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on the February 2011 Report,
Evidence on the Developmental and Reproductive Toxicity of Sulfur Dioxide
authored by the Office of Environmental Health Hazard Assessment (OEHHA)
of the California Environmental Protection Agency

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Introduction and Overview

In February 2011, California EPA (in particular, its Office of Environmental Health Hazard Assessment; OEHHA) released for public comment a report, *Evidence on the Developmental and Reproductive Toxicity of Sulfur Dioxide*. The Report (herein referred to as the Background Document) is intended to provide “comprehensive information on the reproductive [and developmental] toxicity of [sulfur dioxide] SO₂.” The information is to be used by OEHHA’s Science Advisory Board in considering whether SO₂ should be added to the “Proposition 65” list of chemicals “known to the State to cause reproductive toxicity.”¹

In this document, we comment both on the Background Document and on other relevant reports and literature. We hope that our comments will prove useful to the Science Advisory Board and others.

The Background Document reviews and assimilates literature pertaining to associations between sulfur dioxide exposures and reproductive and/or developmental outcomes in men, women, and their offspring (and in male and female laboratory animals and their pups). The Report appears to be comprehensive, citing more than 130 papers and reports, and comprising 258 pages.

OEHHA’s primary finding is that sulfur dioxide causes male reproductive toxicity. In particular, it finds that sulfur dioxide reduces the quality of sperm, and that this reduction is one reason that women attempting to get pregnant do not do so within the first month of their trying to conceive.

At the outset, it might be noted that this is an unprecedented finding. All of the “authoritative bodies” on which OEHHA may rely — namely the U.S. EPA, U.S. FDA, NIOSH, and IARC² — have evaluated the scientific evidence regarding sulfur dioxide and health effects, and none has concluded that the chemical causes reproductive and/or developmental toxicity. The most recent of these evaluations, U.S. EPA (2008), was exhaustive in its review and analysis of the literature regarding SO₂ and all health effects.

¹ By definition, reproductive toxicity and developmental toxicity are different sets of phenomena. Reproductive toxicity refers to harm to eggs, sperm, and/or other tissues, organs, and systems involved in initiating and maintaining pregnancy. Developmental toxicity, in turn, refers to harm to the conceptus, embryo, and/or fetus. In the context of Proposition 65, however, these two types of toxicity are sometimes both referred to simply as reproductive toxicity.

² *Per* the California Code of Regulations, Title 22, Section 12306, OEHHA may rely on these four “authoritative bodies . . . for the identification of chemicals as causing reproductive toxicity.”

OEHHA also finds that SO₂ is associated with preterm birth and with retarded fetal growth. Again, U.S. EPA and other agencies and expert reviewers have evaluated the relevant information and do not so conclude.

Of course, the fact that OEHHA differs from other agencies in its evaluation of the evidence does not *per se* mean that OEHHA is incorrect — especially since OEHHA’s review is the most recent. In what follows, then, we evaluate the evidence presented in the Background Document, along with information not therein presented. We begin with some basic biology of SO₂ — a vital topic that the Background Document ignores. We then discuss the epidemiological and toxicological evidence with regard to SO₂ and reproductive toxicity, preterm births, and fetal growth retardation. We conclude with a brief discussion. As explained throughout, this evidence fails to indicate that sulfur dioxide is toxic to reproduction and/or development.

Endogenous sulfur dioxide, sulfite, and sulfate

Sulfur dioxide is an endogenously synthesized, essential biochemical: indeed, it is at the heart of the sulfur cycle in all animals. *In vivo*, SO₂ is generated *via* the enzymatic oxidation of the sulfur-containing amino acids in protein — primarily cysteine and methionine (Stipanuk, 1986; Feng *et al.*, 2007). The more protein we eat, the more sulfur dioxide we synthesize (Magee *et al.*, 2004). And, the amounts of SO₂ we generate are substantially greater than the amounts we inhale — even in heavily polluted environments.

Once synthesized, SO₂ plays a role not only in allowing the formation of more complex, essential, sulfur-containing molecules; it also has physiologic actions *per se*. In particular, endogenously generated SO₂ acts to signal vasodilation, thus lowering blood pressure (Du *et al.*, 2008; Meng *et al.*, 2009). The aorta naturally contains relatively high concentrations of SO₂, and the dose-dependent ability of SO₂ to relax aortic rings has been demonstrated *in vitro* (Li & Meng, 2009; Meng *et al.*, 2009). In rodent studies, endogenous SO₂ has been reported to protect against myocardial injury (Liang *et al.*, 2010) and to slow the development of atherosclerosis (Li *et al.*, 2011).

Of course, this does not mean that endogenous SO₂ could not also somehow harm reproduction or development, but it must be said that no evidence of such an effect exists. The only functional difference between inhaled SO₂ and endogenous SO₂ is that the former contacts the lungs first, whereas the latter is synthesized intracellularly. Once absorbed into the circulation, they are identical.

The same is true of the bisulfite and sulfite that form *immediately* upon the dissolution of SO₂, whether generated *in vivo* or inhaled via air, in the body’s water; and of the sulfate, which is generated from sulfite *via* the enzyme, sulfite oxidase. The Background Document fails to note that sulfate is an essential nutrient (Food and Nutrition Board, 2005). Many essential biochemicals — such as chondroitin sulfate (a component of cartilage) and cerebroside sulfate (an essential component of the myelin sheath of nerves), bile acids, and catecholamines — can be synthesized only when the body contains sufficient quantities of sulfate.



Interestingly, because the fetus cannot synthesize enough sulfate on its own, maternal blood concentrations of sulfate increase approximately two-fold during pregnancy (rising from 0.3 to 0.6 millimolar), thus nourishing the fetus with sulfate *via* the placenta (Cole *et al.*, 1984, 1985 & 1992). Studies using genetically engineered laboratory mice demonstrate that normal placental and fetal development cannot take place in the absence of these increased circulating concentrations of sulfate. Indeed, an array of placental sulfate transporters (in humans and all other mammals) acts to maintain sulfate homeostasis among the fetus, the placenta, and the pregnant women (Markovich, 2001; Dawson, 2011). Fetal and neonatal brain development cannot successfully take place in the absence of sufficient supplies of sulfate (Feng *et al.*, 2005).

Moreover, even though children and adults biosynthesize much of the sulfate we need, diets depleted of sulfur-containing amino acids and sulfate *per se* result in restricted growth and development (McGarry & Roe, 1973; Price & Jollow, 1989; Hou *et al.*, 2003; and Pecora *et al.*, 2006). These adverse effects can be reversed by returning sulfate ingestion-rates to those provided by healthy diets (Dawson, 2011).

Unlike sulfate, sulfite *per se* is not an essential nutrient (and is clearly toxic at high concentrations), but is also present in our diets and, as noted above, is also generated endogenously. The estimated average U.S. per capita consumption rate of sulfite is 0.2 mg/kg-day (as SO₂-equivalents; LSRO, 1976). Endogenous production of sulfite is much larger than this — on the order of 10 mg/kg-day (as SO₂-equivalents; Magee *et al.*, 2004); and the chronic NOAEL in laboratory rodents of ingested sulfite is on the order of 70 mg/kg-day (as SO₂-equivalents; JECFA, 1999).

Let us put this in perspective. Even in heavily polluted cities such as Beijing — where mean concentrations of SO₂ were on the order of 100 µg/m³ (Xu *et al.*, 1995) — the amounts of SO₂ that people would inhale (about 0.025 mg/kg-day) are on the order of some:

- 8 times smaller than the average amount of sulfite in our diets;
- depending on our protein intakes, some 400 times smaller than the average amount of sulfite that we synthesize endogenously; and
- 3,000 times smaller than the NOAEL for sulfite.

Moreover, the sulfite derived from inhaled SO₂ is simply oxidized to sulfate and excreted in urine *via* the same renal mechanisms that act to maintain blood sulfate concentrations within optimal ranges.

Finally, we note that since protein is the *in vivo* source of SO₂, if SO₂, sulfite, and/or sulfate were toxic to reproduction and/or development, then so too would protein be. To our knowledge, it is not. Throughout the Background Document, the authors provide what they consider to be plausible mechanisms by which SO₂ may harm sperm, fetal growth, or other aspects of reproduction and development. A more balanced analysis might have also provided biologically and physiologically-based reasons for questioning the likelihood of such harm.

Reproductive Toxicity

The OEHHA Background Document indicates that sulfur dioxide harms male reproduction. For this conclusion, Document authors rely primarily on four epidemiological studies. The first of these, by Dejmek *et al.* (2000a), is characterized by OEHHA as “evidence strongly supporting a cause and effect association between exposure to SO₂ and decreased fecundability.” The other three studies (Selevan *et al.*, 2000; Rubes *et al.*, 2005; and Robbins *et al.*, 1999) are interpreted by OEHHA as persuasive evidence that sulfur dioxide adversely affects “semen quality.”

At the outset, it should be noted that all four of the studies at issue were conducted by the same group of researchers working in the same, heavily polluted (at that time) city of Teplice, in the Czech Republic. This city was part of the infamous “black triangle” in Central Europe where, especially during the Soviet era, extensive surface mining, chemical, petrochemical, and materials production, hospital waste incineration, and uncontrolled combustion of poor quality brown coal (especially in the winter) led to elevated concentrations of countless pollutants, both outdoors and indoors (Sram *et al.*, 1996 & 1999; Pinto *et al.*, 1998; Binkova *et al.*, 1996 & 2003; Rubes *et al.*, 2007). The high sulfur content of the local coal made sulfur dioxide a convenient *marker* for pollution, but thousands other pollutants —gases, aerosols, and particles— would have been emitted as well, from a multitude of sources, both industrial and residential. These pollutants (the vast majority of which were never measured) would have been concentrated in ambient air during the same usage and meteorological conditions (such as during the winter) under which SO₂ was concentrated.

OEHHA ascribes adverse associations reported in any of these studies to SO₂ alone, but this is unwarranted. Indeed, data reported in the very studies cited in the Background Document indicate that outdoor air in Teplice contained substantially elevated concentrations of respirable particles and a multitude of associated pollutants, such as polycyclic aromatic hydrocarbons (PAHs) — including the potent mutagen benzo(*a*)pyrene — several of which have been reported to harm fertility in mice and other animals, and/or to be associated with sperm DNA damage and infertility in humans (Ford & Huggins, 1963; MacKenzie & Angevine, 1981; Mattison *et al.*, 1980; Gaspari *et al.*, 2003; Xia *et al.*, 2009; Gu *et al.*, 2010). In studies not cited in the Background Document, the Teplice investigators themselves have generated considerable evidence regarding PAH-induced DNA-changes in study subjects (Binkova *et al.*, 1996; Topinka *et al.*, 1997a,b; Dejmek *et al.*, 2000b; Sram *et al.*, 2006).

Ambient air in Teplice also contained substantially elevated concentrations of numerous metals, including those known or reasonably suspected to harm sperm and other aspects of male reproductive health — such as aluminum, chromium, lead, and nickel (Pinto *et al.*, 1998; Sallmen, 2001; Ashiru & Odusanya, 2009; Bonde, 2010;). Moreover, brown coal was used in households throughout the region as a fuel for hand-fed *indoor* heating stoves, which likely led to greater personal exposures to combustion gases, particles, and countless products of incomplete combustion than exposures resulting from outdoor air pollution (Ghosh *et al.*, 2011).

Thus, at a minimum, even had ambient air pollution in Teplice been reliably demonstrated to harm reproduction, it cannot be reliably concluded that ambient SO₂ was to blame.

The fact that the primary studies were all by the same group, all working in the same region, should also give readers pause. To our knowledge, their central findings have not been replicated by others, or even by the same researchers working elsewhere. Nor has reproductive toxicity been reported in men (or women) working in industries, such as sulfiting of wine and other foods, where exposures to SO₂ are potentially quite large relative to ambient levels.

The Background Document authors state that the primary study — Dejmek *et al.*, 2000a — was “well conducted” and otherwise persuasive, but this is not the case. Dejmek *et al.* (2000a) claim that they sought to determine whether air pollution in Teplice could be associated with subfertility. Dejmek and colleagues grouped new mothers into two categories (1) women who conceived within their first month of trying, and (2) women who “required” more than one month to conceive. All women in the second category (77% of all new mothers) were considered to be subfertile. Of course, they were not.³

The authors then tested whether any of *twenty* different combinations of air pollutants (as measured at one ambient air monitor in the City) and months prior to conception could be associated with the “fecundability” (probability of getting pregnant) of the mothers within their first month of unprotected intercourse.⁴ Nineteen of these combinations were not so associated; one was at the chosen level of statistical significance of 1 in 20! In particular, ambient air concentrations of sulfur dioxide in Teplice, measured two months before conception (but not one, three, or four months before), were associated with the proportion of mothers who became pregnant within one month of trying. From this, the authors inferred that sulfur dioxide reduces fertility. To say the least, this interpretation of the one “finding” in twenty comparisons should cause readers to pause: this result is entirely consistent with the null hypothesis. Moreover, eleven years hence, the result has not been replicated.

The authors go on to infer that this “impaired fecundability” was due to sulfur dioxide-induced damage to the sperm of the women’s mates. For this, Dejmek and colleagues present zero evidence — and *neither their nor anyone else’s studies, before or since, have demonstrated that sulfur dioxide impairs men’s (or women’s) fertility.*⁵ Dejmek and coauthors claim — and the Document Authors repeat — that the “timing” of their result makes sense, because the second month prior “coincide[s] with the sperm maturation period.” In fact, the duration of

³ Although definitions of subfertility are necessarily imprecise, no one considers failure to conceive within one month as evidence of subfertility. As summarized by Gnoth *et al.*, (2005):

Most . . . pregnancies occur in the first six cycles with intercourse in the fertile phase (80%). After that, serious subfertility must be assumed in every second couple (10%) although — after 12 unsuccessful cycles — untreated live birth rates among them will reach nearly 55% in the next 36 months.

⁴ The pollutants were NO_x, PAHs, PM₁₀, PM_{2.5}, and SO₂; the time-periods were the first, second, third, or fourth month prior to successful conception.

⁵ In general, fertility can be reduced due to impaired gametogenesis, transport of gametes in the male or female reproductive tracts, fertilization, migration of the zygote to the uterus, implantation, survival of the conceptus, and/or maintenance of the pregnancy through the delivery of a live birth. Sulfur dioxide has not been demonstrated to harm any of these stages of reproduction.

spermatogenesis in humans is on the order of 74 to 90 days (Amann, 2008; Jafarabadi, 2007). The authors provide no explanation as to why SO₂ concentrations should be determinative in the second month prior and not the first or third. The authors (and OEHHA) simply assume that the “reduced fecundability” of the mothers was due to some damage to their mates’ sperm; indeed, the Background Document does not even mention this study in its section entitled “female reproductive studies.”

The Background Document does not recognize that the investigators’ methods, and hence results and interpretations, were unreliable. Among other flaws, Dejmek and colleagues (2000a) apparently failed to question the new mothers with regard to critical factors, such as how often, and during what times of each menstrual cycle, the women had unprotected intercourse. Obviously, coital behavior is basic to fecundability, and no reliable measure of the latter can be made without knowledge of the former.⁶

Dejmek *et al.* (2000a) also fail to show much of the data that they *did* collect. Obviously, times to pregnancy in any population of women of child-bearing age form a distribution; some women become pregnant within one month of trying, others within two, others within three, and so on. In their methods section, Dejmek and colleagues make clear that they collected these data; there is no excuse for failing to plot or otherwise present these data or summary statistics there from.

We note that the OEHHA authors seem more willing to impute significance to Dejmek *et al.* (2000) than do Dejmek and colleagues themselves. Indeed, Dejmek and colleagues note, “Alternative explanations for our results are also possible.” The authors take note of several of their study’s limitations; OEHHA takes note of none. *Per* the authors, these limitations included:

- (i) the fact that only the mothers, not the fathers, were interviewed (so that some information regarding potentially confounding paternal factors may be missing);
- (ii) the fact that coital frequencies, birth rates, and pollution levels are all known or likely to be seasonally-dependent (and thus correlated with SO₂ concentrations), but, as we have indicated, the first of these were not measured, let alone controlled for; and
- (iii) the heavy reliance by the authors on their own unpublished, and unverified, results regarding “semen quality” (their reference 43).

OEHHA writes that three other epidemiological studies (Selevan *et al.*, 2000; Rubes *et al.*, 2005; and Robbins *et al.*, 1999) support their conclusion that sulfur dioxide is a male reproductive toxin because it adversely affects the quality of semen and/or sperm. As noted above, all of these studies are from the same group of researchers (with the same set of working hypotheses) as Dejmek *et al.* (2000). Moreover, all involved small numbers of subjects who comprised overlapping subsets of men (the women were no longer of interest to the researchers) from Teplice. Each of these studies has significant limitations; the results are of uncertain

⁶ Dejmek *et al.* (2000a) claim, and the Document authors take at face value, that measures of fecundability in just the first month “gives an unbiased estimate of the mean fecundability in the cohort.” For this indicate that this is can only be assumed to be true once one has accounted for coital frequency during each menstrual cycle, which Dejmek *et al.* did not.

health-significance, and results are inconsistent both within and across the studies. Importantly, none of these studies indicates that the study subjects in fact had any difficulty reproducing.

Selevan and colleagues (2000) collected (only) one semen sample from each of 154 men in Teplice and 118 men in another Czech city, Prachatice, which, relative to Teplice, was considerably more rural and much less polluted. The authors hypothesized that some aspects of air pollution would adversely affect one or more indices of the quality of semen and/or sperm. They did not, however, hypothesize that SO₂ *per se* was likely to be harmful. Instead, they noted:

Metabolites of the PAHs present in this industrial air pollution can form protein or DNA adducts in body tissues . . . and thus have the potential to damage germ cell DNA. PAHs also reportedly alter male reproductive function in test species . . . providing additional rationale for this study. Furthermore, metals such as lead and cadmium that are present in the particulate fraction of air pollution have been associated with decrements in human semen quality . . .

The authors recognized that characterizing a man's semen *via* only one sample was a significant limitation of their study, but do not explain why they did not gather multiple samples. Moreover, although the authors accounted for age — in that all study subjects were 18 years old — they did not control for other determinants of sperm quality.

The study results were mixed and difficult to interpret. For example, the median percentage of motile sperm in both sets of men — those in Teplice and those in Prachatice — were reported to be only 31% and 36%, respectively: both of these values are considerably smaller than the international median reference value for this index, which is 61% (Cooper *et al.*, 2009). No explanation for this low fraction of sperm motility is offered and, in any event, it was not observed in a subsequent study using some of the same subjects (described below). Some indices of semen quality — such as sperm concentration and sperm count — were found to not be associated with levels of air pollution. Other indices were associated with “medium” levels of air pollution, but not with “high” levels of air pollution; and many apparent associations with air pollution disappeared when differences in season (an established modulator of many aspects of semen quality) were taken into account. The authors refer to their preliminary findings as “intriguing,” and call for more study.

Additional study was indeed undertaken by the same group of investigators, as reported in Rubes *et al.*, 2005. This time, the investigators gathered up to seven semen samples from 36 of the young men from Teplice who had been reported on by Selevan *et al.* (2000), and again tested the hypothesis that air pollution adversely affects the quality of semen and/or sperm. Air pollution was characterized only as “high” — corresponding to samples gathered in the winter — or as “low” — corresponding to samples taken in September. The results were largely negative. As noted by the authors:



No significant associations were found between exposure to air pollution and any of the routine semen measures (volume, concentration, total count, percentage motile, or percentage normal morphology considering whole sperm or sperm head shape). Similarly, no significant associations were found between exposures and any of the three selected CASA [computer-aided sperm analysis] measures, or total aneuploidy. . . . associations between exposure to intermittent air pollution and sperm morphology or motility . . . found in the earlier study were not replicated in the present study.

These investigators did report an association between air pollution and *one* of the eleven measures of sperm quality they examined — namely increased DNA fragmentation as determined by a sperm chromatin structure assay (SCSA). The authors hypothesized that this association could be due to reactive metabolites of PAHs reaching the testes and damaging sperm DNA; no mention of sulfur dioxide is made. Regardless, the mean values for DNA fragmentation were, according to the authors, “within the range associated with good fertility potential,” so interpreting this result as a manifestation of reproductive toxicity is unwarranted. And, of course, the multiple comparison problem cannot be dismissed.

Members of this group of investigators (Robbins *et al.*, 1999) also evaluated, by means of fluorescence *in situ* hybridization, the cytogenetics of sperm in semen samples that had been collected and archived from 32 of the previously enrolled, nonsmoking study subjects from Teplice. OEHHA’s evaluation is that, “This study [Robbins *et al.*, 1999] found an association between YY8 disomy and exposure to high air pollution levels.” Not in any reliable sense. The study consists of one semen sample provided by each of 13 men during the summer of 1993, and one semen sample provided by each of 19 *other men* during the winter of 1993. The frequencies of four different types of disomy of the sex chromosomes were tallied; the frequencies of three of these types did not vary between the summer sperm and the winter sperm; the frequencies of the fourth type did vary according to season, such that it was more common in the winter samples. Obviously, the results *per se* mean very little, and have not been replicated. Different men provided different samples; no adjustments for any of the many potentially relevant factors (such as ambient temperature and nutrition) that vary between summer and winter were made; the study has not been replicated; and, in any event, interpreting the study as if it provides evidence that ambient SO₂ damages sex chromosomes is an over interpretation, to say the least.

“Related male reproductive studies”

The Background Document claims that three “related male reproductive studies” (Nordenson *et al.*, 1980; Meng *et al.*, 1990; and Yadav *et al.*, 1996) support the conclusion that SO₂ is a reproductive toxin. In fact, none of these is a reproductive study. Each study instead tallies chromosomal abnormalities in peripheral blood lymphocytes (not sperm) in workers exposed to SO₂. OEHHA fails to note the complete lack of evidence that SO₂ adversely affects the chromatin structure of sperm. In contrast to SO₂, substances that are genuinely toxic to sperm, such as ionizing radiation, alkylating cancer chemotherapeutic agents, lead, and cadmium, have been shown to induce DNA strand breaks and other abnormalities in sperm from exposed laboratory mice, rats, and humans (reviewed in Delbes *et al.*, 2010). There is no basis for

assuming, as OEHHA apparently does, that effects seen in chromosomes in circulating lymphocytes bear any relationship to reproductive health, however defined. Indeed, although several of the workers studied in the papers cited by OEHHA were exposed to high concentrations of SO₂, *no evidence of toxicity to reproduction was reported*. Again, OEHHA fails to note this.

Even on the subject of whether occupational exposures to SO₂ are associated with damage to circulating lymphocytes, the evidence is less than persuasive. For example, with regard to the first of these studies, OEHHA writes, without further qualification, “In Nordenson *et al.* (1980), workers at a sulfite pulp factory in Sweden exposed to SO₂ had significantly increased frequencies of chromosomal aberrations in lymphocytes.” This is true, but OEHHA fails to mention the study’s limitations. For example, there were only seven study subjects. These seven were compared with fifteen controls who were matched for sex (all were males) but not for age or other potentially important characteristics. Also, oddly, men who smoked were found to have significantly *fewer* chromosomal aberrations than nonsmoking men, which casts doubt on the reliability of the assay to identify genotoxic exposures. Indeed, the authors themselves recognize that their data do little more than raise the question contained in the paper’s title, to wit, “Is exposure to sulphur dioxide clastogenic?”

OEHHA fails to cite or discuss a paper published two years later, by Sorsa and colleagues (1982), which asked the same question, but found opposite answers. This latter study was also small, comprising only eight study subjects and eight controls. Regardless, the sulfur dioxide-exposed workers were found to *not* have more chromosomal damage than controls; and, in this study, smokers *were* found to have more chromosomal damage than nonsmokers, which is what one would expect.

The second study cited by OEHHA, namely Meng *et al.* (1990), involves workers in a sulfuric acid factory. OEHHA writes, “In a study conducted in China, Meng *et al.* (1990) observed similar findings of significantly increased frequencies of chromosomal-type and chromatid-type aberrations in lymphocytes of workers in a sulfuric acid factory.” But OEHHA fails to note that the authors also report that the smokers in their study were *not* found to have increased frequencies of these aberrations compared with nonsmokers, which result in and of itself renders the study results questionable. Moreover, the controls selected for the study were people working at a university, who were not likely to be similar to the study subjects in all relevant characteristics but for sulfuric acid-working. Indeed, the workers at issue were exposed not only to sulfur dioxide as a gas, but to (apparently) very high concentrations of sulfuric acid mist, which, quite unlike sulfur dioxide, is an established human carcinogen. Depending on the method by which the sulfuric acid was manufactured at this factory, occupational exposures to catalysts and other chemicals may have been involved, but were not quantified.

The third study cited by OEHHA, Yadav and Kaushik (1996), focused on workers at an Indian fertilizer manufacturing facility. In this study, smokers and alcoholics *were* found to have higher, and exposure-duration-dependent, rates of chromosomal damage (again, in their circulating lymphocytes, not their sperm) than nonsmokers and/or nondrinkers. OEHHA notes, correctly, that the workers’ lymphocytes showed increased levels of chromosomal aberrations

(relative to controls, who were matched for some but not all potentially relevant factors). But OEHHA fails to quote from Yadav and Kaushik themselves, who write, “There have been conflicting reports on the genotoxic effects of SO₂ on human beings;” and who cite a study by Mandal (1990; not cited in the Background Document), also in fertilizer factory workers, which found no increases in chromosomal abnormalities. More generally, the use of sulfuric acid to manufacture fertilizer from pulverized phosphate rock obviously results in worker exposures to substances beyond just sulfuric acid mist (and SO₂). Thus, even were these workers to be at excess risk of reproductive toxicity (for which, again, these studies provide zero support), one could not reliably conclude that SO₂ was to blame. At a minimum, one would need to account for particle emissions and, depending on the specific ores in the pulverized rock, for heavy metals such as cadmium, mercury, and lead.

With regard to all three of these papers, OEHHA takes no note of the complexities of conducting and properly interpreting studies of genotoxic biomarkers in circulating lymphocytes. So doing, it fails to recognize that factors such as a subjects’ and controls’ intakes of the nutrients vitamin B₁₂ and folate (not measured in any of the studies at issue), and possibly their rates and extents of alcohol ingestion, must be taken into account in both analyzing and interpreting the data (Battershill *et al.*, 2008).

Studies in Rodents

Next, OEHHA refers to four studies in rodents — Meng *et al.* 2003, 2004, & 2007; and Zhang *et al.*, 2005 — that it terms “male animal reproductive toxicology studies.” The phrase is misleading: these are not “reproductive toxicology studies,” since none examined gonadal function, spermatogenesis, post-testicular processes, fecundity, or any related behavior or outcome. The four studies involved exposing laboratory mice to exceptionally large (on the order of 11,000 to 43,000 parts per billion) and frankly toxic concentrations of sulfur dioxide. The exposures resulted in morphological changes in cell and tissue structure in *all* examined organs — lungs, livers, spleens, kidneys, brains, hearts, and testis. None of the changes indicated that the testis was a specific target-organ. The observed changes were consistent with extensive oxidative damage throughout the body — which is what one would expect, since the animals’ organs were essentially being bleached. Biochemical changes reflective of defenses against oxidative damage were also noted; again, these are what one would expect in response to such extreme exposures. Notably, all four papers are by the same group of investigators, and the results that they report have not been replicated by others.

Remarkably, the Background Document fails to point out that four studies of sulfite in laboratory rats reported no adverse effects on reproduction. The most important of these is a study by Til and colleagues (1972), in which five dose-levels of sulfite were administered over three generations of rats. Sulfite was found to have no adverse effect on fertility or mean number of pups per litter. These indices are, of course, direct measures of the reproductive toxic potential of a chemical and/or its metabolites, as opposed to the (at most) indirect indices reported in the studies reviewed by OEHHA. Three similar, earlier studies of sulfite in laboratory rats (Lockett & Natoff, 1960; Cluzan *et al.*, 1965; and Lanteaume *et al.*, 1965) were apparently also negative. As summarized in a major review by Gunnison, 1981:



In all of these studies sulphite was . . . [administered at] daily intakes of between 0.05 and 2 mmol/kg. Usually several generations of rats were treated and all experiments lasted for at least 1 yr. In these studies, investigators measured the effect of sulphite on such parameters as fertility, the general health and growth rates of offspring . . . and histological appearance of major organs and tumour incidence. In general, no consistent trends attributable to sulphite exposure were apparent in any of these parameters.

OEHHA's failure to take note of these studies or of the review article (Gunnison, 1981) in which they are discussed is disquieting.⁷

Preterm birth

The Background Document seems to suggest that sulfur dioxide is reliably associated with preterm birth.⁸ In particular, the Document cites ten epidemiologic studies examining preterm birth (variably defined); reports that eight of these ten “reported significant findings;” and asserts that many of the studies’ limitations would lead to underestimates of “the true effects of air pollution on preterm birth.” We note that here the Background Document (correctly) implies that the studies cited examine “air pollution” whereas subsequently this is taken to be synonymous with “sulfur dioxide.” In fact, the designs of all of these studies are such that none can separate the possible effects of air pollution from those of sulfur dioxide alone.

There are many problems here, not least of which is the Document’s failure to define what it means by preterm birth. The classical definition refers to births occurring 3 or more weeks prior to term (that is, less than 37 weeks of gestation). But the primary study on which the Background Document relies (Xu *et al.*, 1995; discussed below), which also is the first chronologically, finds that 100 µg/m³ increases in ambient concentrations of SO₂ are associated with a “reduced length of gestation” of only 12.6 hours! Obviously, such an effect *per se* would be of no clinical significance; moreover, as explained below, it is but an artifact.

All of the studies discussed in this section of the Background Document are exploratory data analyses. None state refutable primary hypothesis, and none distinguish post-hoc findings. All

⁷ The absence of this and other important and relevant studies may indicate an underlying problem in the methodology used by the authors of the Background Document. Indeed, this methodology is never presented, but should be. What specific bibliographic search strategies were employed? Were papers limited to those in English-language publications? (Apparently not, in that one, but only one, paper written in Chinese is cited. What about all of the other, arguably relevant, foreign-language publications?) What specific key words and combinations thereof were used? Once important papers were identified and read, were other papers, such as those cited therein, gathered and reviewed? Were important papers read by two or more of the Document authors? What use, if any, was made of the many expert reviews of this topic by other agencies and expert groups? Why, for a chemical as widely-studied and exhaustively reviewed as sulfur dioxide, did the Background Document offer no analysis of the relevant reasoning and conclusions offered by others?

⁸ Oddly, the Background Document classifies preterm birth as a developmental problem, but this is incorrect. Of course, preterm birth can *lead* to (postnatal) developmental problems — this is the very reason that it is dangerous — but not the other way around.

suffer from large numbers of comparisons, which multiplicity is not taken into account in evaluation of statistical significance. More importantly, this problem is ignored entirely in the Background Document, which repeatedly conflates all apparently positive findings from all studies as if they provided consistent, indeed overwhelming, evidence of an SO₂ effect. In fact, they do not.

For example, Brauer *et al.* (2008) used three measures of exposure (nearest monitor, inverse distance weighted, land-use regression), then calculated mean exposures over the first and last 30 days, the first and last 3 months, and the full duration of pregnancy, a total of 15 exposure measures (we ignore here the additional examination of road proximity included in the paper). Moreover, the paper was also examining seven other pollutants (NO, NO₂, CO, PM_{2.5}, BC, PM₁₀, and O₃) so that in total $8 \times 15 = 120$ exposure measures were examined. Further, 5 outcome measures were examined (SGA, LBW, Gestation < 37 wk, Gestation < 35 wk, Gestation < 30 wk). Thus at least 600 statistical tests were performed (actually 1200, since both crude and adjusted odds ratios were presented)! It would be remarkable to *fail* to find some “statistically significant” results in such a large number of tests; although as it turned out, *no association between any outcome measure and any SO₂ exposure measure was considered by the authors to be significant*.

Brauer *et al.* (2008) is the last-but-one paper in chronological order of publication of the ten papers cited by the Background Document (the first of which, as noted above, is Xu *et al.*, 1995, on which the Background Document relies so heavily), yet clearly investigators in the field are still searching for a specific refutable hypothesis to test. Indeed, every paper obtained concentration data sufficient to calculate all the time-averages of concentration used by Brauer *et al.* (2008) (and more besides). It is unclear whether measures other than those explicitly reported were evaluated in the earlier papers, and not reported because they provided no “statistically significant” results in the preliminary data analyses that the earlier papers provide.

The inconsistencies in results across studies was noted by the authors of the most recent of these series of papers (Darrow *et al.*, 2009), but not by OEHHA. Darrow and colleagues note that they have “focused on” three exposure averaging periods based on previous results and “current hypotheses about biologic mechanisms,” but even this study can at best be described as an exploratory data analysis; and readers are provided no indication as to whether *other* exposure averaging periods were also examined but not reported. Darrow *et al.* (2009), apparently applied adequate control for seasonal confounding (see below), and, so doing, found *no discernible effect of SO₂ on preterm birth*. Also shown are results for all 78 analyses that were apparently performed, with three that are marginally “significant” for a deleterious effect subsequently discussed extensively; whereas the one result that shows marginal significance of a protective effect is not further mentioned. But such findings are entirely expected for 78 statistically independent analyses; and there are insufficient data provided to evaluate any dependencies. A probability plot of the distribution of logarithms of relative risk (Figure A), normalized by standard deviations estimated from the confidence intervals, shows nothing suggesting an actual effect; the 78 values obtained are indistinguishable from a sample from the normal distribution expected under a null hypothesis of no effect [$p = 0.2$, Shapiro Wilk test for normality; $p > 0.08$ Kuiper or Kolmogorov-Smirnov test comparing with the expected (0,1) normal distribution].

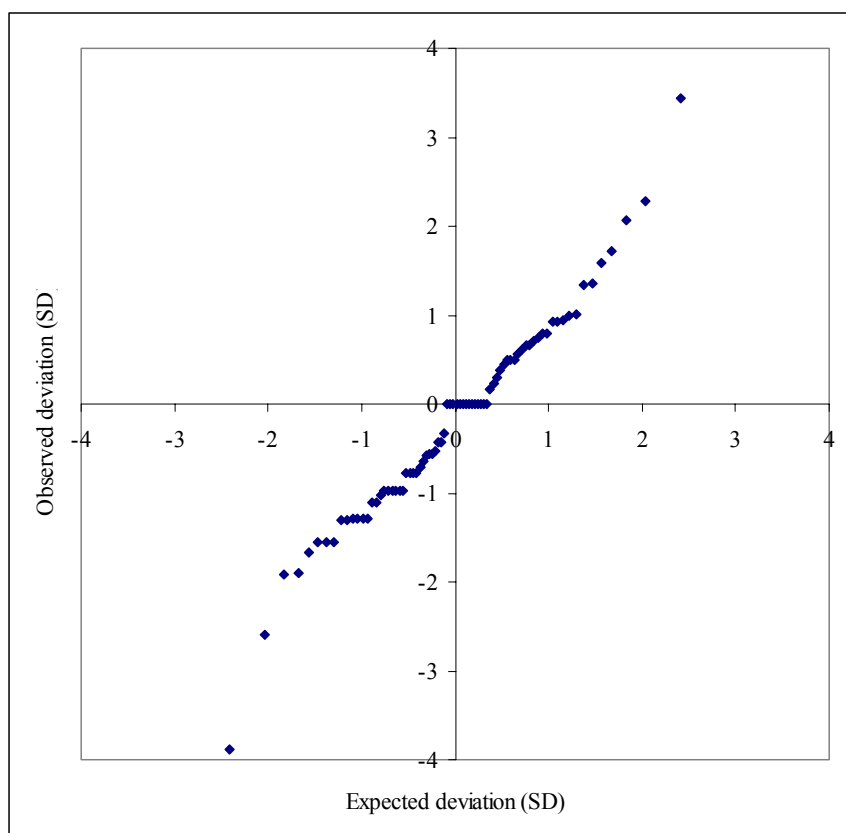


Figure A Probability plot for the 78 results given by Darrow *et al.* (2009). The discreteness and the apparent higher significance on this plot of negative findings (left lower quadrant) is due to the limited precision provided.

The ten studies cited have in common the use of a “time-series” approach, extolled in the Background Document’s Integrative Evaluation (Section E.3, in the context of Xu *et al.*, 1995) as having various advantages. However, these studies are not true time-series studies, and do not have the usual advantages of such studies. What is being examined in these studies is a one-time event that definitely occurs in a narrow time window — the population examined is not equally at risk for that event at all the times examined, and the event (birth) certainly occurs for every member of the populations examined (since that is how the populations are selected). Thus there is no way in which each member of the population examined serves as her own control for the event of interest, the principal advantage cited for conventional time-series analyses, except perhaps in the few days prior to birth.

Further, it is found in multiple populations (e.g. Xu *et al.*, 1995; Darrow *et al.*, 2009; Liu *et al.*, 2003) that there is seasonality (variation on a monthly time-scale) in pre-term birth (either in probability of pre-term birth, or in length of gestation), and long-term trends (with time-scales of years) are also present (e.g. Liu *et al.*, 2003). As noted above, and as is well known, SO₂ concentrations also vary with season and have long-term trends (e.g. Xu *et al.*, 1995; Liu *et al.*, 2003). Importantly, the advantages of time-series analyses can be realized only if both such

effects are adequately compensated in the analysis, so that spurious correlations due merely to unrelated seasonal and long-term trends in both “effect” and potential “cause” are removed.

Of the ten studies cited, only one, Darrow *et al.* (2009) clearly took adequate account of seasonality; this was by using splines with monthly knots. Jiang *et al.* (2007) may or may not have used an adequate approach (again using splines): given the limited description, readers cannot tell. The other eight studies either took no account for seasonality (Sagiv *et al.*, 2005⁹; Mohorovic, 2004), or used only monthly (Brauer *et al.*, 2008; Liu *et al.*, 2003, Bobak, 2000) or “seasonal” control (Jaludin *et al.*, 2007; Leem *et al.*, 2006; Xu *et al.*, 1995), the last generally corresponding to four seasons per year, but occasionally two seasons per year (Xu *et al.*, 1995, Table 3).

The inadequacy of “seasonal” control using four seasons only is readily demonstrated using the monthly average data of Xu *et al.* (1995, their Figure 2; see our Figure B, below). The seasonal anti-correlation between sulfur dioxide concentration and gestation period is evident. Xu *et al.* (1995) used multiple linear regression with control for four seasons (or two seasons, in some analyses) in an attempt to remove this anti-correlation. Figure C, below, shows the effect of such control on the correlation between sulfur dioxide concentration and gestation period as a function of a variable offset between SO₂ concentration and birth date.¹⁰ The coefficients obtained (right-hand scale in Figure C) are similar to those obtained by Xu *et al.* (1995; Table 2), strongly suggesting that the results of Xu *et al.* (1995) are due simply to uncompensated residual seasonal correlations, and not to sulfur dioxide.

⁹ The lack of control is not obvious in the study description. Sagiv *et al.* (2005) included the seasonal effect on number of births, but did not account for the potential seasonal effect on the probability of pre-term birth.

¹⁰ To derive Figure C we have assumed that the SO₂ concentration has the same monthly average values in the previous year, and linearly interpolated between monthly average values to estimate monthly averages offset by fractions of a month.

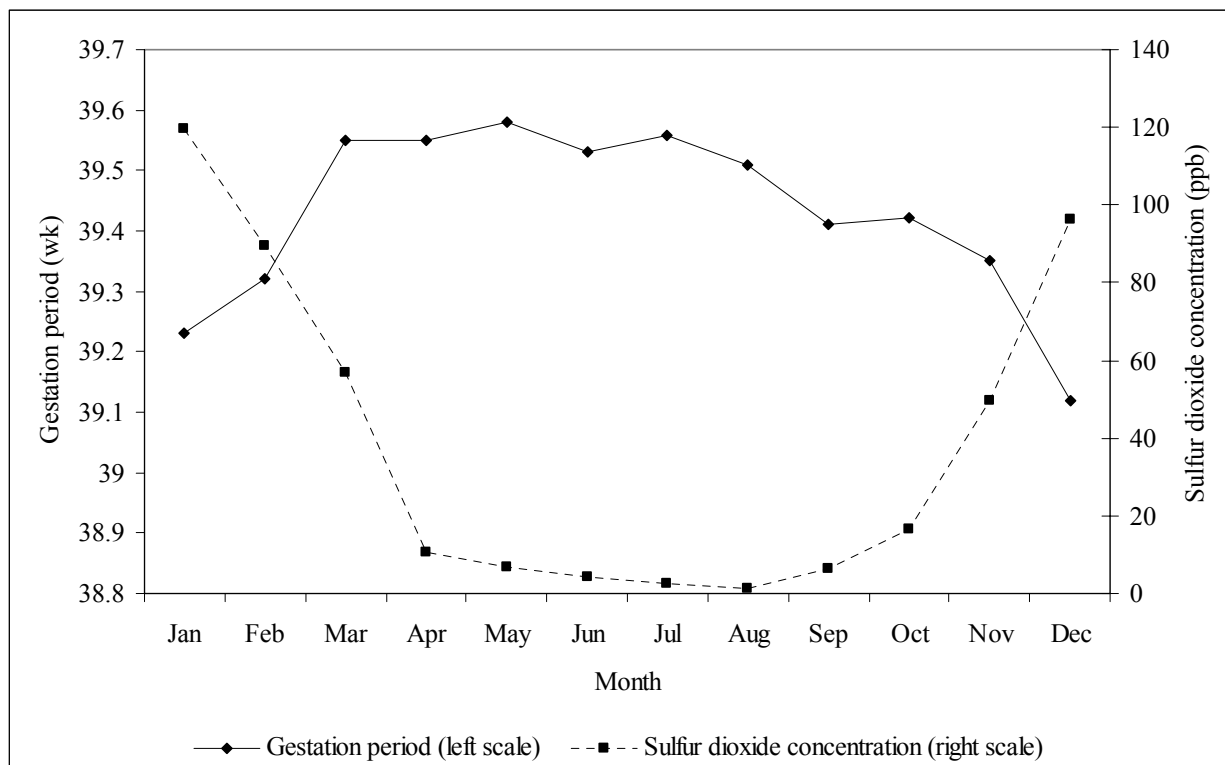


Figure B Monthly average gestation period and sulfur dioxide concentration (Xu *et al.*, 1995).

We have considered the possibility that the results shown in Figure C correspond to the *desired* evaluation of the correlation of small fluctuations of gestational age and SO₂ concentrations around the seasonal trends. Figure D evaluates this possibility: it shows the same analysis using a pure sine wave in place of the SO₂ concentration, with a phase and peak-to-peak amplitude roughly equal to those of the SO₂ concentration. Evidently, since in this case there are no non-seasonal fluctuations with which to correlate, the results shown for the pure sine wave are entirely due to residual seasonal correlation not eliminated by the “seasonal” control. Similar residual seasonal correlation will necessarily contaminate any analysis using just four (or fewer) seasons for “seasonal” control.

The p-values are shown in Figure C and Figure D in order to illustrate the low values attainable even with so few data (12 data points, 7 degrees of freedom since the model includes a coefficient and the four seasonal terms). In Xu *et al.* (1995), individual data are included in the model for gestation period and SO₂ concentration, introducing more variability. However, the large number of individuals averages out the effect of individual variation in gestation period. Xu *et al.* (1995) use average SO₂ concentrations, finding their maximum “effect” with an averaging time of 7 or 8 days, corresponding to ½ of each month’s data (only 14 per month of SO₂ data were evaluated); so there also is little additional (within-month) variability from the concentration data; and again the effect of a large number of individuals is to average such variability. Any way one looks at it, the observations of Xu *et al.* (1995) are entirely consistent with residual confounding.

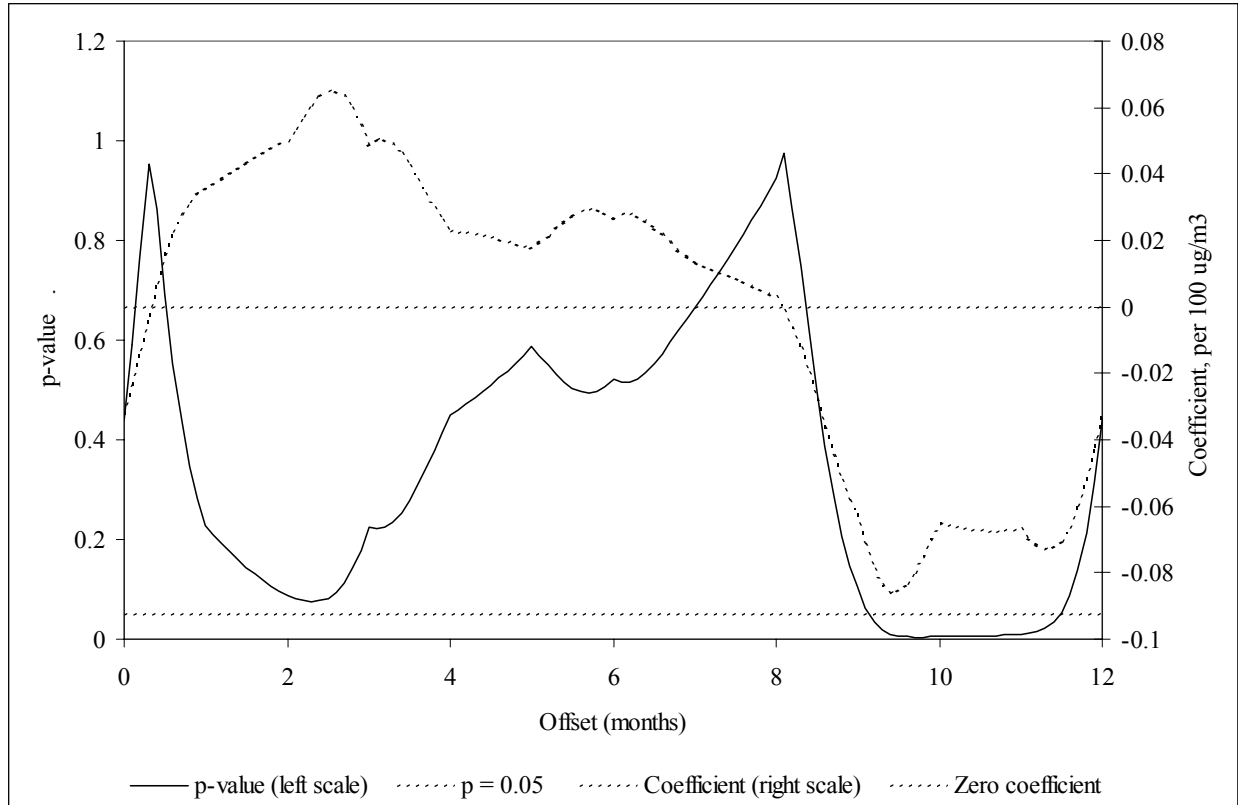


Figure C Correlation between offset SO₂ concentration and gestational age, controlling for season.

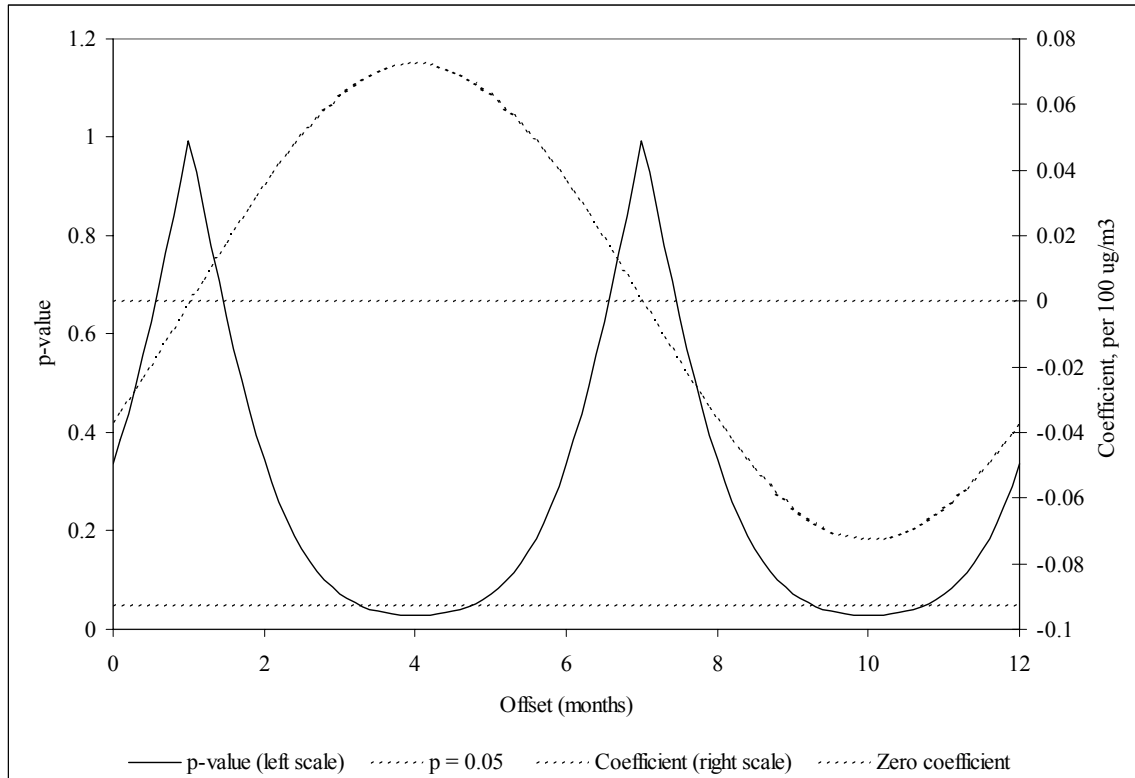


Figure D As for Figure C, using a pure sine wave shape for the SO₂ concentration.

The effect of averaging concentrations with strong annual periodicity over periods ranging from 1 week to 9 months is primarily to introduce an offset in the annual periodic variation, and to reduce the amplitude. The offset is equal to $\frac{1}{2}$ the averaging time, but the amplitude is reduced by only about 1% for a 1 month averaging, 5% for 2 months, 10% for 3 months, 36% for 6 months, and 70% for 9 months. Higher frequency components of the variation are averaged more rapidly, so the averaged concentrations more closely resemble a pure sine wave. The major variation in averaged concentrations thus remains the annual periodic variation, and control sufficient to remove this major variation is essential to prevent confounding by annual periodic variations in birth-related effects that are unrelated to air pollution (with the particular averaging period turning out “significant” selected primarily by the required phase shift to match this unrelated periodic variation).

There are insufficient data provided to evaluate the effect of residual confounding for the other studies that used seasonal or monthly control, or no control for trends at all. Examination of the results reported as “significant” indicates, however, that all are entirely consistent with either random effect (a low p-value by chance among the multiple comparisons) or residual confounding due to seasonality.

One approach is to compare the results presented in different papers for the apparent size of the effect seen, and compare this apparent size with the concentration variation claimed to cause it. We here use the interquartile range (IQR; Table A), estimating it where it is not given (for Sagiv

et al., 2005, we assume a lognormal distribution; for Xu et al, 1995, we interpolate into the monthly averages).

Study	IQR, ppb
Brauer, 2008	1.1
Brauer, 2008	1.3
Jalaludin, 2007	2.2
Liu, 2003	3.5
Sagiv, 2005	6.0
Sagiv, 2005	4.2
Leem, 2006	28.2
Darrow, 2009	6.0
Bobak, 2000	14.5
Jiang, 2007	13.6
Xu, 1995	57.2

Table A Reported or estimated IQRs

Figure E shows a plot of reported excess RR for preterm birth versus IQR for all reported exposure metrics involving averaging over periods from 1 week to 9 months for nine of the ten cited reports (Mohorovic, 2004 is omitted because that paper cannot be interpreted without making unverifiable assumptions). As can be seen, the reported excess RRs are essentially independent of the IQR — which is precisely the effect expected if significant results are the effect either of random effects or residual correlations with unrelated seasonal variations. We note also that the results of Darrow *et al.* (2009) at an IQR of 6 ppb represent the results of the only study with adequate control for seasonal confounding.

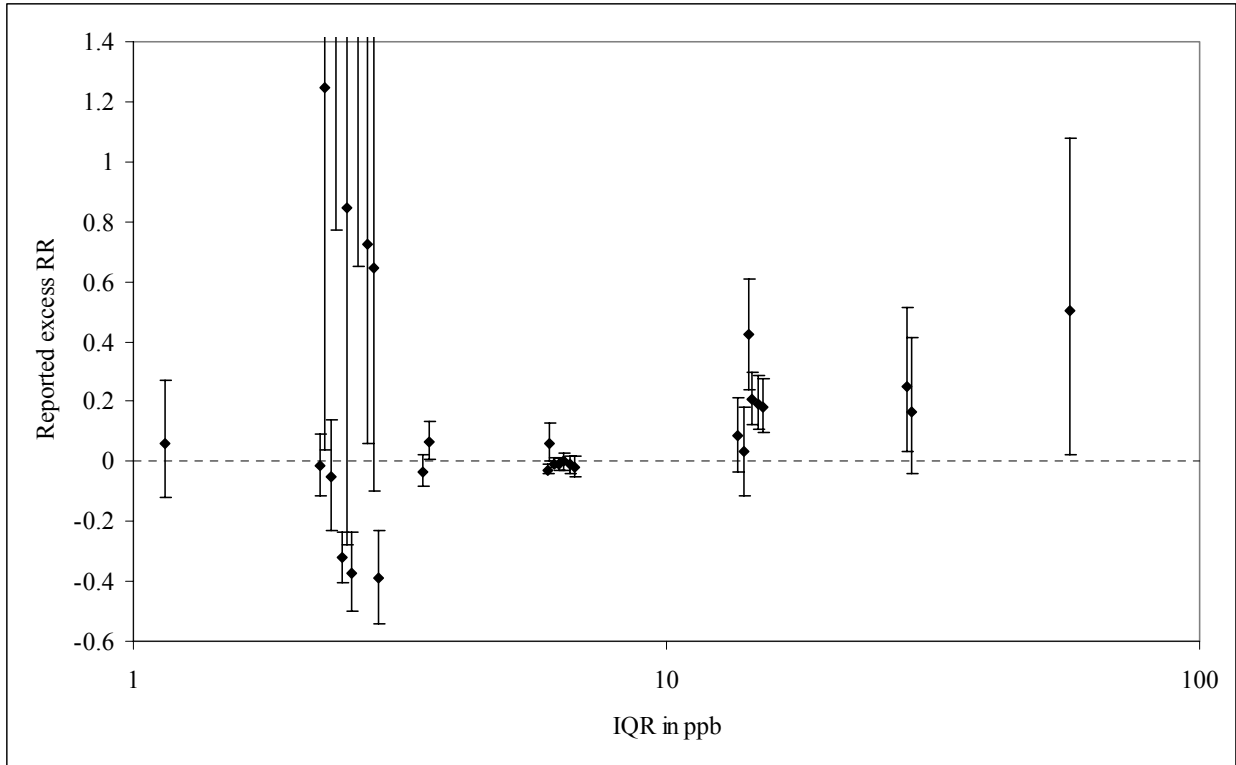


Figure E Incremental RR for preterm birth versus IQR; all reported values with averaged exposures, omitting three very large, imprecise, reported RR estimates (2.94 [0.65,7.04]; 2.98 [0.77,6.81]; 12.3 [7.5,19.4]) at 2.2 ppb IQR from Jalaludin *et al.* (2007). IQRs are slightly offset to show multiple values within a study.

Fetal growth retardation

The Background Document reports that “. . . several [five, apparently] well-conducted studies suggest that SO₂ [in ambient air] is associated with reduced birthweight, independent of co-pollutants.” The Document summarizes these studies as follows (p. 67):

Five of the six studies that clearly assessed exposure both temporally and spatially found SO₂ exposure was associated with poorer fetal growth (Yang *et al.*, 2003; Lin *et al.*, 2004b; Dugandzic *et al.*, 2006; Williams *et al.*, 2007; Hansen *et al.*, 2008). The exception was the study by Brauer *et al.* (2008), which had a mean SO₂ concentration at the limit of detection. Studies that assessed only temporal or spatial variation in SO₂ exposure were less likely to find that SO₂ exposure was associated with restricted fetal growth.

In what follows, we discuss these studies to determine whether and to what extent they are reliable, provide consistent results, and are likely to indicate causal associations.

Yang *et al.* (2003) and Williams *et al.* (2007) hypothesized linear models relating reduction in birth weight to various covariates and to average 1st trimester exposure to SO₂ (Yang *et al.* also examined the 2nd and 3rd trimester, finding no effect). While both sets of authors claimed significant effects, the effect sizes predicted were remarkably disparate: Yang *et al.* (2003) reported 1.36 (95% CI = 0.49, 2.22) grams reduction in birth weight per ppb of SO₂, while Williams *et al.* (2007) reported 55.2 (35.1, 75.4) grams per ppb. Such disparities might well be expected, since Yang *et al.* (2003) adjusted for temporal trends with just a “seasonal” effect (with just two seasons, summer and winter), and Williams *et al.* (2007) made no adjustment at all for temporal trends (nor even for gestational age!). Since the reported associations were quite likely to have been confounded by temporal trends, these associations are unreliable.

Three other studies examined the relative risks for low birth weight (<2,500 g at term). Lin *et al.* (2004b) reported increased relative risks for SO₂ averages over the entire pregnancy and in the third trimester, with suggestions of dose-response for each trimester. However, examination of the methodology and SO₂ concentrations provided (Table 1 of their report) shows that SO₂ concentrations were extremely different between Taipei and Kaohsiung, the two cities included in the study, and even had large gradients with geographic location within Kaohsiung, but had relatively smaller variation with season. The RR estimates are thus almost completely confounded by any contrasts between Taipei and Kaohsiung, or more generally by geography, that were not controlled by the covariates (gestational period, gender, birth order, maternal age, maternal education, and season). Since there are large variations in birth weight with SES, it seems unlikely that these covariates provide adequate control for geographic differences other than SO₂ concentration. The seasonal control in this study was also inadequate, since it used just two seasons (warm/cool).

Dugandzic *et al.* (2006) reported borderline statistically significant RR for 1st trimester average SO₂, but applied no control for seasonal effects, rendering the results uninterpretable (and the RRs unadjusted for birth year in this study are confounded by the significant long-term time trends noted by the authors in both SO₂ concentration and probability for pre-term birth).

Next, as the Background Document notes, the Brauer *et al.* (2008) study was entirely null.

Finally, the only study cited that applied potentially adequate temporal and temperature control (for annual cycles and long-term trends) by an extensive differencing approach using 30-day windows was the exploratory data analysis by Hansen *et al.* (2008). Even such 30-day control might be inadequate, however, and the authors did not evaluate the effect of smaller or larger time windows. This study examined certain fetal ultrasound measurements in weeks 13–26 “as direct estimates of growth,” but without demonstrating or citing any relationship of these measurements to fetal birth weight, which was dismissed without justification as being “an indirect (and delayed) measure of growth,” or to any other measure of full-term fetal health (or even full-term fetal ultrasound measures). The authors investigated four ultrasound measurements (BPD, FL, AC, HC), four individual 30-day exposure periods (the first months), seven distances from the nearest monitor (2, 4, 6, 8, 10, 12, and 14 km), and four pollutants (PM₁₀, NO₂, SO₂, O₃), for a total of 448 (!) estimates of relative risk (clearly these are not independent, not least because of the inclusion of populations for shorter distances within those for larger distances). After pre-selecting the 2 km distance based on all 448 RR estimates, Hansen *et al.* (2008) claimed 7 significant results in the 64 (adjusted) RR estimates. Even were these results not pre-selected, such an observation barely conflicts (p=0.04) with the expected chance number even if all the RR estimates were independent. They are not independent, however, because of the correlation between pollutants.

These six studies thus provide little to no reliable evidence as to any associations between sulfur dioxide in ambient air and birthweight or other measures of fetal growth. Examination of the methodologies of all but one indicates failure to control for confounding factors and other biases; and none of the studies attempts to compensate for multiple comparisons.

The Background Document also reviews studies involving prenatal exposures to sulfur dioxide in laboratory animals and effects on fetuses and offspring. Notably, its review is incomplete and potentially misleading.

In particular, the Document notes (page 113): “. . . two studies [Murray *et al.*, 1979; Singh, 1989] examined fetal growth associated with inhaled SO₂ in mice, and both found reductions in birthweight associated with maternal SO₂ exposure.” But, as explained below, the full results of the studies are not as summarized. More importantly, the Background Document fails to mention that the laboratory animal exposures at issue — 25 ppm of SO₂ and higher — are larger than the exposures demonstrated by Meng and Liu (2007) to cause cellular and tissue damage in mice in all major organs. It should go without saying that dams with such pervasive damage may also be compromised with regard to their abilities to produce normal pups; and that this says nothing about reproduction and development at levels lower than those causing maternal toxicity.

The first of these studies, Murray *et al.* (1979), (i) exposed both mice and rabbits to high concentrations (25 ppm and 70 ppm, respectively) of SO₂; (ii) deprived the pregnant animals of food and water during each 7 hours/day exposure-period; (iii) induced, according to the investigators, “slight toxicity” in the dams of both species; (iv) found no reductions in the weight

of rabbit fetuses; and (v) found reductions in the weight of mouse fetuses only from maternal exposures to high concentrations of both carbon monoxide and SO₂, but not to SO₂ alone.

The second of these studies, Singh (1989), involved exposing pregnant mice to 32 ppm or 65 ppm SO₂. Mean pup weight was significantly reduced in the 65 ppm-exposed group but not in the 32 ppm-exposed group. The authors noted no “visible signs of maternal toxicity,” but, as mentioned above, histopathological examinations (which were not performed) would have found substantial damage.

The Background Document fails to note that a third, similarly designed study in mice (Petruzzi *et al.*, 1996) found no reductions in birth weight — presumably because the highest concentration tested was “only” 30 ppm.

Moreover, the Background Document fails to point out that four studies of sulfite in laboratory rats found no adverse effects on fetal growth or development. These negative studies (Til *et al.*, 1972; Lockett & Natoff, 1960; Cluzan *et al.*, 1965; and Lanteaume *et al.*, 1965) were cited and summarized by Gunnison, 1981— an important review on sulfite toxicity that, as noted above, should have been identified in literature searches performed as part of OEHHA’s work.

Focusing only on the two “positive” studies, the Background Document concludes (p. 113), “Although the epidemiologic evidence has limitations . . . the two studies in mice are consistent with the association between maternal inhaled SO₂ and fetal growth restriction in humans.” This is not a reliable interpretation. Contrast it with the balanced analysis of this literature offered by U.S. EPA (2008; page 3-63):

In summary, epidemiologic studies on birth outcomes have observed positive associations between SO₂ exposure and low birth weight; however, toxicological studies provide very little biological plausibility for reproductive outcomes related to SO₂ exposure. The inconsistent results across trimesters of pregnancy and the lack of evidence regarding confounding by copollutants further limit the interpretation of these studies.

Additional comments on Xu *et al.* (1995)

As noted above, the Background Document places considerable weight on the study of Xu *et al.* (1995). This is the first of the cited epidemiological studies and suffers from the defects noted above. Moreover, several of the statements made in the Background Document on page 108 and in the more detailed discussion on pages 223–226 are incorrect.

On page 108, the claim is made that Xu *et al.* (1995) “control for TSP, an important co-pollutant”. On page 225 it is stated “However, when SO₂ and TSP were included in the model simultaneously, the estimated effects were reduced by 32% for SO₂ (to 0.051 week, or 8.4 hours) and 36% for TSP, but remained statistically significant.” In fact, the original authors do not claim that these coefficients, either individually in the joint model, or both together in that model, are statistically significant. What is stated is “When TSP and SO₂ were included in the model simultaneously, the estimated effects were reduced by 32% for SO₂, ($\beta = -0.051$, standard error [SE] = 0.028) and by 36% for TSPs ($\beta = -0.027$, standard error [SE] = 0.015).” The cited standard errors indicate p-values >0.06 for the coefficients taken individually (two sided tests; all p-values given in the paper are, correctly, two-sided), hence not “statistically significant” according to the usual standards. The authors make no statement about the p-value for the joint model (although we can surmise that the joint effect of the pollutants was likely statistically significant).

The Background Document on page 226 follows the authors in claiming that for active and passive smoking prevalence “these factors would be expected to be independent of daily air pollution levels and in this study would not affect the association between air pollution and preterm births.” However, while that statement is true about prevalence of exposure to active or passive smoking, it does not follow that ETS could not confound any relationship between air pollution and gestational age at birth, as is implied. The authors note that “Few homes in this area are air conditioned, and windows are kept open most of the time from May to September.” It follows that (indoor) ETS concentrations are likely to be highly correlated with outdoor air concentrations of SO₂ and TSP, hence to potentially confound the apparent association between gestational age and air pollution.

The Background Document claims (page 108) “important information such as gestational age was collected prospectively.” But how could gestational age (or even conception date) be determined prospectively?! Possibly what is meant is that the estimated conception date was obtained prior to the birth; however, the methods used by local obstetricians (who apparently obtained these data) to debrief mothers-to-be of the date of “the first day of their last menstrual cycle” are not discussed. This is important, because a very small time-varying bias in this estimate could easily invalidate the estimated association with air pollutants if it had an annual cyclical component (see above).

The Background Document claims (page 108) “advantages of time series analyses which are unlikely to be affected by differences in population demographics.” This may be true in general, but it is not true in this case. Here, any annual cyclical time-varying bias could affect associations with air pollutants, and this observational study does not eliminate such annual

cyclical biases in demographics (*e.g.* cyclical changes in personal habits associated with conception, such as marriage rates or attempts at conception and the environmental conditions under which they are attempted). The advantages of time series analyses are also largely removed by the approach taken in Xu *et al.* (1995), in which they average air pollution concentrations over the days preceding birth. By such averaging they remove the variations that are exactly those required to demonstrate anything beyond the correlations with annual cyclical variations (which are all that are demonstrated in Figure 5 on page 225).

The Background Document does not discuss the limitations of the Xu *et al.* (1995) study. The primary limitation is that this study will necessarily report as associated with gestational age and rate of preterm birth any variables that have an annual cyclical variation, since both gestational age and preterm birth rates have such an annual cyclical variation (Table 1¹¹ and Figure 2 of Xu *et al.*, 1995). *Indeed, the results reported by Xu et al. (1995) are precisely what would be expected if gestational age and preterm birth are entirely causally unconnected with air pollution.*

The Background Document reproduces in Figure 6 on page 226 the gestational age distributions claimed by Xu *et al.* (1995). Those distributions, however, are not accurately drawn, in that they quantitatively contradict other information given in the same paper, and may be misleading in their failure to show uncertainties. The three curves shown in Figure 6 all have more than 7% cumulative weight at <37 weeks, contradicting the statement of 3.2% overall premature births (Table 1 of Xu *et al.*, 1995). The standard deviations of the curves also are 1.7 to 1.8 weeks, exceeding the standard deviations given in Table 1. The cumulative total frequency for the “moderate polluted days” is approximately 110%, which is, of course, not possible. Further, the total counts in the extreme left tail of the curves are relatively small (approximately 3 to 6 count per 5-day frequency bin used to construct these curves¹²), so that the uncertainty ranges in the left tails overlap one another considerably.

All told, Xu *et al.* (1995) provide no reliable evidence of an effect even of air pollution — let alone of sulfur dioxide *per se* — on preterm birth.

¹¹ The precise definition of “season” in Table 1 is never given, although “summer” and “winter” in Table 1 must be distinct from the same terms as used in Table 3 (since the latter covers the whole year). Digitizing the monthly average gestational age in Figure 2 and comparing with seasonal averages in Table 1 suggests that winter was there defined to be December through February, whereas winter in Table 3 was November through April.

¹² That 5-day bins were used follows from the absolute values of the percentage frequencies given. Other bin sizes imply substantially larger deviations from 100% total.

Discussion and conclusion

We have reviewed the evidence and found that it does not indicate that sulfur dioxide causes reproductive or developmental toxicity. Our conclusion comports with that reached by others. These include: (i) U.S. EPA — which agency, in 2008, published its exhaustive and widely vetted *Integrated Science Assessment for Oxides of Sulfur*; (ii) the agencies or groups, such as OSHA, Cal/OSHA, NIOSH, and ACGIH, responsible for protecting workers exposed to potentially quite high concentrations of SO₂; (iii) the FDA, which is responsible for limiting the amount of sulfite (to which absorbed SO₂ immediately converts) added to foods and beverages; and (iv) to our knowledge, all analogous agencies in other countries.

The Background Document is, of course, the most recent of reviews, and it is conceivable that the literature or analysis that it presents — in support of its conclusion that SO₂ causes “decreased fecundability” and preterm birth — is new and compelling. But this is not the case. The two studies upon which the Document authors rely most heavily — Dejmek *et al.* (2000) and Xu *et al.* (1995) — were, as just indicated, published 11 and 16 years ago, respectively, and have been reviewed by many others. Unfortunately, the Background Document’s analysis of these and essentially all other cited studies is superficial and incomplete.

The Background Document lacks any coherent scientific methodology for conducting its assessment. The document “does not contain sufficient documentation on methods and criteria for identifying evidence from epidemiologic and experimental studies, for critically evaluating individual studies, for assessing the weight of evidence, and for selecting studies”¹³ that can be relied upon.

Remarkably, the Background Document’s assimilation of the literature often comes down to statements such as, “Thus, all three studies reported associations between air pollution and semen quality,” and, “Eight of the ten epidemiological studies examining the association between SO₂ exposure and preterm births reported significant findings,” but such statements obscure far more than they reveal, and are hardly the way that cause-and-effect are established. *Of course* virtually all of the papers cited in the Background Document present *some* positive result: this is why they were published in the first place, and were found by the Document authors according to their apparent search strategy. The keys are to determine not only whether each result reported in each paper is reliable *per se*, but whether the *specific* results reported by one group of investigators have been replicated by others, have not been reliably refuted by others — and, in the final analysis, whether the evidence *as a whole* is cohesive and indicative of cause-and-effect.

Unfortunately, although the Document is entitled, “Evidence *on* the Developmental and Reproductive Toxicity of Sulfur Dioxide,” it reads as if its intent were to present evidence *in favor of* such toxicity, rather than evidence both for and against. Readers are repeatedly told why apparently positive results make sense (or are even underestimates of true effects), and why apparently negative results may well be false negatives. This is especially disquieting in that the authors of the very papers on which OEHHA relies are often much more cautious and nuanced in

¹³ This quotation is from the recent NAS (2011) review of U.S. EPA’s IRIS draft formaldehyde document, and applies with equal strength to the Background Document.

their presentations. And, as we have shown above, many problems both within and across papers appear once one digs just a bit deeper.

The Background Document's failure to evaluate SO₂ in the context of endogenous production, and to take account of the extensive studies available on sulfite, is also disturbing. In contrast, the expert bodies on which OEHHA may rely do recognize the importance of this context. U.S. EPA (1982, page 12-2), notes, "[k]nowledge of the chemistry of sulfurous acid and SO₂ is necessary to understand the physiological and toxicological properties of SO₂." U.S. FDA's Select Committee on GRAS Substances evaluates SO₂, sodium sulfite, sodium bisulfite, sodium metabisulfite, and potassium metabisulfite in a common report (U.S. FDA, 1976). Similarly, while NIOSH is primarily concerned with inhalation exposures to SO₂ and adverse effects on the respiratory system, its evaluation of "Absorption, Distribution, Fate and Excretion" (NIOSH, 1974) refers to papers demonstrating the equivalence of sulfite and SO₂ upon absorption. More generally, the importance of endogenous production in evaluations of this nature has been reemphasized by NAS (2011) as an important yardstick with which to measure plausibility.

Using the usual terms, scientific evidence of cause-and-effect may be considered to be (i) inadequate, (ii) limited, or (iii) sufficient. As shown, the evidence that sulfur dioxide compromises reproduction is, at most, barely more than inadequate. Accordingly, as a scientific matter, classifying sulfur dioxide as a "chemical known to the State to cause reproductive toxicity" would be a mistake.

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