Toxicology Letters 254 (2016) 52–62

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

# Assessment of petroleum streams for thyroid toxicity

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## HIGHLIGHTS

- 19 petroleum streams were assessed for thyroid effects, from 349 animal studies.
- 3 studies found effects related to thyroid toxicity, related to high aromatic content.
- Two human studies reported significant but weak associations with thyroid cancer.
- The 19 petroleum streams presented a low potential for thyroid effects or toxicity.

## ARTICLE INFO

*Article history:*
Received 21 September 2015
Received in revised form 22 March 2016
Accepted 2 May 2016
Available online 2 May 2016

*Keywords:*
Thyroid
Petroleum streams
Hydrocarbon
PAH
Polycyclic aromatic hydrocarbons
Hormone

## ABSTRACT

The thyroid gland, and its associated endocrine hormones, is a growing area of interest in regulatory toxicology due to its important role in metabolism, growth and development. This report presents a review of the toxicology data on chemically complex petroleum streams for thyroid hormone effects.

Toxicological summaries and studies from all available published and un-published sources were considered, drawing upon the European REACH regulatory submissions for 19 petroleum streams, with in depth review of 11 individual study reports and 31 published papers on related products or environmental settings. Findings relevant to thyroid pathology or thyroid hormone homeostasis were specifically sought, summarized, and discussed. A total of 349 studies of 28-days or longer duration were considered in the review, including data on mice, rats, rabbits, dogs, humans, and fish. The thyroid was almost invariably not a target organ in these studies. Three rodent studies did find thyroid effects; one on a jet fuel product (JP-8), and two studies on a heavy fuel oil product (F-179). The JP-8 product differs from other fuels due to the presence of additives, and the finding of reduced T4 levels in mice in the study occurred at a dose that is above that expected to occur in environmental settings (e.g. 2000 mg/kg). The finding for F-179 involved thyroid inflammation at 10–55 mg/kg that co-occurred with liver pathology in rats, indicating a possible secondary effect with questionable relevance to humans. In the few cases where findings did occur, the polycyclic aromatic hydrocarbon (PAH) content was higher than in related substances, and, in support of one possible adverse outcome pathway, one *in-vitro* study reported reduced thyroid peroxidase (TPO) activity with exposure to some PAH compounds (pyrene, benzo(k) fluoranthene, and benzo(e)pyrene). However, it could not be determined from the data available for this review, whether these specific PAH compounds were substantially higher in the JP-8 or F-179 products than in studies in which thyroid effects were not observed. Thus, a few products may carry a weak potential to affect the thyroid at high doses in rodents, possibly through secondary effects on the rodent liver or possibly through a pathway involving the inhibition of TPO by specific members of the PAH family.

Human epidemiology evidence found weak and inconsistent effects on the thyroid but without identification of specific chemicals involved. Two studies in petroleum workers, which found a lower rate of morbidity and mortality overall, reported a statistically significant increase in thyroid cancer, but the

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http://dx.doi.org/10.1016/j.toxlet.2016.05.001

small number of cases could not exclude confounding variables as possible explanations for the statistical findings.

Overall, the available data indicates a low potential for thyroid hormone effects from exposure to petroleum streams, especially when the aromatic content is low. Because regulatory studies for most chemicals do not include detailed thyroid function or receptor studies, it remains possible that subclinical effects on this system may exist that were not detectable using conventional pathology or hormone measurements.



## 1. Introduction

Increasing attention is being paid to assessing the potential for endocrine-mediated effects of possible toxicological relevance to human health and the environment to occur for chemicals in commerce globally. The thyroid gland and its associated endocrine action and physiological regulation are among those systems of increasing interest due largely to its influential role in normal early development and metabolism.

With the increasing interest of regulators and the general public over the potential for androgen, estrogen and thyroid effects of exposures to natural and manufactured substances, this review was undertaken to gather the available information on thyroid findings in studies of petroleum streams and to assess these for product stewardship. There are reports that various chemicals (natural and synthetic) can disrupt thyroid hormonal balance directly and/or indirectly through various mechanisms, and the U.S.EPA has concluded that individuals, particularly in younger stages of development, are potentially vulnerable to adverse effects as a consequence of exposure to thyroid-disrupting chemicals (Miller et al., 2009). Although several types of environmental chemicals have been found to interact with thyroid receptors with potential effects on the developing brain (Zoeller, 2007), petroleum stream chemicals have not been specifically reviewed for thyroid effects.

Petroleum streams represent an array of substances, many with common physical/chemical and toxicological properties that arise from the complexity of the natural source material oils and the variety of products that are formed during the refining and downstream processes (McMillen et al., 2001). Humans may be inadvertently exposed to various petroleum stream products, including such every day products as gasolines and motor oils. Although, in general, most petroleum substances are of low toxicity, local and systemic toxicity has been shown to occur with some of these complex materials. For example, studies have shown that some high boiling point substances (HBPS) can produce systemic and developmental effects in rodents at high doses, and some materials are mutagenic *in vitro* (Gray et al., 2013). Gray and colleagues concluded that some of these effects are related to the profiles of aromatic constituents in these substances (Gray et al., 2013). A similar conclusion formed the basis for predictive modeling of developmental toxicity of HBPS by Murray and associates (Murray et al., 2013a). The developmental effects reviewed by Murray and associates are not known to be related to any thyroid hormonal changes, although this possibility was not the focus of the review.

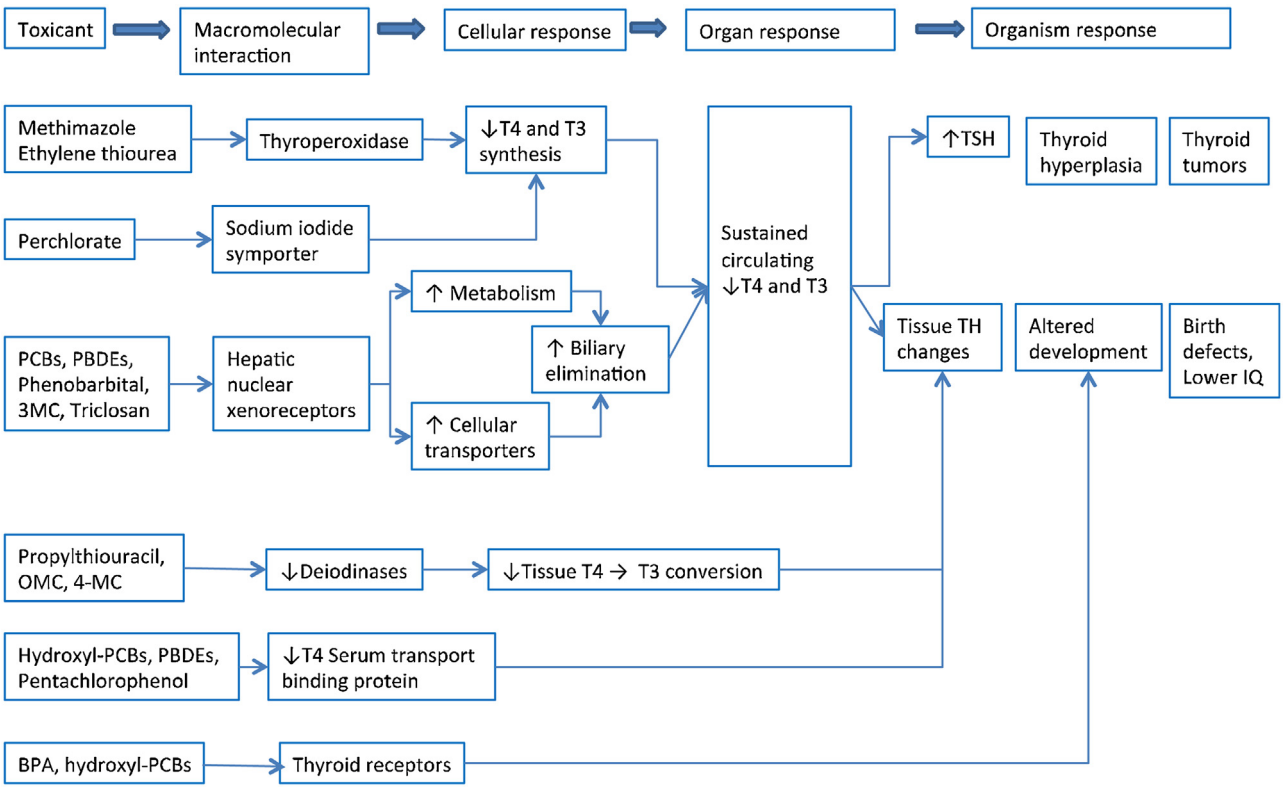


**Fig. 1.** Adverse outcome pathways for toxic effects on the thyroid gland and hormonal system.

Polycyclic aromatic hydrocarbons (PAHs) are present in some petrochemical streams, and these compounds are often studied for their potential mutagenicity, carcinogenicity, and other hazards. A recent study using human thyroid peroxidase (TPO) enzyme activity as a sensitive indicator of potential effect on thyroid hormone synthesis, found conflicting effects of PAHs on TPO activities (Song et al., 2012). Although there is no known mechanism to explain why such effects occur, the authors concluded that certain PAHs, notably pyrene, benzo(k)fluoranthene, and benzo(e)pyrene, inhibited TPO activity. TPO is a key enzyme, upregulated in expression by thyroid stimulating hormone (TSH) and responsible for iodination of tyrosine to form mono, di-, triiodothyronine (T3), and tetraiodothyronine (T4). Dibenzo(a,h)anthracene had an apparent stimulatory effect on TPO in their study, which was also not explainable by any known mechanism (Song et al., 2012). The *in vivo* significance of these changes has not been determined.

This report presents a review of the published and unpublished toxicology data for petrochemical streams and associated downstream products for thyroid hormone effects, identifying any critical studies that allow for the assessment of indirect or direct effects on thyroid pathology or hormonal regulation through an Adverse Outcome Pathway (AOP) approach (Perkins et al., 2013). Fig. 1, adapted from the review by Perkins and colleagues, illustrates the pathways relevant to thyroid cancer, thyroid hormonal effects, and thyroid toxicity. In their review, Perkins and colleagues described adverse outcomes relating to chemical impacts on the thyroid hormone system, which were presented as an example of a complex series of mechanistically unrelated or, in some cases, inter-related events leading to thyroid tumors or developmental toxicity. While the scheme presented is generally relevant to vertebrates, some of the pathways hold more relevance than others to human health. In particular, those thyroid hormone depleting pathways that result in chronic TSH stimulation with ensuing thyroid tumors, are not seen in humans. Additionally, the pathways resulting in developmental toxicity contain several key event steps that vary substantially across species, such that direct generalizations cannot be made across species in terms of doses required or nature and likelihood of effects occurring.

The consideration of AOPs allows for a framework for conducting generalizable assessments across a variety of chemicals that have comparable mechanisms of toxicity. This leads to a weight of evidence assessment as described and advocated by Borgert et al. (2011).

The aim of this paper is to systematically review regulatory and product safety toxicology data for various petroleum product streams, and some of their major components, considering the relevance of different AOPs, and summarizing results into a weight of evidence for the potential for thyroid hormone system toxicity. It should be noted that regulatory studies are not ideal for addressing detailed mechanistic questions. Thus subtle changes in thyroid hormone regulation or receptor binding may conceivably exist but not result in directly observable pathology in the thyroid gland.

## 2. Approach

A comprehensive survey of 19 REACH Chemical Safety Reports (CSRs), representing 19 petroleum substance categories, submitted by the petroleum refining industry to the European Union was undertaken, complemented by a review of 31 published papers from a search using PubMed, Google Scholar, Toxnet, and Web of Knowledge, with keywords including: Petrochemical; Thyroid; T3; T4; TSH; bitumen; hydrocarbon; PAH; polycyclic aromatic hydrocarbon; BTEX; benzene; toluene; ethylbenzene; xylene; lubricant; gasoline; and petrol. Papers relating to thyroid effects in animals or humans of one or more petroleum derived chemical components were obtained. The petroleum streams reviewed are listed in Table 1. These categories and their definition/domain are provided in Appendix A, Supplementary material.

Other published studies that were reviewed included those on petroleum derived materials including: Naphthenic aromatic solvent, high naphthenic solvent, are included in the review. Although not comprehensive, studies on some of the various chemicals downstream of the cracking/refining process (e.g. BTEX, solvents, aliphatic compounds, etc) were included in the review.

It was assumed for the purposes of this report, that acute toxicity studies would impart little value to the evaluation of thyroid effects, and thus were not included in the data review. Instead, the focus of this review is on effects observed in subchronic (minimum 28-day) studies, 13-week studies, and chronic exposures in animal and human epidemiology studies, where measures of thyroid hormone effects and/or pathology of the thyroid gland or the liver would be expected to be more commonly investigated. Reproductive and developmental studies were also included in the review. Eleven individual study reports were reviewed in detail to ensure that potential findings of interest that may have been considered incidental at the time of the report writing, were not overlooked when weighing the evidence of the complete database of information. A total of 349 individual animal studies formed the major basis of the review.

In Fig. 1, the organism responses to various toxicological impacts on the thyroid shown in outcome 1 involve rodent specific mechanisms, whereas those contributing to outcome 2 involve pathways of relevance to both rodents and humans.

## 3. Results

### 3.1. Bitumen

Nine relevant animal studies, including six chronic studies were described in the REACH CSR for Bitumens (CONCAWE, 2010a). Two dermal 28-day studies of vacuum residue bitumen in rabbits

**Table 1**
Categories of petroleum streams and individual substances with chemical safety reports reviewed.

| Bitumen | Oxidized Asphalt (individual substance) |
|---|---|
| Cracked Gas Oils | Paraffin and Hydrocarbon Waxes |
| Untreated Distillate Aromatic Exctract | Petrolatums |
| Foots Oils | Slack Waxes |
| Heavy Fuel Oil Components | Straight Run Gas Oils |
| Highly Refined Base Oils | Treated Distillate Aromatic Extracts |
| Kerosines | Unrefined/Acid Treated Oils |
| Other Lubricant Base Oils | Vacuum Gas Oils, Hydrocracked Gas Oils, and Distillate Fuels |
| Low Boiling Point Naphthas (Gasolines) | Residual Aromatic Extracts |
| Other Gas Oils | |

(5/sex/group) using doses up to 2000 mg/kg bw/day found no treatment-related effects on clinical chemistry, hematology, or pathology to reproductive organs. The only pathological finding reported grossly or microscopically was to the site of first contact in the skin in the form of slight edema (API 1983a; API 1983b). Body weights were slightly reduced at the top dose, which was related to reduced food consumption. One spontaneous white nodule on the thyroid was observed in a female low dose (200 mg/kg BW) rabbit, but no similar findings occurred at the mid or high dose levels, up to 2000 mg/kg BW (API 1983a). There was therefore no evidence of a treatment-related effect on the thyroid in gross or histopathological examination.

The toxicity of similar substances is described in the REACH CSR for Bitumens, as there is variation and compositional overlap with other petroleum streams (CONCAWE, 2010a).

A rat 90-day inhalation study to blended paving bitumen (blend of CAS RN 64742-93-4 (70% air-rectified) and CAS RN 64741-56-6 (30% vacuum residue)) at concentrations of (total hydrocarbon) 100 mg/m$^3$, at 6 h/day, 5 days/week, found high dose males to exhibit slightly elevated serum urea, potassium, and calcium. The authors concluded an effect consistent with slight acidosis or an effect on the kidney (Fraunhoffer Institute, 2001).

High dose female rats exposed to bitumen, by comparison, exhibited a mild increase in mean cell concentrations and in lactate dehydrogenase (LDH) and gamma-glutamyltransferase (GGT) in the broncho-alveolar lavage fluid. Similar, but not statistically significant, effects were seen in the exposed male rats. However, the recovery period for males upon cessation of exposure was 90 h whereas the recovery period for the females was 18 h. The only treatment-related histopathological finding in rats after 90 days was in the nasal and paranasal cavities from the high-dose groups. Thus thyroid effects were not seen while other measures of exposure and/or toxicity were evident, and since this study was conducted according to OECD413, histological examination of the thyroid would have been expected in the study protocol.

A chronic inhalation study using fumes from 70% air-rectified, 30% vacuum residue bitumen at concentrations of up to 100 mg/m$^3$ for 104 weeks in rats, resulted in minimal impacts (Fraunhoffer Institute, 2006). A finding of slightly increased GGT levels in bronchoalveolar lavage (BAL) fluid was of minor significance and overall the findings indicated that the respiratory tract is not significantly impacted by bitumen fumes. No histopathological or hematological findings related to treatment were found, including to the thyroid.

The previously described chronic/carcinogenicity inhalation study using fumes from 70% air-rectified, 30% vacuum residue bitumen at up to 100 mg/m$^3$ for 104 weeks in rats, found no increases in the number of tumor-bearing animals among the groups, nor any statistically-significant increases in organ-related tumor incidences compared to the controls. It was concluded that the fumes from mixed air-rectified asphalt and vacuum residue were not carcinogenic to rats. It was stressed in the Chemical Safety Report (CSR), submitted by the industry to the European Chemicals Agency in 2010, that the inhalation toxicity studies to Bitumen and similar substances reflect only the inhalation hazards from exposure to the fraction that can become volatilized.

The carcinogenicity studies on the bitumen fume material examined numerous non-cancer endpoints and organ/tissue changes, and while some minor serum chemistry changes were noted (slightly increased lactate dehydrogenase in females, and GGT in males and females) no pathological effect on the thyroid was reported.

Because the animal studies on bitumen fumes capture only the volatile components of the material in vapor phase, a number of skin painting studies on the whole bitumen mixture help to illustrate any systemic hazards of the whole mixture. In five chronic, skin painting studies in mice, no reports of thyroid effects were reported, however it is not clear if thyroid pathology was included in these older studies in mice.

Two large epidemiological studies on bitumen fumes were conducted in 2001 and 2009, with a focus on lung cancer. No specific investigations on thyroid parameters were mentioned in these studies, thus the human data do not provide additional insight into the potential of a thyroid effect (CONCAWE, 2010a).

Overall, there is no evidence for an effect of bitumen or bitumen fumes on rodent or human thyroid hormones. Animal data are unequivocally negative for thyroid pathology. Human studies have not found specific effects on the thyroid, but are not necessarily conclusive due to lack of specific focus on the outcome pathways of interest to thyroid toxicity.

### 3.2. Cracked gas oils

Eighteen relevant animal studies were described in the REACH CSR for Cracked Gas Oils (CGO), including one chronic study in rats (CONCAWE, 2010c). In these study summaries, no evidence for thyroid effects was presented. One dermal 90-day study in rats, exposed to 30 or 125 mg/kg BW/day of Beaumont Coker Light Gas Oil, found no evidence of thyroid pathology, even though treatment-related pathologies of various types were reported for 12 organs or tissues examined histologically (Mobil 1991). The CGO compounds therefore have a low hazard potential for thyroid effects.

### 3.3. Aromatic extracts

REACH CSR reviews for Untreated or Treated Distillate Aromatic Extracts, and Residual Aromatic Extracts include 18 studies from rats, mice, and rabbits, including chronic studies (CONCAWE, 2010f; CONCAWE, 2010g; CONCAWE 2010h). None of the studies using any of the various streams reported thyroid pathology or hormonal effects.

### 3.4. Naphthenic solvents

An inhalation study on a naphthenic aromatic solvent blend of 38% aromatics, 37% naphthenics, 25% paraffins in male rats at a concentration of 10 mg/L of for 13-weeks found very few signs of toxicity, and no micropathological lesions related to treatment in any organ, including the thyroid (Carpenter et al., 1977a).

A similar study on rats and dogs exposed to a high naphthenic solvent (69% naphthenics, and 29% paraffins), found microscopic regenerative lesions in the kidneys of rats, but no other organ, including the thyroid (Carpenter et al., 1977b). It should be noted that the kidney pathology in male rats was likely due to male rat-specific alpha-2u-microglobulin formation; a finding now known to carry no relevance to human health.

Ninety-day inhalation studies on light catalytically cracked naphtha in rats and mice, which examined thyroids histologically, found no treatment-related effect on any examined tissues up to the top concentration of 8000 mg/m$^3$ (Dalbey et al., 1996). A developmental toxicity screen in rats by this same group found no treatment-related developmental toxicity to this material.

A 2-year dermal study in mice exposed to light catalytically cracked naphthas found no evidence of thyroid pathology at concentrations of up to 50% (approximately equivalent to 140–150 mg/kg BW) (API, 1985, 1989).

No human data were identified to specifically evaluate the effect of naphthenic solvents on the thyroid. However, given the lack of effects in animal studies, including rats that are known to be more susceptible to thyroid effects, naphthenic solvents and light

catalytically cracked naphtha do not appear to pose a hazard to the thyroid gland and its associated hormonal system.

### 3.5. Heavy fuel oil

Murray and colleagues summarized 68 developmental toxicity studies on high boiling point petroleum streams and reported that of the various petroleum streams tested, only one fuel oil product, F-179, at a dose of 55 mg/kg caused thyroid inflammation, reported as lymphocytic thyroiditis in male and female rats (Murray et al., 2013b) (Table 2). The authors concluded that the presence of aromatic substances in the tested mixtures was a relevant predictor of thyroid effects. Overall however, with 67 of 68 high boiling point products showing no evidence of thyroid specific effects, these substances cannot be considered as a class to be hazardous to the thyroid, and the significance of this one mild pathological effect in the one product is unlikely to be an effect of generalizable concern. It is not known what component(s) or impurities were responsible for the finding, but the compositional distribution of F-179 was markedly different, having a higher% aromatic content, particularly with the 3, 4, and 5-ring constituents compared with the other materials tested. Specifically, the F-179 material was two-thirds aromatic materials, and consisted of: 0% of 1-ring, 0.7% of 2-ring, 10% of 3-ring, 30% of 4-ring, 20% of 5-ring, 6% of 6-ring, and 0% of seven and larger-ring substances (Murray et al., 2013a).

A similar finding was reported in a study report of a 90-day dermal toxicity study in rats using F-179 (ARCO, 1993). Lymphocytic thyroiditis was seen at dose levels that also resulted in significant hepatotoxicity and thymic atrophy, with the effect occurring at doses of greater than 0.01 mL/kg (approximately 10 mg/kg BW) with a NOAEL of 0.001 mL/kg (1 mg/kg BW). Liver histopathology and weight differences were seen at 0.05 mL/kg or greater. Therefore, even though the thyroid finding in this study occurred at a reasonably low dose level, liver findings also occurred at similar doses in a dose-dependent manner, indicating a possible involvement of the liver and a secondary effect on the rat thyroid. Such a mode of action would be consistent with that shown for 3-methylcholanthrene (Fig. 1). There was no compositional information on the test material in this study, other than a purity statement of “100% aromatic petroleum hydrocarbons”.

The REACH CSR for Heavy Fuel Oils includes fifteen 28-day studies, and three 90-day studies in rats (CONCAWE, 2010b).

Overall, despite the one developmental and 90-day toxicity studies with mild thyroid inflammation findings, the weight of evidence indicates that Heavy Fuel Oils generally present a low level of hazard to the thyroid.

**Table 2**
Composition of jet fuel JP-8.

| Hydrocarbon type | Weight% |
|---|---|
| Dodecane | 22.54 |
| Tetradecane | 16.87 |
| Decane | 16.08 |
| Hexadecane | 12.22 |
| Butylbenzene | 4.72 |
| Cyclooctane | 4.54 |
| 1,2,4,5-Tetramethylbenzene | 4.28 |
| Tetraline | 4.14 |
| m-Xylene | 3.95 |
| Isooctane | 3.66 |
| Methyl cyclohexane | 3.51 |
| 1-Methylnaphthalene | 3.49 |

Adapted from Agency for Toxic Substances and Disease Registry (ATSDR), 1998.

### 3.6. Highly refined base oils

The REACH CSR for Highly Refined Base Oils includes 21 relevant studies in animals (rats, mice, and dogs), including four chronic studies (CONCAWE, 2010i). No treatment-related findings were reported in the thyroids of any species in any study. The chronic dietary study in rats on White Oil, found no treatment-related gross or histopathology on the thyroid after 24 months at doses of up to 1200 mg/kg BW/day (Trimmer et al., 2004). Similarly, there was no evidence in the REACH CSRs or found in the published literature that Highly Refined Base Oils have any effect on the rodent or human thyroid.

### 3.7. Heavy gas oil

A published 13-week dermal study in rats exposed to heavy gas oil bitumen upgrading product (B-HGO-2) found increases in thyroid pathology in males and females, with effects starting at 8 mg/kg/day (Poon et al., 1996). Treatment-related pathology included reduced follicle size, increased epithelial height, and reduced colloid density. In this study, while the thymus and thyroid were sensitive target organs, no mechanism of action or hypothesized mode of action for the thyroid pathology was discussed. The components of the oils responsible for these effects are unknown. As thyroid hormones themselves were not measured in this study, it cannot be determined if these findings were linked to any thyroid hormonal influence of heavy gas oil components.

### 3.8. Kerosines

The REACH CSR for Kerosines describes 26 relevant studies on various compositions, including 7 chronic studies, with no indication of thyroid effects (CONCAWE, 2010d). Some of the shorter duration studies did not include pathological assessment of the thyroid (e.g. ARCO, 1987). Keil and colleagues studied the immunotoxic effects of jet propulsion fuel (JP-8) on pregnant mice exposed by gavage, and found that adult T4 levels decreased in relation to treatment of 2000 mg/kg, while T3 levels remained unchanged (Keil et al., 2003). No effect on thyroid hormone levels was seen at a dose of 1000 mg/kg, indicating that the thyroid effect occurs only at high doses, and possibly due to a secondary effect on the liver. A 90-day and reproduction study on rats exposed by gavage to JP-8, did not report examination of thyroid glands for pathology (Mattie et al., 2000). A mode of action was not proposed by Keil and colleagues, but the hormone changes reported in mice are consistent with secondary effects from induction of specific liver enzyme changes (Zabka et al., 2011). The compositional information available in the study by Keil for JP-8 is descriptive, and it should be noted that, in addition to a significant number of additives, the material contains approximately 81% alkanes, primarily in the C8–C17 range. The remaining portion consists of 10–20% aromatics, with relatively low levels of benzene, toluene, and xylenes (Keil et al., 2003). A summary of JP-8 composition is presented in Table 2, data available by the Agency for Toxic Substances and Disease Registry (ATSDR) (1998).

The overall weight of evidence points to a low hazard potential to the thyroid from kerosines and jet fuels, although the thyroid hormone finding in the JP-8 material may warrant closer inspection as to compositional differences with other similar fuels.

A relevant study was described by McKee et al. (2014). In this study, repeated exposures to straight run heavy naphthas resulted in minor thyroid effects. A pathology examination attributed the findings to a secondary effect of increased metabolizing capacity in the liver with a corresponding effect on thyroid hormone

homeostasis. The authors provided additional discussion in support of this hypothesis.

### 3.9. Gasolines

The REACH CSR for Gasolines describes findings from 87 relevant studies in mice, rats, and rabbits, including 11 chronic studies, with no specific thyroid findings (CONCAWE, 2010c) (Table 2). A 60-day inhalation study (8 h/day, 5 days/week) of unleaded petrol in rats, measured serum T4 but found no treatment-related effect at the single concentration of 2000 mg/m$^3$. Systemic effects, including reduced body weight and alterations in some organ weights were also observed (Vyskocil et al., 1988).

### 3.10. Occupational epidemiology studies in petrochemical industry

There is an extensive epidemiology literature regarding mortality and cancer morbidity among petroleum workers. Several comprehensive reviews of results from these epidemiologic investigations, which have involved petroleum workers in the U. S., U.K., Canada, Australia, Finland, Sweden and Italy, have been published (Wong and Raabe, 2000; International Agency for Research on Cancer (IARC), 1989). Collectively, these studies have not identified thyroid cancer as a malignancy related to petroleum exposures, although specific reporting for thyroid cancer may be limited in some studies. A comprehensive review of risk factors for thyroid cancer concluded that no chemical has been clearly identified as a thyroid carcinogen in any epidemiologic studies, and the only risk factor identified to date for thyroid cancer is ionizing radiation exposure (Ron and Schneider, 2006). Finally, extensive reviews of epidemiology studies for other occupations, along with petroleum-related occupations, have not identified thyroid malignancies as a concern (Siemiatycki et al., 2006).

While there is no clear epidemiologic evidence of petroleum-related exposures and thyroid malignancies, there have been a few reports of increased thyroid cancer. A cohort study on oil production and pipeline workers by Texaco found lower than expected SMRs for all cancer deaths in production workers; the only statistically significant increase being in thyroid cancer. However, in this study, the increase was only seen in production workers, and the result was due to only 4 cases, all of whom had worked more than 5 years in the Pumper-Gauger job description, including workers who operate oil pumps and manifolds, with 2 of the cases working more than 5 years also in the Roustabout category, which included workers who set up and maintain oil well heads and fix lead lines to stock tanks. (Divine and Barron, 1987). The small number of cases diminishes the likely significance of this finding, although no confounding factor was identified in the paper.

A study of workers at a petrochemical research facility in Illinois found an overall lower risk of cancer, consistent with the above and other similar occupational studies of this type, yet did find, as above, that thyroid cancer was one significantly increased type of cancer; 7 cases observed, vs 2.6 expected (Sathiakumar et al., 2001). The increase in thyroid cancer was not associated with particular occupational subgroups or locations within the facility, and the authors concluded that the finding could have been a result of one or more unaccounted for confounding factors.

A small study of 42 petrol station workers and 37 control subjects reported thyroid effects in the form of decreased T3, increased T4, and decreased TSH levels as a function of years worked (Uzma et al., 2008). These authors concluded that the solvent vapors from long term exposures at or near the petrol station were likely the cause of the thyroid effects, although a specific hypothesis with biological plausibility was not presented.

### 3.11. Community or population based epidemiology studies

Some empirical evidence exists for a possible association between polycyclic aromatic hydrocarbon (PAH) exposure and thyroid effects in non-occupational surveys. Zhu and colleagues measured urinary, creatinine-adjusted PAH, including 1-hydroxynaphthalene (1-N), 2-hydroxynaphthalene (2-N), 1-hydroxypyrene (1-P), and 2-hydroxyfluorene (2-F) in spot urine samples, and correlated these against blood T3, T4, and TSH in Chinese adult males. These authors found that the higher tertiles of 2-F were associated with increased TSH. No other associations with the remaining PAHs were found with significant $p$-values for trends (Zhu et al., 2009).

The potential role of environmental pollutants to cause developmental toxicity in humans from modest changes in thyroid function was reviewed by Gilbert and colleagues (Gilbert et al., 2012). This review concluded there was evidence associating PCBs and PBDEs with thyroid effects, and further study was recommended to determine if these effects could have any link with brain development. Petroleum streams or analytes were not found to be of significance in this review, although it is not clear if these were a specific focus for assessment.

A reported cluster of Hashimoto's Thyroiditis in community members living in proximity to a petrochemical complex refining naphtha into polyethylene and polypropylene was investigated by the Brazilian Epidemiological Surveillance Center (De Freitas et al., 2010). Their study of 847 residents within the area of concern compared with the same sample size in a control area found an increase of 2.39-fold for HT (OR 2.39; 95%CI = 1.42-4.03), and 1.78-fold for anti-thyroid antibodies (OR 1.78; 95% CI = 1.23-2.60). The study authors concluded that there was a higher prevalence and risk of developing thyroiditis and anti-thyroid antibodies among residents of areas surrounding the petrochemical complex.

### 3.12. Interspecies comparative studies

One significant thyroid hormone disrupting mode of action involves the inhibition of thyroperoxidase (TPO) enzyme, which critically catalyzes the conversion of tyrosyl moieties in thyroglobulin to monoiodothyronine, diiodothyronine, T3, and T4. Paul and associates studied the species differences between porcine and rat models across 12 test substances in order to inform risk assessment relevance to humans (Paul et al., 2013). In their studies, 12 chemicals were tested, with varying associations with TPO activity, but none of these compounds were related to petroleum streams.

### 3.13. Ecological studies

Some publications have reported thyroid hormone effects in wildlife species associated with exposures to petroleum wastes or contaminants, such as PAHs. A study of two species of fish (Shiner Surfperch, and Staghorn Sculpin) thyroid hormones in relation to various environmental pollutants in five areas of the San Francisco Bay, and Tomales Bay, found strong negative correlations of T4 and T3 with increasing polychlorinated biphenyls (PCBs), but a comparatively mixed (positive or negative) and weak associations with only a few PAH compounds, with only 3 of the 14 PAH congeners studied showing a significant negative correlation, two others having positive correlations with T3 and T4 (Brar et al., 2010).

In contrast to the findings reported in fish, Gentes and colleagues reported increased T4 and T3 hormones in tree swallows exposed to oil sands process material. In this study, soil PAH content was associated with thyroid hormone differences between test sites. Again in this study, no putative mechanism was

**Table 3**
Summary of study summaries reviewed for thyroid effects.

| Material Common Name | Representative Composition or Identifier | Thyroid effect in test species | Species | Human relevant AOP? | Key studies (No. of studies) | Max Dose or Concentration Tested or NOAEL/LOAEL (if thyroid effect) | Reference |
|---|---|---|---|---|---|---|---|
| Bitumen | Various | No effect on thyroid | Rat, Mouse, Dog, Rabbit, Humans | Yes | 28-d (2) 2-yr (6) 90-d (1) Epi (2) | 2000 mg/kg 100 mg/m3 | CONCAWE, 2010a |
| Heavy Fuel Oil | 64741–64-2 64741–45-3 | No effect on thyroid | Rat | Yes | 90-d (3) 28-d (15) | 2710 mg/kg/d | CONCAWE, 2010b |
| Heavy Fuel Oil | F-179 | Thyroid Inflammation | Rat | Unknown | Dev (1) | LOAEL = 55 mg/kg | Murray et al., 2013b |
| Heavy Fuel Oil | F-179 | Thyroid Inflammation | Rat | Unknown | 90- d (1) | LOAEL = 0.01 mL/kg | ARCO, 1993 |
| Heavy Fuel Oil | Various formulations | No effect on thyroid | Rat | Yes | Dev tox (68) | 2000 mg/kg/d | Murray et al., 2013b |
| Heavy Gas Oil | B-HGO-2 45.9% Aliphatic; 46.3% Aromatic; 4.6% polar | Reduced follicle size, increased epithelial height | Rat | Unknown | 90 d (1) | LOAEL = 8 mg/kg/d (dermal) | Poon et al., 1996 |
| Cracked Gas Oils | 150-411C bp | No effect on thyroid | Rat, Rabbit | Yes | 2-yr (1) 90-d (3) 28-d (5) Terato (6) 2-gen (3) | 1250 mg/kg/d | CONCAWE, 2010c |
| Kerosines | Jet Fuels | No effect on thyroid | Rat Mouse | Yes | 2-yr (7) 90-d (3) 28-d (10) 1-gen (4) terato (2) | 750 mg/kg/d (oral) 1000 mg/m3 | CONCAWE, 2010d |
| Jet Fuel | JP-8 | Decreased T4; no effect on T3 | Mice | Unlikely | Dev (1) | NOAEL = 1000 mg/kg; LOAEL = 2000 mg/kg (oral) | Keil et al., 2003 |
| Catalyically Cracked Light Cycle Oil | 64741–59-9 | No effect on thyroid | Mice | Yes | 2yr (1) | | CONCAWE, 2010e |
| Untreated Distillate Aromatic Extracts | BP = 250-640C | No effect on thyroid | Rat, Rabbit | Yes | 2-yr (1) 90-d (2) 28-d (1) terato (1) | LOAEL = 125 mg/kg/day (oral); < 30 mg/kg/day (dermal) | CONCAWE, 2010f |
| Naphthenic Solvents | 38% Al; 25% P; 37% N | No effect on thyroid | Mice, Rat | Yes | 90-d (2) | 380 ppm | Carpenter et al., 1977a,b |
| Light Catalytically Cracked Naphtha | 64741–55-5 | No effect on thyroid | Rat, Mice | Yes | 90d (2) Dev (1) | 8000 mg/m3 | Dalbey et al., 1996 |
| Residual Aromatic Extracts | 64742–10-5; 8042–47-5 | No effect on thyroid | Rat Mouse | Yes | 2-yr (5) 90-d (4) 2-gen (2) 1-gen (2) | 2000 mg/kg/d | CONCAWE, 2010g |
| Treated Distillate Aromatic Extracts | | No data available (read-across data only) | – | – | – | – | CONCAWE, 2010h |
| Gasolines | Low Boiling Point Naphthas 86290–91-5; 86290-81-5; 64741-68-0; 64741-41-9; 65813-02-0 | No effect on thyroid | Rat Mouse Rabbit | Yes | 2-yr (11) 90-d (12) 28-d (52) 1-gen (3) 2-gen (1) terato (8) | 750 mg/kg (dermal) 500 mg/kg (oral) | CONCAWE, 2010i |
| Unleaded petrol | Octane = 80.5, 27% A | No effect on thyroid | Rat | Yes | 60d (1) | 2000 mg/m3 | Vyskocil et al., 1988 |
| Ethylbenzene, o,p-xylene, hexane, 1,3,5-trimethylpentene | | No effect on thyroid | Human | Yes | 223 MCS cases; 190 controls | Solvent cases had no effect on TSH | Baines et al., 2004 |
| Highly Refined Base Oils | Liquid hydrocarbons BP 218–800C | No effect on thyroid | Rat Mouse Dog | Yes | 2-yr (4) 90d (7) 28-d (2) 1-gen (2) 2-gen (1) terato (5) | 1815 mg/kg/day | CONCAWE, 2010j |
| Foots Oils | Lubricant oil – BP > 158 – 800C | No data available (read-across data only) | – | – | – | – | CONCAWE, 2010k |
| Other Lubricant Base Oils | Solvent-refined base oils CAS 64742-54-7; 64742-52-5; 64742-56-9 | No effect on thyroid | Rat Mouse Rabbit | Yes | 2-yr (9) 90-d (3) 28-d (4) 1-gen (2) terato (1) | 1000 mg/kg/d | CONCAWE, 2010l |
| Other Gas Oils | 64742–46-7 | No effect on thyroid | Rat Rabbit | Yes | 2-yr (1) 28-d (4) | 1000 mg/kg | CONCAWE, 2010m |
| Oxidized Asphalt | 64742–93-4 | No effect on thyroid | | Yes | | | |

**Table 3** (*Continued*)

| Material Common Name | Representative Composition or Identifier | Thyroid effect in test species | Species | Human relevant AOP? | Key studies (No. of studies) | Max Dose or Concentration Tested or NOAEL/LOAEL (if thyroid effect) | Reference |
|---|---|---|---|---|---|---|---|
| | | | Rat<br>Mouse<br>Rabbit | | Epi (3)<br>2-yr (9)<br>90-d (1)<br>28-d (3)<br>1-gen (1) | 300 mg/m3<br>2000 mg/kg (d) | CONCAWE, 2010n |
| Paraffin and Hydrocarbon Waxes | 848301–87-1 | No effect on thyroid | Rat | Yes | 2-yr (1)<br>90-d (5) | 2% dietary | CONCAWE, 2010o |
| Petrolatums | 8009–03-8 | No effect on thyroid | Rat<br>Mouse | Yes | 2-yr (3) | 5% dietary | CONCAWE, 2010p |
| Slack Waxes | 64742–61-6 | No effect on thyroid | Mouse | Yes | 2-yr (3) | 15 mg, 3x/week | CONCAWE, 2010q |
| Straight Run Gas Oils | 68915–97-9; 64741–44-2; 64741-87-9; 64741–43-1 | No effect on thyroid | Rat<br>Mouse<br>Rabbit | Yes | 2-yr (3)<br>90-d (1)<br>28-d (2)<br>terato (4) | 2000 mg/kg | CONCAWE, 2010r |
| Unrefined, Acid-Treated Oils | 64741–50-0; 64741–51-1 | No effect on thyroid | Rabbit<br>Mouse | Yes | 2-yr (1)<br>28-d (1) | 2000 mg/kg | CONCAWE, 2010s |
| Vacuum Gas Oils; Hydrocracked Gas Oils and Distillate Fuels | Diesel Fuel 68334–30-5 | No effect on thyroid | Rat<br>Mouse<br>Rabbit | Yes | 2-yr (1)<br>90-d (3)<br>28-d (8)<br>terato (6) | 1.71 mg/L<br>1000 mg/kg/d<br>5 mL/kg/d | CONCAWE, 2010t |
| PAHs | Acenaphthene, acenaphthylene, anthracene | decr T4, incr T3 | Sculpin | Questionable | Ecotox (1) | NA:<br>R = −0.54 (T4), R = +0.77 (T3) | Brar et al., 2010 |
| PAHs | Acenaphthene, Fluorene, Phenanthrene | incr T3 | Surfperch | Questionable | Ecotox (1) | NA:<br>R = 0.64 | Brar et al., 2010 |
| Crude Oil (water soluble fraction) | BP supplied crude | 2-3x Incr T4 | Turbot | Questionable | Ecotox (1) | 25% WSF<br>2-fold incr T4 | Stephens et al., 1997 |

presented, the findings were correlations only, and the role of PAHs in the observed thyroid changes was speculative (Gentes et al., 2007).

These ecological studies suggest a weak effect of PAHs on thyroid hormone regulation, but since a mechanism of action has not been established or even proposed, the correlative studies in non-human wild species by themselves do not provide sufficient evidence to indicate a particular hazard property of PAHs.

A summary of the available data from summarized reviews on the specific petroleum stream groups reviewed in this report is shown in Table 3. It should be noted that many OECD Guideline toxicology studies measure thyroid gross and histopathology, and, less commonly, thyroid hormone levels, but do not measure molecular interactions with the thyroid hormone receptor, or associated binding proteins. Representative CAS numbers or representative identifiers illustrate the chemical group identification. Key study findings or NOAELs and LOAELs are included also from several key individual studies. Data from four test mammalian species and three fish species in addition to human epidemiological research were reviewed.

## 4. Discussion

This review has considered the evidence for thyroid effects from petroleum substances as a grouping of chemical classes. Unpublished study summaries and published toxicology journal articles were obtained and reviewed. Thyroid effects that would be expected to be of relevance to human health, using an AOP approach, were sought. Nineteen CSR dossiers submitted for REACH registration were reviewed for evidence of toxicity to the thyroid gland or for reports of thyroid hormonal effects (CONCAWE, 2010a, 2010b, 2010c, 2010d, 2010e, 2010f, 2010g, 2010h, 2010i, 2010j, 2010k, 2010l, 2010m, 2010n, 2010o, 2010p, 2010q, 2010r, 2010s, 2010t). The CSRs contained studies conducted largely in the rat and mouse, which are known to be more sensitive than primates to disturbances of thyroid hormone homeostasis. The CSRs together combined for 349 individual studies that included 117 28-day studies, 63 90-day studies, 77 chronic 18-month to 2-year studies, 19 reproduction studies, and 73 developmental toxicity studies. None of the unpublished studies (0/349) reported specific pathological findings in mice or rats in the thyroid gland. Published studies on one heavy gas oil and one heavy fuel oil product reported thyroid pathology of unknown significance. Even though it should be mentioned that measurement of thyroid hormones is not standard in all subchronic toxicity tests, pathological assessment of the thyroid is standard in 28-day, 90-day, and chronic toxicology tests. Thyroid hormones are also often measured in OECD Guideline 90-day or chronic studies. This being the case, if there were an effect on the thyroid, even minor interactions with the thyroid receptor, for example, from common components of petroleum streams, we assume that this should have been apparent in the form of observable pathology from the vast assemblage of the studies summarized in the dossiers reviewed. However, the nature of regulatory toxicology study designs leave the possibility open that low level, subclinical interactions with the thyroid receptor may exist. We cannot rule out the possibility that effects may occur but do not result in frank pathological findings.

The pathways shown in Fig. 1 that result in developmental toxicity contain several key event steps that vary substantially across species, necessitating careful consideration when extrapolating doses or effects that could occur in humans.

Some published experimental studies on animal or cell culture systems report evidence of thyroid-specific effects from certain polycyclic aromatic compounds (Paul et al., 2013; Stephens et al., 1997; Brar et al., 2010). The pathway(s) for these effects is not established, although the one study by Paul and colleagues (Paul et al., 2013) reporting alterations in TPO from specific PAH compounds provides an indication of a possible mechanism of thyroid effects for these compounds. The relevance of the altered TPO activity findings to human health depends on the likelihood of

human exposures to these specific PAHs achieving dose levels that approach the *in vitro* concentrations used.

Chemicals that have been documented to influence circulating thyroid hormone levels in rodents include those that act indirectly through induction of Phase I and/or Phase II liver enzymes (Zabka et al., 2011). The relevance of these effects on circulating thyroid hormone turnover to human health is questionable, as illustrated by Fig. 1. However, while the CSR dossiers reviewed here include liver findings from high doses of petroleum substances, thyroid effects, including indirect pathological lesions, are almost entirely absent, and the liver enzyme inductions reported do not generally follow the patterns reported to chronically deplete rodent thyroid hormone levels (i.e. phenobarbital type induction of CYP2B10 and sulfotransferase 1A1). However, even if such liver and thyroid effects were seen in rodents, the relevance of such findings to humans could be questionable due to the specifics of rodent physiology.

Toxicity studies in rodents, particularly rats, form the standard basis for risk assessment of many substances. However, when considering the relevance of rodent data on the thyroid gland, it is well known that the rodent thyroid, both in terms of its physiology and histology, differs substantially from the primate thyroid, and the management of the thyroid hormones themselves is radically different, resulting in a much more rapid thyroid hormone turnover rate (McClain, 1995). The binding of thyroid hormones to thyroid binding globulin (TBG) results in their protection from degradation and elimination (Lewandowski et al., 2004). However, rats do not express TBG until over 7 months of age, effectively making them much more susceptible to agents that may indirectly enhance the elimination rate of thyroid hormones from circulation. This important interspecies difference results in substantially different susceptibility to changes in thyroid hormone homeostasis. Additionally, the cells that make up the human thyroid gland proliferate slowly. In humans, thyrocytes divide, on average, about every 8.5 years, or about 5–8 times during a typical lifetime (Maier et al., 2006). Rats, by comparison, have a compressed timeframe for a similar lifetime turnover rate of cell division in the thyroid (i.e. about every 3 months) (Jameson and Weetman, 2010). In their study on rats and mice, Maier and colleagues found a surprisingly high rate of spontaneous mutation in the thyroid, and suggested that this could be due to oxidative stress resulting from the relatively higher rate of generation of thyroid hormones (Maier et al., 2006).

Human and rat thyroids also differ in their genetic susceptibility to radiation induced tumors (Gandhi and Nikifurov, 2011). In their study, Gandhi and Nikifurov found that the key genetic elements, RET/PTC (Rearranged During Transfection/Papillary Thyroid Carcinoma), varied between rodents and humans in chromosomal location and genetic distance, resulting in greater sensitivity of rodents to mutagenic events occurring in the thyroid and raising concerns about the suitability of rodent models to predict human thyroid cancer from radiation exposures.

According to a review performed by the USEPA, thyroid hormone kinetics and distribution in the body differ substantially between rats and humans (Choksi et al., 2003; Lewandowski et al., 2004). Table 4 illustrates some of these stark differences.

The implications of these differences are that rats are generally more susceptible to agents that perturb thyroid hormone homeostasis, particularly when any indirect effect increases the already high metabolic rate of turnover of thyroid hormones resulting in a circulating T3 or T4 deficit and consequent TSH increase. This is supported empirically by an analysis of 343 NTP and NCI studies in rats, which found thyroid proliferative lesions in 27/318 studies in male rats, compared with a slightly lower rate 20/320 studies in females (McConnell, 1992).

With these well documented species differences in thyroid physiology, the rodent, and particularly the male rodent, is clearly not an ideal predictive model for human health effects stemming from effects on thyroid hormone homeostasis.

Nevertheless, most of the toxicological data available for risk assessment are developed using rodent models. Thus, any conclusions reached about hazard and risk to the thyroid must continuously hold this highly significant caveat in mind.

For this reason, epidemiology studies are also useful in informing the discussion about thyroid effects of petroleum streams. Two epidemiology studies on petroleum workers found statistically significant increases in thyroid cancer (Divine and Barron, 1987; Sathiakumar et al., 2001). These studies included workers who had various exposures and employment histories. Due to the small number of actual thyroid cancer cases, the role of confounding variables could not be ruled out. There was no indication of non-cancer thyroid illness in either studied group. These two studies do not carry sufficient strength to conclude that the thyroid is a significant target organ of petroleum streams.

A small number of correlative studies in human epidemiology or ecological studies have suggested that the thyroid gland and/or thyroid hormonal regulation may be impacted by constituents of petroleum streams, but these findings were few in number, and did not identify a relationship with specific compounds (Uzma et al., 2008; De Freitas et al., 2010). These are thus inconsistent with the overall toxicology database on petroleum compounds. Furthermore, although a recent *in vitro* study has indicated inhibition of TPO by select PAH compounds in rats, it is not clear if the same effect would be seen in humans, due to the very different thyroid hormone turnover kinetics. PAHs in sediment and soils have been associated with thyroid hormone ratio differences in some ecological studies in fish and birds. These studies are suggestive only and do not indicate a causal role of PAH compounds in thyroid homeostasis. It is unclear if these associations carry relevance to humans. However, such associations could indicate a potentially

**Table 4**
Species differences in thyroid hormone homeostatic parameters.

| Paramater | Human | Rat[a] |
|---|---|---|
| Half-life of T4 | 5–9 days | 0.5–1 day |
| Half-life of T3 | 1 day | 0.25 day |
| Thyroxine-binding globulin levels | High | Absent |
| TSH levels (ng/mL) | 0.05–0.5 | 0.6–3.4 |
| T3 levels (ng/dL) | 147 | 25–100 |
| T4 levels (μg/dL) | 7.2 | 3–7 |
| Amount of T4 required in absence of function gland | 2.2 μg/kg bw/d | 20 μg/kg bw/d |
| T4 production | 1X | 10X |
| Sex difference in serum T4 | No difference | Adult males higher than females |
| Thyroid Colloid | Plentiful | Limited |

Adapted from USEPA 1998, as described by Choksi et al., 2003 and Lewandowski et al., 2004.
[a] Adult rats.

significant effect on wildlife. It could not be determined from the study summaries reviewed which of the streams would be most likely to contain the few PAHs that were found to be active in TPO inhibition.

Overall, thyroid effects were rarely seen in studies on petroleum streams and the role of liver enzyme induction in positive rodent studies could not be ruled out. The few positive findings in human and animal studies do not allow for an AOP approach for evaluating the potential for thyroid effects to occur from petroleum streams generally.

## 5. Conclusions

This review of unpublished petroleum product toxicology and epidemiology studies, and published literature, found negligible evidence in animal studies to suggest that petroleum streams, in general, pose a specific hazard to thyroid hormone homeostasis or to the functioning of the rodent or human thyroid gland. There is no evidence of thyroid toxicity from materials comprised of aliphatic, olefin, naphthenic, and most distillates where the aromatic content is low. Some experimental evidence for thyroid effects in the form of inhibition of TPO activity *in vitro*, and altered T3:T4 ratios in rodents exist from studies on specific polycyclic aromatic compounds, comprised of 3 or more aromatic rings, found in trace levels in some petroleum streams. Mild inflammation in the thyroid was also seen in two rat studies on a heavy fuel product (F-179), but the significance of this effect on human or rat thyroid function is unknown, particularly since over 95% of studies from similar fuel streams found no effect on the thyroid. One Jet Fuel product, JP-8, was found to reduce circulating T4 in the offspring of pregnant mice exposed during gestation to the high dose of 2000 mg/kg/day, whereas no effect was seen at 1000 mg/kg/day. The vast majority of petroleum product streams gave no evidence of any thyroid effect in sensitive rodents described in the chemical safety reports and in numerous individual study reports. A strength of this review is the inclusion of a comprehensive database of a large number of high quality animal studies in multiple species and across the full spectrum of exposure durations. Caution should be used in interpreting negative findings from the dermal studies due to the limited systemic absorption expected for many of the constituents involved. Rodent studies, generally, are not ideal for characterizing the risk of human thyroid effects. Finally, the use of regulatory toxicology studies, and reliance on histopathology results alone is not ideal for addressing mechanistic questions. Thus it remains possible that low-level interactions with the thyroid receptor exist that do not result in thyroid pathology.

Human epidemiology evidence found weak and inconsistent effects on the thyroid but without identification of specific chemicals involved. Two studies in petroleum workers that found a lower rate of morbidity and mortality overall, reported a statistically significant increase in thyroid cancer, but the small number of cases could not exclude confounding variables as possible explanations for the statistical findings.

Overall, the available evidence indicates the potential for thyroid hormone effects from exposure to petroleum streams is low, with some *in vitro* experimental results in rats of potential relevance to reduced TPO activity from specific PAH compounds (i.e. possessing three or more rings) which could be relevant to an AOP. Additionally, some published thyroid findings in rats exposed to heavy fuel oil and jet fuel, which may contain specific PAH constituents, could be associated with this mechanism. The larger data set suggests that the lower molecular weight aromatics (one and two ring compounds) increase liver weight by inducing metabolizing enzymes but do not cause pathological effects at environmentally relevant doses. The aromatics with more rings can cause severe pathological changes. We conclude that the effect on metabolizing enzymes will increase with increasing aromatic constituents to a maximum, then dropping off as toxicological properties affect liver function. These effects, though weak, could conceivably have relevance to human thyroid pathology and function upon repeated high dose exposure to petroleum streams that are found to contain the specific PAH or related impurity or component involved. However, these effects are likely not relevant to typical occupational or consumer exposures. Future epidemiological cohort studies which utilize biomonitoring for specific PAH metabolites or adducts in relation to thyroid function may be of some value in determining if such effects exist in humans.

## Acknowledgements

The author would like to thank the CONCAWE Health Toxicology SubGroup for funding this review and for the provision of access to all needed technical materials to complete the detailed toxicological assessment.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.toxlet.2016.05.001.

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