Public Comment
Recycled Water Policy
Deadline: 12/22/08 by 12 noon

commentletters - Comments on the proposed recycled water policy & Draft Staff Report

From: Edo McGowan <edo_mcgowan@hotmail.com>
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Date: Sunday, December 21, 2008 7:45 PM
Subject: Comments on the proposed recycled water policy & Draft Staff Report

To: Members---State Water Resources Control Board commentletters@waterboards.ca.gov
Fm: Dr Edo McGowan

Re: Comments on Draft Staff Report, its inconsistencies, weaknesses, and thus impacts on your decision process.

Below I discuss several issues within the Draft Staff Report and attempt to show your Board where there are fairly major errors in judgment. I think, from my review, that this stems from the discipline-oriented bias that accrues to asking of your staff what it can not deliver or perhaps even understand. That is a weakness which may pervade the entire staff and it is a weakness that will have potentially disastrous implications for both public health and the environment. As you read through this, my meaning will, hopefully, become clear.
I appreciate your daunting task in reading all these and other's comments. Accordingly, I have attempted to break them into smaller units to help overcome ennui while avoiding the label of ennuyer.

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As noted in the material presented below, endocrine disrupting materials are bioaccumulated in soil dwelling organisms and these then enter the food web. Thus for the Draft Staff Report (DSR) to indicate the following, stating that----------as relate to-------

BIOLOGICAL RESOURCES

Recycled water could potentially be used to develop land that is habitat for candidate, sensitive, or special status species. Predicting any potential impacts from such development would be speculative. However, any development would be analyzed under CEQA and subject to regional plans, polices and regulations.
b. Recycled water could be used to develop land within a riparian habitat. However, predicting any potential impacts from such development would be speculative. Any development would be analyzed under CEQA and subject to regional plans, polices and regulations.
c. It is unlikely that a consequence of the proposed Policy will be the discharge of dredge or fill material into a wetland. The proposed Policy's management practices will minimize any hydrologic impacts to less than significant levels

A recycled water irrigation site could be proposed to be located within a migratory corridor. See 4a. above for discussion of potential impacts.

And, thus from the above, the DSR states------------

--**Less than significant**

Have a substantial adverse effect, either directly or through habitat modifications, on any species identified as a candidate, sensitive, or special status species in local or regional plans, policies, or regulations, or by the DFG or USFWS?	☐☐	☐☐	☐☐ ☹	☐☐
b) Have a substantial adverse effect on any riparian habitat or other sensitive natural community identified in local or regional plans, policies, regulations or by the California Department of Fish and Game or US Fish and Wildlife Service?	☐☐	☐☐	☐☐ ☹	☐☐
c) Have a substantial adverse effect on federally-protected wetlands as defined by Section 404 of the federal Clean Water Act (including, but not limited to, marsh, vernal pool, coastal, *etc.*) through direct removal, filling, hydrological interruption or other means?	☐☐	☐☐	☐☐ ☹	☐☐
d) Interfere substantially with the movement of any native resident or migratory fish or wildlife species or with established nati resident or migratory corridors, or impede the use of native wildlife nursery sites? --**Less than Significant**				

Comments--------------------

As noted below, it becomes obvious the call by staff, relating to biological resources, that such i

The following will help your Board appreciate this. While these articles below may speak of sew
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Following the path of contaminants from your bathroom to the birds
Fawn Pattison
14 Mar 2008

This is a story about sludge, worms, and songbirds, and it starts in your
bathroom cabinet.


When we treat our wastewater to remove "biosolids" -- a
polite term for our human waste -- all sorts of other things end up in the
leftover sludge, including the drugs we take and the "personal care
products" like lotion, shampoo, makeup, and cologne that we slather on our
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bodies, which have been absorbed through our skin and then excreted in our waste. The treated wastewater is usually discharged into the local river, and the sludge that's been removed from it frequently becomes fertilizer for agricultural production.

Researchers at the U.S. Geological Survey have found that the hungry earthworms who feed on this sludge in farm fields contain concentrated levels of our drugs and personal care products in their bodies. In fact, a USGS study published in February found that the compounds bioaccumulate in earthworms, meaning that the worms bear higher levels of these pollutants than the surrounding soil does. The USGS researchers note that worms could become monitoring species to help us determine the relative pollution levels in soil, but state that the pollution in these worms have "unknown effects" for wildlife (read the story in Science News).

"Unknown" maybe in that particular study, but researchers in the U.K. published a disturbing study about a week later that provides some insight into what happens to the polluted worms: Birds eat them.

This particular study examined European starlings in the wild, who like to forage in farm fields where fertilizer from sewage sludge has been applied, because the soil is rich in earthworms and other organisms who are busy feasting on the nutrients in the fertilizer. But they're also feasting on the contaminants in the fertilizer, and those contaminants have an impact on the foraging birds (story in The New York Times).

The contaminants in sewage sludge can contain hormone-mimicking compounds that act like estrogen in the birds' bodies. (Following the thread here? Those compounds are the drugs and personal care products that the USGS was examining in the earlier study.)

The U.K. researchers found that the contaminants boosted development in the part of the male birds' brains that control their songs, making them sing longer and more complex songs. The researchers also found that female starlings preferred the long, complex songs of the contaminated male starlings.

The bad news is ... they're contaminated. The same endocrine-disrupting compounds in the male starlings that made them attractive as mates make them unfit as fathers, because the compounds suppress the birds' immune systems and make them sick. While that might be good news for American birders who aren't fond of invasive starlings, it's rather bad news for birds everywhere who like to eat worms. While that fat earthworm might taste good and improve a male songbird's chances of attracting a pretty lady bird, it could actually be crippling his chances of producing a healthy brood of babies.

This might seem like just a scientific curiosity if the same kinds of effects hadn't also been noted in many other species, including fish, reptiles, and amphibians. Sort of makes you think twice about that nice body spray in your bathroom cabinet that's supposed to make you more attractive to a mate, doesn't it?

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You can print out the whole study for free at this link:

http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0001674

Pollutants Increase Song Complexity and the Volume of the Brain Area HVC in a Songbird

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Abstract
Environmental pollutants which alter endocrine function are now known to decrease vertebrate reproductive success. There is considerable evidence for endocrine disruption from aquatic ecosystems, but knowledge is lacking with regard to the interface between terrestrial and aquatic ecosystems.

Here, we show for the first time that birds foraging on invertebrates contaminated with environmental pollutants, show marked changes in both brain and behaviour. We found that male European starlings (Sturnus vulgaris) exposed to environmentally relevant levels of synthetic and natural estrogen mimics developed longer and more complex songs compared to control males, a sexually selected trait important in attracting females for reproduction. Moreover, females preferred the song of males which had higher pollutant exposure, despite the fact that experimentally dosed males showed reduced immune function.

We also show that the key brain area controlling male song complexity (HVC) is significantly enlarged in the contaminated birds. This is the first evidence that environmental pollutants not only affect, but paradoxically enhance a signal of male quality such as song. Our data suggest that female starlings would bias their choice towards exposed males, with possible consequences at the population level. As the starling is a migratory species, our results suggest that transglobal effects of pollutants on terrestrial vertebrate physiology and reproduction could occur in birds.

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Introduction
Numerous examples exist of the detrimental effects of environmental pollutants on the survival or reproductive success of wild organisms e.g.[1]-[3]. In particular, both natural and synthetic endocrine disrupting chemicals (EDCs) act to alter the function of the endocrine system [4], causing gross anatomical changes [5]-[7], as well as changes to behaviour [5] in a range of taxa, including fish, reptiles and amphibians. EDCs' potential to alter physiological function has led to concerns that they could be potent physiological disruptors for wild organisms [7] or, more controversially for humans [8].

According to sexual selection theory [9], male secondary sexual traits have evolved as a result of female preferences and may act as indicators of male quality. Bird song is under strong sexual selection [10] and song production is controlled by discrete neural pathways in the brain which develop and operate under endocrine control of the nervous system [11]. Although the exact roles of testosterone and estrogen in controlling song production are still much debated [12], estrogens are known to be necessary for the masculinisation of the avian song centres in the developing male songbird brain [13]. Furthermore, aromatization of testosterone to estradiol has a neurotrophic effect in male song sparrows (Melospiza melodia) and is implicated in controlling the degree of neural plasticity seen in adult songbirds [14]. Many songbird species show seasonal development of their neural song system due to photoperiodic influences on hormone production [15]. This leads to the possibility that neural development in adult birds, which is strongly controlled by the endocrine system may be susceptible to changes in endocrine function.

Natural and synthetic estrogens are known to both occur in concentrated amounts in sewage effluent [16]. As part of sewage treatment processes worldwide, effluent is trickled over filterbeds rich in organic sediment, thereby supporting a complex community of micro and macro-invertebrates [17]. These commonly occurring environments provide an essential foraging environment for a range of wild songbird species, including for one of the most invasive bird species on a global scale, the European starling (Sturnus vulgaris), particularly during the winter [18]. The effects of EDC exposure on adult songbird behaviour and physiology are largely unknown, although a recent observational study has documented that neural centres associated with song production may be detrimentally affected by exposure to dichlorodiphenyltrichloroethane (DDT)[19]. This study, which correlated egg levels of a range of DDT metabolites and isomers with neural development in chicks of the American robin (Turdus migratorius), found that nestlings with higher total DDT exposure showed reduced forebrain volumes and reduced volume of the robust nucleus of the archpallium (RA). DDT is a recognised endocrine disrupter which has complex effects on estrogen receptor activity, but the persistent DDT metabolite p,p′-DDE is a recognised estrogen antagonist and has been shown to inhibit the binding of estradiol both in vivo and in vitro[20]. Interestingly, in this study the strongest effects on neural development were seen in relation to p,p′-DDE levels in male birds[19].

In the present study, we sought to test the effects of EDC exposure on immune function, song production and neural development in wild birds. Due to the established toxic effects of a range of EDCs on immune function, including changes in antibody production, nitric oxide synthesis, cytokine synthesis, as well as changes to the allergic response [21], we predicted that birds foraging on sewage filterbeds would show immunosuppression. Although EDCs such as DDT can show toxic effects on neural development [19], a range of endocrine disrupters can function as estrogen mimics, potentially having a neurotrophic effect on the development of HVC. Due to the functional association of estrogens with brain masculinisation and neural plasticity [11], [13], [14], we therefore predicted that exposure to EDCs which act as estrogen mimics would, in contrast to exposure to estrogen antagonists[19], cause an increase in both song production and song complexity. We tested these predictions experimentally by exposing wild-caught starlings to ecologically relevant doses of known EDCs and quantifying the effects on immune function and song behaviour. In order to calculate exposure levels of wild starlings, we identified the EDCs present in invertebrate prey and assessed the intake rate of birds observed foraging at these sites. Since we found substantial levels of both natural and synthetic estrogenic compounds [22], we then tested the effects of ecologically-relevant dose levels of either i) 17-β estradiol alone (E2) or ii) a mixture of all the estrogenic compounds found, including E2, on the behaviour and immune function of wild starlings in captivity. Specifically, we predicted that we would see a stepwise decrease in immune function and stepwise increase in song production, song complexity and neural development across the treatment groups, in the order control, E2, mixture treatment.

Results and Discussion

Wild-caught male starlings (n = 36) were randomly allocated to three experimental treatments: 1) control group 2) E2 group or 3) mixture group which received all the known endocrine disrupters identified from field sampling (see Materials and Methods). All dose levels were calculated following field observations of foraging starlings and analysis of invertebrate samples from sewage treatment filterbeds.

EDC exposure significantly reduced both cell-mediated immune function (Fig. 1a) and the humoral immune response of male starlings (Fig 1b). Treatment did not have an effect on body mass (ANOVA, $F_{2, 32} = 0.334$, $P = 0.718$) nor on haematocrit (% packed red blood cell volume) (ANOVA, $F_{2, 30} = 1.338$, $P = 0.278$) or testosterone titre (ANOVA $F_{2, 32} = 0.66$, $P = 0.524$), as measured at the end of the experimental period.

Figure 1. Immune function in male starlings exposed to chemicals.

The immune function of male starlings in three treatment groups; control (open bars); E2 dosed (hatched bars); and the chemical mixture dosed (black bars) (a) Cell-mediated immune function was measured as wing web swelling of both wings, 24 hours after injection with phytohaemagglutinin (PHA). Treatment had a significant effect on cell-mediated immune function (ANOVA, $F_{2, 32} = 12.16$, $P<0.001$). Bonferroni pairwise comparison post-hoc tests showed that the immune function of males in both chemically dosed groups (E2 or mixture) was significantly lower than that of the control males (E2 versus control $P<0.001$, mixture versus control $P = 0.001$) but there was no significant difference between males in the E2 and mixture groups ($P>0.05$). (b) The secondary humoral response following an intraperitoneal injection of sheep red blood cells (SRBC). Treatment had a significant effect on the secondary humoral response to SRBC (ANOVA, $F_{2, 32} = 10.98$, $P<0.001$). Bonferroni pairwise comparison post-hoc tests showed that the mean response of the males in both dosed groups (E2 or mixture), was significantly lower than the mean of the control males (E2 versus control $P<0.001$, mixture versus control $P = 0.001$), but there was no significant difference between the E2 treated and the mixture treated males ($P>0.05$). Graphs show means+s.e.m. ** indicates $P<0.001$.

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Treatment had a significant effect on the song output of the male starlings (Fig. 2). Males in the group which received the mixture of chemicals spent more time singing, sang more song bouts, sang longer song bouts and had a larger repertoire size, a measure of song complexity, than males in the control group. The mechanism for this effect is clear as when examining the underlying neurobiology. There was a significant effect of treatment on HVC volume, the principal nucleus in the songbird brain associated with the production of complex songs [12], [23], such that the HVC volume of the males in the mixture group was significantly larger than in males in the control group (Fig. 3a, b). There were no significant differences in the HVC volume between males in the E2 and control groups or between males in the E2 and mixture groups (Fig. 3a).

Figure 2. Song production in male starlings exposed to chemicals.

The song production of male starlings in three treatment groups: control (open bars); E2 dosed (hatched bars); and the chemical mixture dosed (black bars) (a) Total time spent singing (sec/h). (b) Number of song bouts per hour. (c) Song bout duration (s) d) Repertoire size. Graphs show means+s.e.m. There was a significant effect of the experimental manipulation on the time spent singing between the treatment groups (ANOVA, $F_{2, 24} =$

6.15, $P = 0.007$). Bonferroni pairwise comparison post-hoc tests showed that the males that received the mixture of chemicals spent significantly longer singing than the control males ($P = 0.009$) and the E2 group ($P = 0.028$). There was a significant effect of treatment on the number of song bouts sung by the males (ANOVA, $F_{2, 23} = 9.16$, $P = 0.001$). Males in the mixture treatment group sang more song bouts than the control males ($P = 0.004$) and the E2 males ($P = 0.002$). Mean song bout duration was significantly longer for males in the mixture treatment group compared to the control males (ANOVA, $F_{1, 11} = 5.842$, $P = 0.034$). Finally, there was a significant effect of the experimental manipulation on the repertoire size of male starlings (ANOVA $F_{2, 16} = 4.39$, $P = 0.030$). The males in the mixture group had significantly greater repertoire size than males in the control group (Bonferroni pairwise comparison post-hoc tests $P = 0.042$). * = $P<0.05$; ** = $P<0.01$.

doi:10.1371/journal.pone.0001674.g002Figure 3. HVC size in male starlings exposed to chemicals.

a) HVC volume (mean+s.e.m.) in the three treatment groups; control (open bars); E2 dosed (hatched bars); and the chemical mixture dosed (black bars) (ANOVA, $F_{2, 32} = 4.46$, $P = 0.019$). HVC volume of the males in the mixture group was significantly larger than in males in the control group (Bonferroni pairwise comparison post-hoc tests $P = 0.032$), but there were no significant differences in the HVC volume between males in the E2 and control groups ($P>0.05$) or between males in the E2 and mixture groups ($P>0.05$) * = $P<0.05$. b) Photomicrograph of an HVC from (i) a chemical mixture treated male and (ii) a control male. Arrows indicate the borders of HVC. Scale bar = 200 µm.

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Finally, consistent with the changes in repertoire size and underlying neural structure, in mate choice preference tests female starlings showed a significant preference for song playback from males dosed with the mixture of chemicals in comparison to control males (Fig. 4). In addition, song from males exposed to the mixture of chemicals was preferred over song from the E2 dosed males, although no difference was found between the preferences for song from E2 dosed or control males.

Figure 4. Song preferences in female starlings.

The percentage of time spent by females on the perch adjacent to song playback from male starlings in the three treatment groups; control (open bars); E2 dosed (hatched bars); and chemical mixture dosed (black bars). Playback from the mixture group was preferred over playback from E2 dosed males ($t_{10} = 2.42$, $P = 0.035$); Playback from the mixture group was preferred over song from control males ($t_9 = 2.57$, $P = 0.029$). There was no significant preference between control and E2 dosed playback ($P>0.05$); Graphs show mean+s.e.m. * = $P<0.05$.

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To the best of our knowledge, our study provides the first experimental test of the effects of ecologically relevant dose levels of endocrine disrupters on avian neural development and behaviour. Our dose levels have been carefully determined following field observations and sampling [22] and assume that starlings in winter take approximately half of their food intake from the sewage filterbeds. The fact that EDC exposure can have detrimental effects on immune function [21] is supported by the consistent immunosuppression across the treated groups. The higher EDC dose of the mixture group did not cause further increased levels of immunosuppression, above that of the E2 group, although there are a number of potential interpretations of this result.

Steroid hormones influence the initial sexual differentiation of the songbird brain [11] and 17-β estradiol is specifically known to affect the plasticity of the avian brain [14], [24], suggesting that estrogen mimics and natural estrogens can directly influence seasonal development of HVC. Males that received the mixture of EDCs showed both increased song output and increased song complexity, almost certainly due to changes in the size of the HVC. We saw no such effects within the group dosed solely with E2. This could be due to the fact that the physiological response to EDC exposure is dose-dependent, such that the higher total dose of EDCs in the mixture group produced effects that E2 alone would not. Alternatively, these effects could be due to the combination, or even a subset, of the pollutants administered in the mixture treatment. Within the brain of the songbird, testosterone is converted into estrogen, which is then released into the blood stream at physiologically significant levels [25]. Paradoxically, in birds where male is the default sex, estrogens are known to be both necessary for the feminisation of the sexual organs during early development, and also for the masculinisation of the avian song centres in the brain [13]. Song complexity determines male attractiveness in many songbird species [10], and has been shown intraspecifically to correlate with the volume of the HVC [23]. Within the cerebral song system pathways only the HVC has estrogen receptors [12], [26] suggesting this region is likely to be one of the most susceptible to the effects of EDCs. Our study has demonstrated the vulnerability of the HVC to disruption by estrogen mimics. In addition, our results also highlight the continued plasticity of the adult songbird brain.

From an ultimate, evolutionary perspective our results suggest that exposure to endocrine disrupters may alter the selective forces acting on songbird populations. It is established that female starlings show active preferences for males which have greater song output and larger repertoire sizes [27]. Our results show that females prefer the song output from males exposed to the complete mixture of endocrine disrupters, despite the fact that such males are immunosuppressed. If female starlings bias their reproductive investment towards males in poor physiological condition then hatching and/or fledging rates could decline with detrimental consequences at the population level.

Our findings document for the first time that invertebrates living on sewage filterbeds take up a range of environmental pollutants. The levels of these chemicals in aqueous sewage effluent leaving the percolating sewage filterbeds in UK have been found to vary: e.g. E2 50 ng/L[16]-100 ng/L [28] or bisphenol A 500 ng/L [28]. Although liquid and solid samples are not analogous, the concentrations of both E2 and bisphenol A identified by our study in 1 g of earthworms therefore greatly exceed (up to 1000 fold) [22] those previously reported in 1 ml of sewage treatment effluent. Our study therefore highlights the potential for such pollutants to have detrimental physiological effects at various trophic levels.

Birds are transglobal vectors for disease and our results highlight the potential for them to demonstrate intercontinental effects of pollution exposure. As the starling is a migratory bird species, our findings may suggest that pollutant exposure on the wintering grounds could affect reproductive success at the breeding sites. Starling populations in the UK have suffered a 50% decrease in the last forty years and consequently the starling is listed as a bird of high conservation concern [29]. Many issues contribute to this decline [29], but reduced reproductive success as a result of EDC exposure may be a factor that has yet to be recognised. Our study has shown that ecologically-relevant levels of EDC intake affect immune function, neural development and behaviour in male starlings and may therefore contribute to their population decline. Further work is needed to quantify the importance of these effects in wild bird populations.

Materials and Methods

Quantification of contamination levels

We observed starlings foraging in the winter of 2003/4 at 20 sewage treatment works in the south west UK, and their prey species were identified. We collected and analyzed duplicate 10 g samples of the earthworm Eisenia fetida, which was the prey item observed to be taken at the greatest biomass. The EDC content of the collected earthworm tissue was quantified using gel permeation chromatography and GC-MS [22]. The mean±s.e of each chemical in the earthworm samples across five sewage filterbed sites were: 9.85±6.7 ng/g of 17 β estradiol (E2), 6.2±2.19 ng/g dibutylphthalate, 26±12.6 ng/g dioctylphthalate and 4.28±2.6 ng/g bisphenol A. We found that E. fetida from garden soil contained significantly lower levels of these chemicals excluding E2 which was only found in the earthworms from the sewage treatment sites [22]. Starlings were observed to take in single E. fetida (mean mass 0.3 g) at a rate of 1/min, with a mean patch residence time of 16 mins/hr observation and the intake rate was constant with increasing food patch residence time (P>0.05). We therefore calculated from our observations that the individual starlings in our study take in on average 14.4 g/day wet weight of invertebrates from the sewage treatment filterbeds. As the daily food intake of invertebrates (wet weight) for adult starlings is approximately 30 g/day [18], intake from filterbeds represents 48% of their daily food intake during the winter months (100.8 g wet weight/week). The daily dose levels used in the captive experiment were based on the chemical content of the filterbed samples and this intake calculation.

Dosing and physiological responses of captive birds

One year old starlings were allocated to three treatment groups: 1) control group: each bird received daily one mealworm Tenebrio molitor with 10 µl of peanut oil, injected into the body cavity as the carrier substance, 2) E2 group: each bird received a mealworm with 200 ng 17- β estradiol (E2) in 10 µl of peanut oil, or 3) mixture group: each bird received a mealworm with 200 ng E2, 520 ng dioctylphthalate, 80 ng bisphenol A, and 120 ng dibutylphthalate dissolved in 10 µl of peanut oil. All the birds were caught as juveniles and housed for one year in outdoor aviaries prior to the start of the experiment. During the experiment the birds were housed in single-sex trios (1 from each treatment group) in outdoor aviaries each measuring 2 m×1 m×1 m and maintained in the same groups throughout the experiment. Birds were dosed 5 days per week from October 2004 until April 2005, to mimic their foraging period on sewage filter beds. All the starlings were maintained on an ad lib diet of an insect paté (Orlux™) and had constant access to water and one nestbox per bird. At the end of the experiment (April 2005), the birds were also weighed and blood sampled for haematocrit levels and testosterone levels. The cell-mediated immune response of the birds was tested in March 2005, by using an injection of phytohaemagglutinin (PHA) into both wings webs [30]. The thickness of both wing webs was measured (mean 3 measurements) at the same location of the wing before injection and 24 hours after injection, using callipers (Moore and Wright™; to 0.1 mm). PHA (Sigma L-8754) in phosphate buffered saline (PBS; 0.45 µg in 50 µl [30], [31] was injected into both wings webs of each bird. The mean response of both wings was calculated and used in all analyses. A control injection of PBS alone, to control for any injection trauma was not carried out, as this has been shown to be unnecessary [32]. After more than 24 hours post-injection, the swelling subsided. The humoral response of the birds was tested in April 2005 using intraperitoneal injection of sheep red blood cells (SRBC) [33]. A control blood sample was drawn before the start of the test. SRBC in Alsever's solution (TCS Microbiol Ltd, Claydon, UK) washed and resuspended 1× PBS to form a 2% solution. 500ul was injected twice intraperitoneally 14 days apart and blood samples were drawn to test for the primary and secondary humoral response. Plasma samples were heat-treated at 56°C for 30 minutes and stored at −20°C for three weeks before testing using a standard haemagglutination test[33].

Song analysis

In March-April 2005 the song output of individual male starlings was recorded as follows: On day 1 a male was moved into a separate outdoor cage with a novel female. Each male was housed with a different female to avoid pseudoreplication. The song output of the male was recorded twice for 3 hours: once in the afternoon of day 1 and once on the morning of day 2. The recordings were made using a Marantz solid state recorder PMD 670 and a Sennheiser K6 microphone body, with a Sennheiser (MKE 2-60 Gold C) sub-minature microphone attachment, mounted ontop of the nestbox. We calculated the following measures: (i) the total amount of time spent singing; (ii) the number of song bouts; (iii) the duration of each song bout and iv) repertoire size. Song bouts were defined as continuous song and were separated from each other by at least 1 s [27]. Repertoire size was estimated from a cumulative plot of the novel phrase types appearing in 20 song bouts [27]. These measures were averaged over both recording periods. Birds that did not sing during either recording attempt were not included in the song analysis.

Testosterone analysis

Testosterone concentrations were estimated from plasma samples in 2 assays using anti-testosterone antiserum (code 8680-6004, Biogenesis, U.K.) and [125I]-testosterone label (code 07-189126, ICN, U.K.) [34]. The mean 50% binding for the assays was 0.355 ng/ml. Samples were run in either duplicate 10ul or 20ul samples and the detection limits were of 0.01 ng/ml or 0.02 ng/ml respectively. The interassay CV was 10.4%.

Neural analysis

Male starlings were killed by decapitation on 22nd April 2005 and their brains were removed immediately by dissecting them out of the skull. Brains were frozen over liquid nitrogen and stored at −80°C until analysis. Brains were cut on a cryostat (Leica) into 30 µm sagittal sections. Sections were mounted onto Superfrost Plus slides (Menzel Gläser, Germany) in four different series. One series was Nissl-stained with thionin and cover slipped. Slides were analysed under bright-field illumination with a microscope (Leitz Aristoplan). For area measurements, brain regions were video-digitised on a PC equipped with an image analysis system (Meta Morph, Visitron, Germany) and measured by the built-in measurement tools. Volumes were calculated as the sum of the area sizes multiplied by section interval and section thickness.

Throughout, all statistical analysis was conducted using Systat v 10. As the males were held in trios, trio group was entered as a covariate in all models, but not found to be significant in any case ($P>0.05$).

Song preference

Wild-caught female starlings ($n = 11$) were placed in a long aviary (810×180×200 cm) with a perch 20 cm away from each of the speakers at each end. A Sony SRS-A37 speaker was hidden behind a cloth at each end of the arena. The speakers were connected to a Sony Walkman portable compact disk player operated by the experimenter.

Five males were used from each treatment group to provide the song stimuli and were randomly paired in the combinations of playback from the different treatment groups. Two different song files were created using Avisoft-SASLAB Pro for each male, each containing three song bouts, for a total clip length of 30 s. These songs were matched for amplitude and used to create 5 minutes song loops, which were then recorded 6 times to create 30 minutes of song stimulus. The playback from a particular male was experienced by either 2 or 3 females and averaged across females. Choice stimuli tests were counterbalanced for each song pair type and side of presentation (right or left speaker) in the testing apparatus and playback was simultaneous at each end of the aviary.

We tested each female with three comparisons of male song stimuli in succession: control versus males treated with the mixture of chemicals;

control versus E2 treated males; and E2 treated versus mixture of chemical males. Each test consisted of two playback blocks. In each block the song playback was played for 5 minutes prior to data gathering. The song stimuli were played for 30 minutes. The amount of time that the female spent on the perches within 20 cm of the speaker was recorded. In the second block the protocol was repeated but the song playback was reversed to control for any side biases. Data were averaged over blocks 1 and 2. The playbacks of the treatment comparisons were carried out sequentially and the order of the pairwise song stimuli choice test was randomly assigned to each female.

Acknowledgments
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Author Contributions
Conceived and designed the experiments: KB SM DP. Performed the experiments: KB SM SB CM. Analyzed the data: SL SM CM. Contributed reagents/materials/analysis tools: KB. Wrote the paper: KB SM. Other: Provided financial support for PDRA as a grant PI: CC. Obtained the funding: KB.

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December 20th, 2008;

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Antidepressants make for sad fish

The drugs are becoming more common in river waters and can play dangerous head games with fishBy Janet Raloff December 20th, 2008; Vol.174 #13 (p. 15)

AWKWARD POSE

Some hybrid striped bass exposed to Prozac eventually began hanging vertically in the water â€" a highly anomalous pose â€" and stopped eating. Clemson Universityâ€™s Institute of Environmental Toxicology TAMPA, Fla. â€"

In the fish world, baby is just another word for lunch. So it behooves aquatic larvae to be ever vigilant. Yet those who as embryos or hatchlings encountered water polluted with trace concentrations of an antidepressant are much more likely to become lunch.

Tons of medicine ends up in the environment each year. Much has been excreted by patients. Leftover pills may also have been flushed down the toilet. Because water treatment plants were never designed to remove pharmaceuticals, water released into rivers by these plants generally carries a broad and diverse array of drug residues.

In 2006, a pair of chemists reported that antidepressants downstream of water treatment plants were making it into the brains of fish.

RIDING HIGH

Normally a bottom-dwelling species, this antidepressant-exposed bass started swimming at the surface, partially out of the water. Below, its putative meal of minnows swam with impunity. Clemson Universityâ€™s Institute of Environmental Toxicology Meghan McGee of St. Cloud State University in Minnesota studies larval fathead minnows.

Recently she set out to see
whether exposure to specific antidepressants would affect the fish. Fish exposed as embryos or hatchlings to trace concentrations of the antidepressant venlafaxine, marketed as Effexor, didnâ€™t react as quickly as normal to stimuli signaling a possible predator. This laid-back reaction could prove to be a â€œdeath sentence,â€ she observes.

McGeeâ€™s is one of many studies probing behavioral impacts on aquatic wildlife from pharmaceutical pollution, especially antidepressants. Emerging data from these studies were reported in Tampa, Fla. November 16-20 at the North America annual meeting of the Society of Environmental Toxicology and Chemistry, or SETAC. Overall, the studies show that antidepressants can impair a fishâ€™s ability to eat, to avoid being eaten â€" and perhaps even to attract a mate.

And venlafaxine: Itâ€™s one of many antidepressants found in river waters. Indeed, McGeeâ€™s team selected concentrations for this study based on values measured downstream of water treatment plants.

PREDATOR CALLING

Researchers placed a cell phone set to vibrate beneath a dish of water holding a larval fish. Fish tend to interpret such vibrations as signaling an approaching predator and will initially curl into a â€œCâ€ and then dart off in a new direction. Minnows exposed to antidepressants reacted only half as quickly as unexposed minnows. Clemson Universityâ€™s Institute of

Environmental Toxicology â€œI was surprised how often I was seeing these antidepressants,â€ recalls Melissa Schultz of the College of Wooster in Ohio, one of the chemists who documented that antidepressants reach fish brains. â€œPretty much any water sample in the vicinity of a wastewater treatment plant will test positive for some group of antidepressants,â€ she finds.

The most common ones showing up in water: venlafaxine, bupropion â€" marketed as Wellbutrin, and citalopram â€" sold as Celexa. What showed up in fish brains were both the drugs and their metabolites, or breakdown products. â€œThe most common ones we saw were metabolites of Prozac [fluoxetine] and Zoloft [sertraline],â€ Schultz says. The second most abundant were the parent compounds: Prozac and Zoloft. â€œSo profiles of these drugs in the brain werenâ€™t matching the profiles we were seeing in the water.â€ Why remains a mystery.

The St. Cloud State researchers exposed minnows to venlafaxine alone or as a mix of four antidepressants and quantified how quickly fish reacted to a stimulus signaling a possible predator. For the stimulus, the researchers chose to send a vibration into the fishâ€™s water. â€œMy wifeâ€™s cell phone got hijacked for the task,â€ explains study leader Heiko Schoenfuss. An electronic chip that allowed the phone to vibrate was removed and placed beneath the dish in which each tiny hatchling was placed. Pressure sensors along the sides of fish detect vibrations, which can signal an approaching big fish.

FAST FOOD

This frame shows a hybrid striped bass quickly gobbling up four minnows. Fed only once every three days, the bass tend to become quite aggressive about downing their meals. After being exposed to high concentrations of Prozac, however, some bass took up to two minutes to capture their first minnow and didn't finish all four with the allotted 25 minutes. Over the nearly month-long experiment, a few bass lost their appetites altogether. Clemson University's Institute of Environmental ToxicologyIncubating eggs were exposed to the drugs for five days before hatching, then the larvae spent 12 days in clean water before testing. In another set of experiments, new hatchlings swam in drugged waters for 12 days before encountering the chip's vibrations.

Only venlafaxine slowed the time it took minnows to recognize and respond to the vibrations. The mix of antidepressants slowed the velocity at which fish fled. When the response time and swimming velocity were accounted for, the new study found that drugged fish reacted slowly to avoid predators. Untreated fish â€œresponded about twice as fast as the pharmaceutically exposed larvae,â€ McGee says.

That wouldnâ€™t be so bad if predators were comparably slowed by these similarly low concentrations of antidepressants â€" billionths of a gram per liter of water. But such nanogram concentrations of fluoxetine didnâ€™t slow the speed at which hybrid striped bass scarf down fathead minnows, according to preliminary data reported at SETAC by Joseph Bisesi Jr. and his colleagues at Clemson Universityâ€™s Institute of Environmental Toxicology in Pendleton, S.C.

To see what concentrations would affect feeding, Bisesiâ€™s group upped water concentrations to between 10 and 40 micrograms per liter â€" values 100 to 1,000 times higher than needed to affect minnow-escape responses in the St. Cloud study. Only then did some of the normally aggressive and hungry bass start to lose their voracious appetites.

Each bass was offered four minnows once every three days. Any not eaten in

25 minutes were removed. Prior to drug exposures, young-adult bass quickly devoured prey, sometimes all four within 10 seconds, Bisesi notes. But six days into a 27-day exposure to fluoxetine, several fish in the higher concentration groups began to show behavior changes. Some waited a minute or two before going after their first fish. Some spit a minnow out after capturing it or failed to eat more than two. A few developed severely aberrant swimming patterns â€" such as hanging vertically in the water or resting at the surface, dorsal fin exposed â€" as minnows cavorted beneath them.

Many of the antidepressants tested work in people by altering levels of serotonin, a neurotransmitter, in the brain. However, Schoenfuss reported at SETAC that fluoxetine also functions like an estrogen â€" at least in adult male fathead minnows. It triggered the minnowsâ€™ production of vitellogenin, a yolk protein normally made only by egg-laying females. The drug also diminished the macho facial bumps and coloration that females prize in their mates.

So clearly these drugs may have multiple modes of action, Schoenfuss says, particularly â€œonce they enter the water and are taken up by nontarget organismsâ€□ â€" like fish. Ironically, his feminized male minnows actually proved more aggressive at guarding nests than did unexposed males. Joanne Parrott of Environment Canada and her colleagues reported at the meeting on a similar boost in malesâ€™ nest-protecting aggression among fathead minnows exposed to venlafaxine.

Of course, all these experiments are quite artificial. Explains Schultz: â€œWhen a fish is exposed to wastewater, itâ€™s not just getting a dose of antidepressants, itâ€™s also encountering lots of other thingsâ€□ â€" including other drugs. In the future, she says, â€œweâ€™ll have to look at how these might all interact.â€□

....

Comments
It continues to be a source of surprise that nobody is studying the effect on humans of these anti-depressants relative to humans failing to flee or fight when it is appropriate.

For example, how has the great American experiment with anti-depressants has helped get us into the current financial insanity. I think that quite a few people have seen it coming, become angry, depressed, disturbed, and instead of dealing with it the way people did in times past - by demanding changes - took anti-depressants.

Inappropriate affect now firmly enforced by drugs, (legal ones) Americans head back to their white-collar jobs where they continue to row the boat toward Niagara falls, accepting defeat, able to not be agitated, angry or depressed.

Let us all appreciate the scenery behind us as we come closer and closer to the edge rather than actually deal with anything! John ToradzeNov. 26, 2008 at 5:54am

The DSR would have you believe that issues relating to contaminants of environmental consequence (CECs) can await for yet more analyses. The U.S. EPA has just come out with a pronouncement that cocktails of CECs as compared to the study of a single material is where the emphasis must be. Below is an abstract that indicates that as currently designed, sewer plants are not able to stop these materials from reaching the environment. However, knowing that and then deliberately setting up a system that will deliver these CECs into communities is in which families of people live vastly different from both a scientific and moral perspective. As noted below, these seem to build up in sediments---what about soils in communities and back yard gardens? Take, for example the following-------------the group known as polybrominated diphenyl ethers (PBDEs), which are flame-retardantsretardants, were recently found to serve as a "**potent neurodevelopmental toxin**" in humans according to Alexander Suvorov and Larissa Takser, at the Département Obstétrique Gynécologie, Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Quebec.

Polybrominated diphenyl ethers in an advanced wastewater treatment plant. Part 2: Potential effects on a unique aquatic system

Authors: Ikonomou, Michael G.; Rayne, Sierra
Source: Journal of Environmental Engineering and Science, Volume 4, Number 5, 1 September 2005 , pp. 369-383(15)
Abstract:
Concentrations of the mono- through deca-substituted polybrominated diphenyl ether (PBDE) flame retardants were determined in the aqueous effluent from a tertiary-level wastewater treatment plant (WWTP) that uses post-filtration ultraviolet light disinfection. The WWTP is located in a semi-arid region of British Columbia. Subsequent limnological modeling of receiving waters examined the potential long-term effects of various PBDE-loading scenarios on this unique aquatic system. Over the three decades from 2002 to 2031, total PBDE concentrations in the water column and in suspended and surficial sediments are expected to increase to $>120\ pg \cdot L^{-1}$ and $\sim 1\ ng \cdot g^{-1}$ wet weight, respectively. Following implementation of a hypothetic halt on PBDE releases into the aquatic system, individual PBDE congener concentrations in the water column and sediments declined by <35% over the ensuing 17-year modeling period after the ban, illustrating the potential long-term problem arising from continued PBDE inputs into aquatic systems worldwide. The results also suggest that PBDEs represent one of the single largest halogenated aromatic loadings to Canadian lakes, rivers, and streams from wastewaters, and their worldwide use continues to increase exponentially. *Key words*: polybrominated diphenyl ethers (PBDEs), flame retardants, municipal wastewater treatment effluent, contaminant fluxes, limnologic modeling.
**:

NOW TO DISCUSS MORE ISSUES WITHIN THE DRAFT STAFF REPORT

HAZARDS and HAZARDOUS MATERIALS

Would the project:	□□	□□	□□	□□ ☹

a) Create a significant hazard to the public or the environment through the routine transport, use, or disposal of hazardous materials?				
b) Create a significant hazard to the public or the environment through reasonably foreseeable upset and accident conditions involving the release of hazardous materials into the environment?	☐☐	☐☐	☐☐	☐☐ ☹
c) Emit hazardous emissions or handle hazardous or acutely hazardous materials, substances, or waste within ¼ mile of an existing or proposed school?	☐☐	☐☐	☐☐	☐☐ ☹

The Draft Staff Report reads------------A consequence of adoption of the proposed Policy may be t
b.-h. The implementation of the proposed Policy is not expected to directly impact hazards and hazardous materials other than as discussed in the above paragraph.

Comment------------------------

From the above, it becomes obvious that certain toxins will bioaccumulate in soils, biomagnify within soil organisms, and these will see adverse impact to terrestrial and aquatic organisms, potentially including man. Thus the call by staff that there are no impacts will misguide the Board. The impacts may be significantly adverse and these issues need a through analysis within the EIR. There are technologies that will mitigate some of this but these are not discussed (where is the discussion of mitigation?). In addition, where is the discussion of the Alternatives. Thus from a series of perspectives, your Board is being given poor guidance. The EIR needs to spell out these impacts, potential mitigation measures and alternatives---presently, none of these options are properly presented to your Board.

<u>HYDROLOGY and WATER QUALITY.</u>

Would the project: a) Violate any water quality standards or waste discharge requirements?	☐☐	☐☐ ☹	☐☐	
b) Substantially deplete groundwater supplies or interfere substantially with groundwater recharge such that there would be a net deficit in aquifer volume or a lowering of the local groundwater table level (*e.g.*, the production rate of pre-existing nearby wells would drop to a level which would not support existing land uses or planned uses for which permits have been granted)?	☐☐	☐☐	☐☐	[
1. Substantially alter the existing drainage pattern of the site, including through alteration of the course of a stream or river, or substantially increase the rate or volume of surface runoff in a manner that would:	☐☐	☐☐	☐☐ ☹	

1. result in flooding on- or off-site	☐☐	☐☐	☐☐ ☹	
1. create or contribute runoff water that would exceed the capacity of existing or planned stormwater discharge	☐☐	☐☐	☐☐ ☹	
1. provide substantial additional sources of polluted runoff	☐☐	☐☐	☐☐ ☹	
1. result in substantial erosion or siltation on-or off-site?	☐☐	☐☐	☐☐ ☹	
1. Otherwise substantially degrade water quality?	☐☐	☐☐ ☹	☐☐	
1. Place housing or other structures which would impede or re-direct flood flows within a 100-yr. flood hazard area as mapped on a federal Flood Hazard Boundary or Flood Insurance Rate Map or other flood hazard delineation map?	☐☐	☐☐	☐☐	[

1. Would the change in the water volume and/or the pattern of seasonal flows in the affected watercourse result in:

1. a significant cumulative reduction in the water supply downstream of the diversion?	☐☐	☐☐	☐☐ ☹	

1. a significant reduction in water supply, either on an annual or seasonal basis, to senior water right holders downstream of the diversion?	☐☐	☐☐	☐☐ ☹	
1. a significant reduction in the available aquatic habitat or riparian habitat for native species of plants and animals?	☐☐	☐☐	☐☐ ☹	
1. a significant change in seasonal water temperatures due to changes in the patterns of water flow in the stream?	☐☐	☐☐	☐☐	[
1. a substantial increase or threat from invasive, non-native plants and wildlife	☐☐	☐☐	☐☐	[
1. Place within a 100-year flood hazard area structures which would impede or redirect flood flows?	☐☐	☐☐	☐☐	[
1. Expose people or structures to a significant risk of loss, injury, or death involving flooding, including flooding as a result of the failure of a levee or dam?	☐☐	☐☐	☐☐	
1. Be subject to inundation by seiche, tsunami, or mudflow?	☐☐	☐☐	☐☐ ☹	

The intent of the proposed Policy is to ensure attainment of water quality objectives. The proposed Policy may increase the use of recycled water and, hence, the salt / nutrient loadings on groundwater basins. The proposed Policy, however, mitigates this effect by requiring the development of regional salt/nutrient management plans that would consider all sources of salts and nutrients and that would prescribe requirements for meeting groundwater quality objectives for all dischargers within a basin.

b. If the proposed Policy results in an increased use of recycled water, this use may be a substitute for groundwater use. Hence, the proposed Policy may help prevent the reduction of groundwater supplies. Groundwater recharge reuse projects directly augment groundwater supplies.

Comment-------------------

As seen in the above text, water quality can be adversely impacted. Previous comments by this writer discuss the adverse impacts upon ground water that may accrue to the use of recycled water---please refer to those comments. The Draft Staff Report indicates that with mitigation the policy's implementation would not violate any water quality standards with proper mitigation. There is this statement by staff alluding to mitigation but no discussion of what that mitigation is or how it will be accomplished. It is agreed that the water can be cleaned up to an extent, but again that depends on the type of mitigation and whether the costs can be kept with in reason for the value of the water for its intended use. None of this is discussed. Further, the provisions of state law in the Health and Safety Code discussing contamination, public health, nuisance as found in code sections bear comment here. The proposed Policy states that that State Water Board shall use the authority provided in Water Code sections 13550 *et seq.* *This is essentially a statement that the State Water Board **shall** implement existing law.* State law here is a double edged sword which the staff seems to conveniently ignore. Since One of the primary conditions on the use of reclaimed water is protection of public health (Water Code Sections 13521, 13522, 13550 (a)(3)). Section 13522 requires abatement of contamination. H&SC 5410 defines contamination as impairment of the quality of the waters of the state to a degree which creates a hazard to public health through poisoning or **spread of disease.** H&SC 5410 (d) further defines contamination and H&SC 5411 states that no person shall discharge sewage or other waste effluent of treated sewage or other waste in any matter which will result in contamination, pollution or nuisance. Nuisance is defined by H&SC 5410(f) as anything which is injurious to health and occurs during or as a result of the treatment of wastes.

Thus, accordingly, mitigation measures would need to remove the contaminants to a level of no impact and that level in a complex cocktail needs considerable scientific study. Thus, I fail to see where the staff report gives any kind of green light for this proposal since mitigation can not be spelled out.

The Draft Staff Report notes-----------that there would be less than significant impacts from **substantial additional sources of polluted runoff**. This statement can not be made, especially based on the above discussion and especially if constructed wetlands are being considered. Additionally, there seems to be a fairly large gray area as to what really constitutes incidental runoff. Further, there seems to be no discussion of movement via drain tiles. All these avenues need to be fleshed out and the way to do this is via a comprehensive EIR. Thus staff's contention of less than significant is in reality potentially significant and potentially adverse.

Otherwise substantially degrade water quality?---------------Again we have the issue of what constitutes water quality and what constitutes contamination (what are contaminants?). This question must be run by the provisions of both the Water Code and the Health & Safety

Code sections that have been discussed by this commentator. The issue of what really constitutes mitigation again raises its head, but again, there is no discussion of mitigation---- merely a vague reference that it may exist----but as what? Thus, again these issues are potentially significant and thus need further analyses within a comprehensiv.e EIR

a significant reduction in the available aquatic habitat or riparian habitat for native species of plants and animals?

This issue is directly related to the consequence of pollutants and contaminants found within recycled water, i.e., endocrine disrupters, pharmaceuticals such as those discussed above and other pollutants that are currently not removed from recycled water. Thus through the impact of these pollutants and contaminants, available habitat may be substantially reduced. Accordingly, the staff's call that these impacts are less than significant can not be made based on any scientific basis. Thus there is the potential for adverse and substantial impacts which will need to be discussed in the EIR.

a substantial increase or threat from invasive, non-native plants and wildlife

As noted above, fish were unable to defend themselves and birds to unable to effectively communicate or parent. Thus without maintaining territorial status, invasive species are able to move into habitats. How this may play out is unknown, but the real potential for such is well discussed in the material above. Consequently, the call by staff that there is no impact can not be supported by scientific evidence. In fact, just the opposite may be the case. Thus, this issue needs to be throughly reviewed in the EIR, for as by relying upon current staff guidance, your Board will be mislead.

POPULATION AND HOUSING

Would the project:

a) Induce substantial population growth in an area either directly (*e.g.*, by proposing new homes and businesses) or indirectly (*e.g.*, through extension of roads or other infrastructure)?

The above is found to be less than significant within the Draft Staff Report. Let us, for a moment reflect upon the purpose of the entire Recycled Water Policy.

The Draft Staff Report notes-----------**Increasing the use of recycled water is a major part of the state's plan for addressing its water supply needs. Drought, the health of the Delta ecosystem and reduced snowpack caused by global warming, present challenges that will likely limit the state's developed water supply. The state needs to encourage the development of recycled water projects to address the water demands of its population, businesses, industries and agricultural producers**.

By off setting potable supplies for uses other than drinking, washing, cooking and similar direct human contact uses, considerable savings will accrue to the current supplies of potable water. This is as it should be, if that alternative water is actually safe and not a threat to man or the environment. However, as it now stands, with rare exceptions, most supplies of reclaimed or recycled water can not claim that high ground. Nonetheless by offsetting volumes of potable-use, this will free up large volumes of potable water and that can then be applied directly to new development. Assume for a moment that in Southern California where most of the population is and the water isn't, this strategy will allow for considerable new development. That development will come at a cost for increased infrastructure (school, roads, emergency facilities, etc) and this will see an increased impact on air quality from expanded energy use and expanded transportation needs. Sure, the cars may be getting greater mileage but this is offset by increased number of cars. Additionally with increased cars, the aerosols from exhaust and tire dust will have increased impacts on human health (lots of good science to back this up). Thus for staff to say that there is no impact on land use planning----or that matter on air quality, noise, population/housing, public services, recreation needs, transportation, or utility needs can not be made on any valid rational basis or on science. To accept these staff findings here merely opens the real possibility that your Board will be badly misguided. Thus the issues of land use planning air quality, noise, population/housing, public services, recreation needs, transportation, or utility needs warrant considerably more analyses within a comprehensive EIR because impacts within each of these areas may be significantly adverse and thus require discussion, mitigation and thus an array of alternatives based on how successful the mitigators are.

Consequently the MANDATORY FINDINGS OF SIGNIFICANCE that the proposal will have less than significant impacts with mitigation, without considering the above and specifying what various types of mitigation would be needed and thus various alternatives, can not be defended by the current levels of analyses within the staff report. To get at these answers a very comprehensive EIR will be needed, but that is not what has been presented to you. Consequently, your Board risks making a fairly serious error if the staff report, as presented is accepted.
