 0.0 L/day (breast fed) and to ingest 30 mg of soil per day (Health Canada, 1998).

[2] Based on the mean concentration of aniline, 0.36 ppb in 31 samples of breast milk from women residing in Ontario (DeBruin *et al*., 1999), a daily consumption of 750 g breast milk per day for infants and the assumption that infants in Canada are exclusively breast-fed (Health Canada, 1998).

[3] For exclusively formula-fed infants, intake of water is only that required to reconstitute formula. Based on the limit of detection of 0.5 µg/L reported in a survey of drinking water conducted in 1991 in 17 municipalities in Quebec, in which aniline was not detected (St Martin, 1992).

[4] The dietary intake is based on consumption of 0.3 litres of water and up to 1.18 kg of food daily. This intake pattern is presented as a hypothetical extreme case and does not reflect recommended infant feeding practice.

[5] Assumed to weigh 15.5 kg, to breathe 9.3 m^3 of air per day, to drink 0.7 L of water per day and to ingest 100 mg of soil per day (Health Canada, 1998).

[6] Assumed to weigh 31.0 kg, to breathe 14.5 m^3 of air per day, to drink 1.1 L of water per day and to ingest 65 mg of soil per day (Health Canada, 1998).

[7] Assumed to weigh 59.4 kg, to breathe 15.8 m^3 of air per day, to drink 1.2 L of water per day and to ingest 30 mg of soil per day (Health Canada, 1998).

[8] Assumed to weigh 70.9 kg, to breathe 16.2 m^3 of air per day, to drink 1.5 L of water per day and to ingest 30 mg of soil per day (Health Canada, 1998).

[9] Assumed to weigh 72.0 kg, to breathe 14.3 m^3 of air per day, to drink 1.6 L of water per day and to ingest 30 mg of soil per day (Health Canada, 1998).

[10] Based on the overall mean reported concentration of aniline (0.011 ug/m^3) in ambient air reported by Zhu and Aikawa 2004 in two regions of eastern Ontario and an estimated 3 hours per day spent outdoors (Health Canada, 1998)

[11] Based on the overall mean reported concentration of aniline (0.011 ug/m^3) in the indoor air of 62 homes in two different locations in eastern Ontario in which cigarette smoking did not occur (Zhu and Aikawa, 2003) and an estimated 21 hours per day spent indoors (Health Canada, 1998).

[12] Based on the limit of detection of 0.5 µg/L reported in a survey of drinking water conducted in 1991 in 17 municipalities in Quebec, in which aniline was not detected (St Martin, 1992).

[13] Based on the mean reported concentration of aniline in composite samples of raw apple in which aniline was detected (0.277 mg/kg), and the detection limit of 0.010 mg/kg for other fruits and vegetables tested from Canada (Cao *et al.* 2009). For canned apple products, a concentration of 0.277 mg/kg was used and for apple pie, a concentration of 0.092 mg/kg was used. The mean concentrations of aniline measured in soft drinks and the concentration of aniline in hard candies were used (Lancaster and Lawrence, 1992). The data set considered includes reports by Neurath *et al*., 1977, by Yu and Wu 1989, and by Hernando *et al*., 1999. For breast milk-fed babies, see footnote 2. Amounts of foods consumed on a daily basis by each age group are described by Health Canada (Health Canada, 1998).

[14] Based on the limit of detection (0.3 mg/kg) of aniline in a survey of agricultural soil from nine provinces in Canada in which aniline was not detected (Webber and Wang, 1995).

Table 2: Consumer product exposure scenarios

Consumer Product	Scenario	Estimated intake
Marker pen Acute* exposure	Child marking skin with ink. Exposed area is 50 cm^2; ink transferred to 50 cm^2 is 0.05 grams. Concentration of aniline in ink is 0.022 % (Hansen et al 2008). Assume 100% absorption by ingestion after mouth contact with the inked skin. Aniline intake per event: 50 mg ink * 10E3 µg /mg*0.00022 g aniline per g ink /15.5 kg = 0.71 µg/kg-bw/event	0.71 µg/kg-bw/event
Marker pen Chronic* exposure	Child marking skin with ink. Exposed area is a 25 cm line; ink transferred to 25 cm is 3.35 mg. Concentration of aniline in ink is 0.022 % (Hansen et al 2008) and ink deposition rate is 134 µg/cm (ACMI 2009). Assume 100% absorption by ingestion after mouth contact with the inked skin. Aniline intake per event: 3.35 mg ink *10E3 µg /mg *0.00022 g aniline per g ink /15.5 kg = 0.047 µg/kg-bw/event Assume child uses marker pens daily.	0.047 µg/kg-bw/day
Polyamide cooking utensils	Preparation of soups and sauces using polyamide tools with aniline migration rate of 30 µg/litre/hour (Kantonales Laboratorium 2006). Assume that the utensil remains in the soup or sauce for one hour at 100 degrees Celsius. Using a detailed intake of foods (Health Canada, 1998), the estimated exposure to aniline arising from use of polyamide utensil from which aniline migrates to soups and sauces only, is Age — µg/kg-bw/day 0 – 6 mo — 0.053 6 mo – 4 yr — 0.14 5 – 11 yr — 0.079 12-19 yr — 0.044 20-59 yr — 0.042 60 + yr — 0.04	0.04 to 0.14 µg/kg-bw/day

* A recent Health Canada study (Health Canada, 2010) suggests that the concentration of aniline used in inks for the current assessment are conservative. Children's exposure to aniline fom the use of markers and pens available on the Canadian market was overestimated. Aniline was not found to be present above the limit of quantification in markers intended for use by Children.

Table 3: Tumourigenic doses (TD_{05}s and TDL_{05}s) for aniline based on the incidence of splenic tumours in male and female CD-F rats (CIIT, 1982)

Tumour type	Aniline dose (mg/kg-bw per day)	Tumour incidence	TD_{05} (TDL_{05}) (mg/kg-bw per day)	Parameter estimates
Males				
Stromal sarcoma	0	0/123	46 (35)	Chi-square = 0.08
	7.2	0/129		Degrees of freedom = 1
	21.6	1/128		p-value = 0.78
	71.9	21/130		
Hemangiosarcoma	0	0/123	75 (61)	Chi-square = 0.17
	7.2	0/129		Degrees of freedom = 2
	21.6	0/128		p-value = 0.92
	71.9	6/130		
Fibrosarcoma	0	0/123	94 (72)	Chi-square = 0.08
	7.2	0/129		Degrees of freedom = 2
	21.6	0/128		p-value = 0.95
	71.9	3/130		
Osteogenic sarcoma	0	0/123	94 (72)	Chi-square = 0.08
	7.2	0/129		Degrees of freedom = 2
	21.6	0/128		p-value = 0.96
	71.9	3/130		
Capsular sarcoma	0	0/123	136 (89)	Chi-square = 0.03
	7.2	0/129		Degrees of freedom = 2
	21.6	0/128		p-value = 0.98
	71.9	1/130		
Females				
Hemangiosarcoma	0	0/129	136 (89)	Chi-square = 0.03
	7.2	0/129		Degrees of freedom = 2
	21.6	0/130		p-value = 0.99
	71.9	1/130		

Table 4. Summary of genotoxicity data of aniline (Based on information identified after June 1993).

Species, Strain, Sex etc	Endpoint	Dose, route of exposure etc.	Results	Reference
		In vivo		
ddY mouse and Wistar rat; male; 4/group	DNA damage (Comet Assay)	Mouse – single oral dose of aniline (100 mg/kg) Rat – single oral dose (150 mg/kg)	Positive – DNA damage in the colon, liver, urinary bladder, lung, brain, and bone marrow. Positive - DNA damage in the stomach, colon, liver, kidney, urinary bladder and lung.	Sekihashi *et al.*, 2002.
Mouse ddY male	DNA damage (Comet assay)	Single oral dose of aniline (1000 mg/kg)	Positive - DNA damage in liver, bladder, lung, brain, bone marrow Spleen was not investigated.	Sasaki *et al*., 1999.
Mouse (strain not identified in secondary source)	Chromosomal aberration assay	Intraperitoneal; 220, 300, 380 mg/kg; twice; 24 hour interval. Sampling: 16, 20 and 24 hours after second treatment.	Negative in mouse bone marrow cells. All doses induced clinical symptoms, no cytotoxic effects were induced.	Bayer AG, 2001b, cited in ECB, 2004.
Mouse; CBA; male; 5/group	Chromosomal aberration.	Intraperitoneal 220, 300, 380 mg/kg of Aniline HCl (equivalent to aniline base; two doses separated by a 24-hour interval.	Negative.	Jones and Fox, 2003.
Mouse; B6C3F1; male; 5/group	Micronucleus induction (bone marrow)	Oral; 12, 23, 47, 120 and 470 mg/kg of aniline in corn oil; gavage; two doses; 24-hour Interval	Weak positive response (increase in micronuclei polychromatic erythrocytes in 23 or 470 mg/kg groups, but not well correlated with dose).	Ress *et al.*, 2002.
Rat (strain not Identified)	Micronucleus induction (bone marrow)	Single oral dose 500 mg/kg; sampling done at 48 hours.	Negative.	Bayer AG, 2001a, cited in ECB, 2004.
Rat; PVG; male; 7/group	Micronucleus Induction	Single oral dose of aniline HCl	Positive – dose-related induction	Bomhard, 2003.

	(bone marrow) Study in compliance with OECD principles of GLP (revised 1997).	(equivalent to 0, 300, 400 and 500 mg/kg-bw of aniline base). (samples obtained 24 and 48 hours after treatment).	of micronuclei observed at the 24-hour sampling time, but not following 48-hour.	
Mouse; B6C3F1; male and female	Micronucleus Induction (peripheral blood)	Oral; 500, 1000 and 2000 ppm aniline HCl; 90 days.	Positive (in males and females) in polychromatic and normochromatic erythrocytes.	Witt *et al.*, 2000.
In vitro				
Escherichia coli IC203 and IC188	Mutagenicity (WP2 Mutoxitest)	Aniline HCl; 1000 µg/plate	Negative	Martínez *et al.*, 2000.
Salmonella typhimurium (TA 98, 100)	Ames test	Aniline; 317, 325, 1250, 2500, 5000 µg/plate	Negative	Aßmann *et al*., 1997.
Salmonella typhimurium (TA 98, 100)	Ames test	Aniline; 1, 10, 30, 100, 300, 1000, 3000 µg/plate	Negative (with or without S9 mix)	Chung *et al*., 1995; 1996.
Sacchromyces cerevisiae (strain, RS112)	DEL recombinagenic activity) (generation of oxidative free radical species)	Aniline; 0, 5, 10, 12 mg/ml	Induction of recombination only at 12 mg/ml	Brennan and Schiestl, 1997.
Chinese hamster ovary cells (CHO)	Chromosomal aberrations	Aniline; 444, 888, 1176, 2664 µg/ml	Positive (in the absence of hepatic activation system).	Chung *et al*., 1995; 1996.
Chinese hamster lung cell line (CHL/IU)	Chromosomal aberrations	Aniline; 500, 1000, 1500, 2000, 2500 µg/ml	Positive	Matsushima *et al*., 1999.

APPENDIX A: SEARCH STRATEGY

To identify new critical exposure and toxicological data for aniline, an updated literature search was conducted up to March 2009 using the strategy of searching by name or CAS registry number in databases or websites of various organizations including: Chemical Carcinogenesis Research Information System (CCRIS), ChemIDplus, The Carcinogenic Potency Database (CPDB), The Dictionary of Substances and their Effects (DOSE), Integrated Risk Information System (IRIS), Toxic Substance Control Act Test Submission Database (TSCATS), Genetic Toxicology Data Bank (GENETOX), Hazardous Substances Data Bank (HSDB), International Uniform Chemical Information Database (IUCLID), Registry of Toxic Effects of Chemical Substances (RTECS), Toxicology Literature Online (TOXLINE), Agency for Toxic Substances and Disease Registry (ATSDR), European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), European Chemicals Bureau (ECB), Health Canada (HC), National Industrial Chemicals Notification and Assessment Scheme (NICNAS), National Toxicology Program (NTP), Organization for Economic Cooperation and Development (OECD), United Stated Environmental Protection Agency (USEPA), World Health Organization (WHO), International Agency for Research on Cancer (IARC), International Programme for Chemical Safety (IPCS)

An updated literature review (up to June 2008) of production, importation, use and environmental release data was based on a search of information in the National Pollutant Release Inventory (Environment Canada, 2008), the Toxic Release Inventory (U.S. EPA, 2000), the Pesticide Management Regulatory Agency of Health Canada (Health Canada, 2005) and the Use Patterns and Controls Implementation Section of Environment Canada (Environment Canada, 2000). Information presented in the European Chemicals Bureau Risk Assessment Report Aniline, (European Chemicals Bureau, 2004), which included data on exposure and effects of aniline, the CEH Marketing Research Report Aniline (Bizzari and Kishi, 2007), and information received from ACMI were also reviewed.