
TECHNICAL MEMORANDUM

TO: C. GRABHAM (WQ)
FROM: B. HOPE
SUBJECT: AQUATIC TOXICITY OF STEROLS & STANOLS
DATE: 10/25/10
CC: J. WIGAL (WQ)

Whole Effluent

Whole municipal effluent contains a mixture of natural and synthetic xenobiotics, household and agricultural chemicals, pharmaceuticals, hormones, and other compounds. Studies have correlated exposure to whole effluent with a range of traditional toxicity endpoints (mortality, growth, etc.), as well as with adverse hormonal and reproductive alterations in adult and juvenile fish (Liney et al., 2006). These latter effects have been associated with natural and synthetic steroids, alkylphenol polyethoxylates, phthalates, and pesticides. The majority of work on non-traditional effluent effects has focused on steroid estrogens and certain alkylphenols known or suspected to have endocrine disrupting properties (Folmar et al., 2001; Jeffries, 2008, 2010; Peng et al., 2008; Quinn et al., 2006). The question is whether any of these effects, both traditional and non-traditional, could be ascribed specifically to the four sterols and stanols on the SB 737 persistent pollutants list.

ANIMAL STEROLS and STANOLS

Coprostanol (CASRN 360-68-9)

Coprostanol was listed on the Priority Persistent Pollutant List (P3 List) on the basis of a toxicity estimate made by the U.S. EPA ECOSAR model. Because its LogK_{ow} is > 8 (8.82), coprostanol could have been eligible for a solubility exclusion had not its estimated chronic value (ChV) for fish ($0.04 \mu\text{g L}^{-1}$) been less than its solubility ($0.3 \mu\text{g L}^{-1}$) at saturation. This suggested that, despite its low solubility, enough of it could get into solution to cause adverse effects in fish. Although the literature is limited, it has been reported that coprostanol could reduce lipid peroxidation¹ (Gagné et al., 2006), as well as induce estrogenic effects, in mussels (Gagné et al., 2001). An unpublished Canadian study (L. Falk, *undated*) suggested that coprostanol, because of structural similarities, may compete with estrogen for receptors, lessening the estrogenic response. It has also been found to bioaccumulate in mussels (Gagné et al., 2002; Sherwin et al., 1993). Thus the limited work done to date suggests that coprostanol may pose a threat to fish. This threat may be small relative to those posed by other, more

¹ Lipid peroxidation (LPO) refers to how lipids are degraded by free radicals. Because lipids are key components of cell membranes, this degradation can lead to cell damage. In addition, the end products of LPO may cause mutations or cancer.

active, chemicals present in effluent. Conversely, it's possible that its effects are being obscured by those associated with these other chemicals.

Cholesterol (CASRN 57-88-5)

Cholesterol was listed on the P3 List on the basis of a toxicity estimate made by the U.S. EPA ECOSAR model. Because its LogK_{ow} is > 8 (8.74), coprostanol could have been eligible for a solubility exclusion had not its estimated ChV for fish ($0.06 \mu\text{g L}^{-1}$) been less than its solubility ($95 \mu\text{g L}^{-1}$) at saturation. This suggested that, despite its low solubility, enough of it could get into solution to cause adverse effects in fish. However, there appears to be no published research on the aquatic toxicity of cholesterol itself to provide perspective on this model estimate, either because it has not been thought of as a toxic pollutant or because assessments of its toxicity have produced uninteresting results. At best, cholesterol is implicated circumstantially because all steroid hormones, including several studied as endocrine disruptors in effluent (e.g., estrone, estradiol (Peng et al., 2008)), are synthesized from the same cholesterol precursor. These steps cannot occur in the ambient environment, only within an organism.

Cholesterol is commonly abundant in natural coastal and marine sediments and seawater, as well as in freshwater. Addition of cholesterol to aquatic systems could remove one of the limitations on the growth and reproduction of certain aquatic invertebrates, thereby potentially altering the population density and structure of aquatic communities (Hassett, 2004). But, from a toxicological perspective, it may pose a minimal threat relative to those posed by other, more active, chemicals present in effluent.

PLANT STEROLS and STANOLS

Phytosterols and phytostanols are a group of steroid alcohols and esters that are structurally related to cholesterol, but differ in the structure of the side chain. They occur naturally and exclusively in plants and are therefore normal constituents of the human diet. They are typically added, in free or esterified form, to foods to reduce absorption of cholesterol in the human gut and thereby lower blood cholesterol levels. It is now generally accepted that sterols and stanols have the same cholesterol lowering efficacy. They appear in municipal effluent as by-products of domestic and commercial human activity (digestion, food preparation) and in industrial effluent as by-products of food processing or wood processing (primarily from pulp and paper mills). Studies of the effects of specific plant steroids on fish and aquatic life are limited (see below). However, there have been several studies involving mixtures of plant steroids (and sometimes other chemicals) that have reported adverse impacts on the hormonal and reproductive systems of fish (Lethinen et al., 1999; Mattsson et al., 2001; Nakari & Erkoma, 2003).

Stigmastanol (CASRN 83-45-4)

[Sitostanol, β -Sitostanol, (3 β ,5 α)-Stigmastan-3-ol] This chemical was listed on the P3 List on the basis of a toxicity value obtained from a 1999 NCASI document, received in response to

DEQ's request for comments on the proposed initiation levels. That value ($75 \mu\text{g L}^{-1}$) is the concentration at which there were no effects on gonad somatic indices, liver somatic indices, condition factors, egg production, egg size, or egg hatchability of adult fathead minnows during a 56-day life cycle test (NCASI, 1999). This value is higher than that estimated with ECOSAR ($0.008 \mu\text{g L}^{-1}$). Because its LogK_{ow} is > 8 (9.73), sitostanol could have been eligible for a solubility exclusion had not its estimated ChV for fish ($0.008 \mu\text{g L}^{-1}$) been less than its solubility ($0.03 \mu\text{g L}^{-1}$) at saturation. No other research on the effects of sitostanol alone on fish or aquatic life could be found.

Sitosterol (CASRN 83-46-5)

[β -Sitosterol, (3 β)-Stigmast-5-en-3-ol] This chemical was listed on the P3 List on the basis of a published toxicity value. That value ($25 \mu\text{g L}^{-1}$) is the concentration at which there was a statistically significant increase in vitellogenin and a statistically significant decrease in plasma pregnenolone in immature rainbow trout (Tremblay and Van Der Kraak, 1999). These responses suggest that this chemical is having an effect at the biochemical level on an organism's hormonal and reproductive systems. The comments were primarily critical of DEQ's consideration of these "non-traditional" endpoints. This value is higher than that estimated with ECOSAR ($0.01 \mu\text{g L}^{-1}$) or that used by U.S. EPA for an NPDES discharge permit in Idaho ($1 \mu\text{g L}^{-1}$). Because its LogK_{ow} is > 8 (9.65), sitosterol could have been eligible for a solubility exclusion had not its estimated ChV for fish ($0.01 \mu\text{g L}^{-1}$) been less than its solubility ($0.04 \mu\text{g L}^{-1}$) at saturation. There is also evidence that β -Sitosterol may affect the behavior of fish (Clotfelter and Rodriguez, 2006).

There is limited evidence to implicate any individual plant sterol or stanol for adverse effects. There is some evidence to suggest that plant sterols and stanols could pose a threat to fish, particularly if discharged to water bodies in larger than "natural" quantities. Both sitosterol and stigmastanol have potential to induce "non-traditional" adverse effects in fish. However, limited research has been performed in this regard.

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