Dear Ms. James,

Center for Food Safety appreciates the opportunity to comment on the use of imidacloprid in California, with respect in particular to the ongoing pollution of the state’s well water with this insecticide, on behalf of itself and its more than 100,000 California members. Center for Food Safety (CFS) is a public interest, nonprofit membership organization with offices in Washington, D.C., San Francisco, California, and Portland, Oregon. CFS’s mission is to empower people, support farmers, and protect the earth from the harmful impacts of industrial agriculture. Through groundbreaking legal, scientific, and grassroots action, CFS protects and promotes the public’s right to safe food and the environment.

Imidacloprid contamination of well water in California

Imidacloprid has been found contaminating wells in California at least since 2014. From 2014 to 2017, 14 of 137 wells tested had imidacloprid concentrations ranging from 0.054 to 5.97 ppb (excluding wells with imidacloprid levels below the reporting limit of 0.05 ppb (CA DPR 4/10/18, Appendix 1). The average and median concentrations were 0.612 and 0.122 ppb. Multiple wells have tested positive for imidacloprid every year from 2006 to 2018, with concentrations generally rising over that period (CA DPR 2021, Figure 9).

These are frequent detections and high levels relative to other states. Minnesota did not detect any imidacloprid in 108 public drinking water wells in 2015; while just 2% of 1,103 private drinking water wells turned up positive in 2019, with the maximum level detected 0.17 ppb (MN DoH 2020). In Iowa, limited groundwater testing in the context of a field experiment found at most 0.12 ppb imidacloprid (Hladik et al. 2017). Three percent of 1,313 samples taken from 1,120 private potable wells in Wisconsin tested positive for imidacloprid from 2011 to 2017, with mean and maximum contamination levels of 0.47 and 1.59 ppb, respectively (Bradford et al. 2018, Table 2). Relative to Minnesota and Wisconsin, where imidacloprid use is a small fraction of California’s, California does not appear to be testing many wells.

We would also like to know if any of the imidacloprid-positive wells tested positive for any other neonicotinoid pesticide. More generally, given the common mechanism of neurotoxicity of neonicotinoids, it would make sense for CA DPR to report neonicotinoid
contamination as a group rather than separately. This is standard practice by other states, for instance Wisconsin and New Jersey (Bradford et al. 2018, Millemann et al. 2020).

The California Department of Pesticide Regulation (CA DPR) does not see a health threat from consumption of water contaminated with imidacloprid, even at the maximum detected concentration of 5.97 ppb; and in fact believes that imidacloprid can be safely consumed at concentrations in water up to the reference level of 283 ppb (CA DPR 4/13/21).

Center for Food Safety disagrees with this assessment. As discussed below, the safety threshold (reference level) that CA DPR established for imidacloprid is far too high to protect the health of Californians exposed to this toxic insecticide. While we do not have specific recommendations for restricting any particular agricultural or other uses to reduce exposure at this time, the first step must be to set a truly health-protective exposure threshold, the focus of these comments. This would then provide a concrete basis for deciding whether usage restrictions are needed to bring exposure below that new threshold; if so, then discussion could follow as to which restrictions or prohibitions make the most sense.

**Deficient, unacceptable Bayer study used to set human safety threshold**

CA DPR states that exposure even to 5.97 ppb of imidacloprid in well water does “not pose acute or chronic health risks to humans,” since it falls below the “reference level of 283 ppb” previously established by CA DPR’s Human Health Assessment Branch (CA DPR 4/13/21, p. 2). However, this reference level (i.e. safety threshold) is based on a blatantly deficient study, and is set far too high, as explained below. As a result, imidacloprid at levels detected in California well water may well be hazardous.

The key input to establishing this reference level is an unpublished, developmental neurotoxicity study (DNT) in rats designated “Sheets, 2001” (Ibid., pp. 3, 9). EPA also reviewed this study (EPA undated). While the April 13th, 2021 memorandum does not specify who conducted the study, it was the Agricultural Division of the Bayer Corporation, the primary manufacturer of imidacloprid.


The purpose of the study was to assess potential adverse effects of imidacloprid on the neurological development of fetal and infant rats. To this end, three groups of female rats were fed different doses of imidacloprid for 41 days: 20 days during pregnancy (gestation days 0 to 20), and 21 days after birth (post-natal day or PND 1 to 21), with young rats exposed *in utero* and then via mother’s milk until weaning at PND 21. The doses were 8, 19 and 54.7 mg/kg/day, plus an untreated control group.¹ The young rats were evaluated for developmental

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¹ This means 8, 19 or 54.7 milligrams of imidacloprid per kilogram body weight of the rat were administered in the diet each day to low, intermediate and high-dose rats, with 30 rats in each group.
neurotoxicity from birth until 75 days of age by means of various neurological tests and brain measurements.

Imidacloprid had two major effects, which were observed primarily in the high-dose rats. It reduced motor and locomotor activity in male and female pups at PND 17, and in female pups at PND 21. And it altered the dimensions of two critical parts of the brain in female pups: decreasing the width of the caudate putamen by 6% at PND 11 and by 2% at study termination; and reducing the thickness of the corpus callosum by a substantial 27% at PND 11 (CA DPR 2006, pp. 52-53; EPA undated, p. 24).² CA DPR reviewers cite scientific literature to show that the morphometric brain changes could well be linked to the decreased motor and locomotor activity (CA DPR 2006, pp. 53-54).

The reviewers also note that “the study was deficient” because Bayer did not report brain measurements for any of the rats in the low- and mid-dose groups, but rather only for the high-dose rats (Ibid, p. 54). Thus, it is impossible to determine either the lowest dose that causes these brain changes (lowest observed effect level, LOEL), or the highest dose that does not cause them (no observed effect level, NOEL). Since the NOEL is required to establish the human safety threshold, and “[t]his study could not be used to determine the developmental NOEL” (Ibid., p. 54), CA DPR should have rejected it out of hand, absent submission of the missing data. Indeed, this was EPA’s response. The Agency said the study did not satisfy the requirements for a developmental neurotoxicity study in rats, and could only be upgraded upon submission of the morphometric brain data for the other female groups, along with other data (EPA undated, p. 3).³ CA DPR’s earlier 2004 review of this study is similar, except the author seemed to have reason to believe the additional brain data were not available: “Study is not acceptable, and appears not to be upgradeable” (CA DPR 2006, pp. 165-166). To our knowledge, the additional needed data were never submitted to either CA DPR or EPA.

Instead of rejecting a study its own scientists found to be “deficient,” “not acceptable,” and incapable of establishing a developmental NOEL or a human safety threshold, CA DPR contradicted itself and used the study for precisely that purpose anyway. First, the ONLY dose for which brain measurements were available was improperly designated the “LOWEST adverse effect level,” which is pure speculation without brain data for animals in the two lower-dose groups.⁴ Second, after explicitly rejecting the study as unsuitable for determining an NOEL, CA DPR went ahead and established an “estimated NOEL (ENEL)” by arbitrarily applying a default factor of 10 to the high dose, 54.7 mg/kg/day, which was improperly designated the LOEL, to arrive at an ENEL of 5.5 mg/kg/day (Ibid., p. 54).

This unacceptable study is apparently still being used, 15 years later, to set the safety threshold for imidacloprid in well water: “The critical acute point of departure (POD) was a no-observed-effects-level (NOEL) of 5.5 mg/kg/day from a developmental neurotoxicity study in rats (Sheets, 2001)” (CA DPR 4/13/21, p. 3); the human health reference level (HHRL) of 283

² CA DPR and EPA largely concur in their evaluations of the study, but differ slightly in details.
³ According to the protocol for this study, morphometric brain data would be collected initially for the high-dose and control groups; but if effects were seen in the high-dose group, then the brains of animals from lower dosage groups would be examined, which of course did not occur here (EPA undated, p. 10).
⁴ In other words, the LOEL could very well be either the intermediate or the low dose.
ppb imidacloprid in drinking water is explicitly based on “the NOEL of 5.5 mg/kg/day” from that study (Ibid., pp. 5-6).\(^5\)

Imidacloprid’s effects on the rat brain may well have significance for humans. The caudate putamen is a central component of the basal ganglia, and performs critical functions in the integration of information, including motor control, cognition and emotion; its degeneration in humans contributes to reduction of motor activity in Parkinson’s disease, while its complete degeneration in Huntington’s disease is accompanied by a large increase in motor activity (Schröder et al. 2020).

The corpus callosum connects the right and left hemispheres of the brain. In humans, it is comprised of more than 200 million nerve fibers, the largest connective pathway in the brain. Its functions are related to coordination and complex problem-solving. Forming between 12 and 16 weeks after conception, it matures at age 12. If the corpus collosum has not grown properly during fetal development, then it never will (Seymour 2017).

We also question CA DPR’s setting of the LOEL and NOEL in the rat teratology study. The study authors found maternal toxicity at 10 mg/kg/day based on 10% reduced food intake and 4% reduction in body weight gain at that dose, as well as what appear to be statistically significant, dose-dependent declines in both food intake and body weight gain from low to high dosage groups (CA DPR 2006, pp. 46-47). CA DPR not only rejected 10 mg/kg/day as the LOEL, it jumped a dosage group and made 100 rather than 30 mg/kg/day the LOEL. Reduced body weight gain and food intake are properly regarded as toxic effects, and the LOEL should be reclassified as 10 mg/kg/day.

**Issues related to the Food Quality Protection Act**

The Food Quality Protection Act requires establishment of an additional 10-fold margin of safety to account for pre- and post-natal toxicity, unless convincing evidence shows that the young are no more susceptible or sensitive to the toxic effects of a pesticide than adults. The brain effects in the Bayer study were found in young animals. And CA DPR scientists discuss the abundant evidence that pre- and postnatal exposure to nicotine, which is structurally related to imidacloprid (a *neonicotinoid*), and also targets nicotinic acetylcholine receptors in the brain, is associated with numerous adverse effects in young rats and humans (including Sudden Death Syndrome) (CA DPR 2006, pp. 91-92). They also note the striking fact that females are more affected in these nicotine studies, just as imidacloprid preferentially affected female brains and neurological function in not only the Bayer study discussed above (Sheets 2001), but at least two other Bayer neurotoxicity studies, Sheets 1994a and 1994b (Ibid., p. 92).

CA DPR ignored their scientists’ call to consider the greater susceptibility of the young to the toxic effects of nicotine “when assessing the effects of imidacloprid on the developing organisms” (Ibid. p. 92). Application of a safety factor to protect the young is necessary when, as here with imidacloprid, the neurological development of the young is impacted.

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\(^5\) Note how CA DPR entirely drops the “estimated NOEL (ENEL)” nomenclature in these recent references to the Bayer study, which inadvertently or not serves to obscure the fatal flaws in that study and the numerical gymnastics undertaken to use it for establishing the human safety threshold despite those flaws.
Aggregate exposure to imidacloprid

In developing its human health reference levels for acute and chronic exposure in drinking water, it does not appear that CA DPR factored in aggregate exposure to imidacloprid via all other routes, which include residues in food, residential uses, occupational activities and ambient air. Interestingly, when CA DPR conducted its hazard assessment of imidacloprid in 2006, the agency concluded that drinking water was not “a major contributor to the total dietary exposure,” suggesting a proportionally greater exposure from food (Ibid., p. 93). With much greater use today, exposures via both food and water as well as other routes will have increased greatly. CA DPR must ensure that in assessing risk, all routes of exposure are considered; and likewise that any reference levels set for drinking water (or any other individual route of exposure) account for co-exposures via other routes.

Cumulative toxicity from exposure to neonicotinoids

Imidacloprid shares its mechanism of toxicity with other neonicotinoid pesticides, including acetamiprid, dinotefuran, thiacloprid, thiamethoxam and clothianidin. They all bind to nicotinic acetylcholine receptors (nAChRs) in the brain, thereby continuously stimulating neurons, resulting in death as well as sublethal effects (Simon-Delso et al. 2015). Neonicotinoids are more highly toxic to invertebrates than vertebrates because the former have a larger number of nAChRs with high affinity to these insecticides. Neonicotinoids target primarily the nAChR subtype α4β2 in insects and mammals, and mammalian toxicity correlates with agonist action and binding affinity at these receptors, their primary target in the brain (Tomizawa and Casida 2005).

This shared mechanism of toxicity demands cumulative risk assessment of these neonicotinoids for the risks they pose to humans. Cumulative assessment is further supported by the similarity in neurological effects they cause in animal studies submitted to EPA by registrants, and by the efforts of independent scientists to assess cumulative dietary exposure to neonicotinoids by employing relative potency factors (for discussion and references, see CFS 2020).

CA DPR conceded the need to assess neonicotinoids as well as nicotinoids cumulatively in 2006, but cited modest agricultural use and sparse monitoring data as obstacles to doing so; however, the agency also stated that “the risk of concomitant dietary exposure to multiple neonicotinoid and nicotinoid pesticides would be addressed when residue data become available” (CA DPR 2006, pp. 93-94). Agricultural use of imidacloprid in California has more than tripled since 2006, and was roughly 350,000 lbs. in 2018 (CA DPR 2021, Figure 1). Overall neonicotinoid use in 2018 (including non-agricultural uses) stood at 640,000 pounds (see table below). However, these figures exclude seed treatment uses, which have the potential to double these amounts, according to a recent analysis of neonicotinoid use in California (Mineau 2020). In addition, much more monitoring data is presumably available. California should thus conduct a full cumulative risk assessment of neonicotinoids.
### Neonicotinoid Pounds Applied in CA, excl. seed treatments (2018)*

<table>
<thead>
<tr>
<th>Neonicotinoid</th>
<th>Pounds Applied in CA, excl. seed treatments (2018)*</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetamiprid</td>
<td>63,542</td>
<td></td>
</tr>
<tr>
<td>Clothianidin</td>
<td>15,965</td>
<td></td>
</tr>
<tr>
<td>Dinotefuran</td>
<td>32,028</td>
<td></td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>466,268</td>
<td>Represents nearly half of national use, as per USGS</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>61,506</td>
<td></td>
</tr>
<tr>
<td>TOTALS</td>
<td>639,309</td>
<td></td>
</tr>
</tbody>
</table>


### Endocrine and metabolic disruptors

Imidacloprid is also well known to be a reproductive toxin and an endocrine disruptor (Mikolic and Karaconji 2018). CA DPR notes that imidacloprid like other neonicotinoids activates the nAChRs that in turn regulate the endocrine system; that structurally related compounds like nicotine are known to have neuroendocrine effects by interacting with brain nAChRs; and that the State of California classifies nicotine as a reproductive toxin. CA DPR furthermore cites various developmental and reproductive effects identified in registrant studies (including the morphometric changes in brain structures in Sheets 2001) as potentially the result of imidacloprid’s endocrine disrupting activity (CA DPR 2006, p. 94).

There is far more literature available today on this subject. For instance, Pandey and Mohanty (2015) demonstrated that exposure of a wild bird (*Amandava amandava*) to a fairly low level of imidacloprid (0.155 mg/kg/day) for 30 days in food resulted in disruption of the pituitary-thyroid axis, including adverse effects to the thyroid gland and alterations in the levels of T4, T3 and thyroid stimulating hormone.

Many scientists now place endocrine disruptors in a broader category of compounds that include disruptors of metabolism, often via interactions with nuclear receptors (Heindel et al. 2015). Imidacloprid is one such metabolic disruptor. Park et al. (2013) found that imidacloprid increased lipid accumulation during differentiation of 3T3-L1 adipocytes in cell culture, while Sun et al. (2016) demonstrated that imidacloprid is an obesogen that potentiates the obesity-promoting effects of a high-fat diet in male mice, at doses as low as 0.06 mg/kg/day for 12 weeks (Sun et al. 2016, Figures 1A and 1B excerpted below; see also Egusquiza and Blumberg 2020). Interestingly, Sun et al. chose the low dose of 60 ug/kg/day to match CA DPR’s reference dose, based on the Sheets 2001 study (CA DPR 2006, Table 21, p. 84). CA DPR’s acute dietary exposure estimate shows that intake of this amount or greater is at the high end of the possible, based on tolerance-level and maximum detected residue data available to it in 2006 (Ibid., Tables 19 and 20, pp. 81-83). We would note that regular consumption of well water with 6 ppb imidacloprid would result in exposure of 0.6 ug/kg/day for a 10 kg toddler, assuming intake of 1 liter per day, and that this amount represents nearly 10% of the chronic exposure estimated for 1-2 year olds (Ibid., Table 20).
CA DPR should carefully consider the potential for imidacloprid to disrupt the endocrine system and metabolic regulation, including non-traditional endpoints such as obesogenic or metabolism-disrupting activity – especially with the huge increase in usage of imidacloprid and other neonicotinoids since the 2006 hazard characterization.

Alternate points of departure

The ENEL of 5.5 mg/kg/day from Sheets (2001) and the derived reference level of 0.06 mg/kg/day are clearly unacceptable as benchmarks for assessing the adverse health effects of imidacloprid. Moreover, by using this deficient study to establish a critical human health safety threshold, CA DPR is telling registrants they are free to abstain from performing critical measurements or tests, or at least from reporting them, with no consequences. Indeed, by making Sheets (2001) the point of departure, CA DPR is rewarding Bayer for this egregiously unacceptable behavior.

This episode underscores the need for CA DPR to consult the peer-reviewed scientific literature, preferably by authors not affiliated with pesticide companies, not only for framing the interpretation of registrant study findings, but for the serious business of actually setting safety thresholds to protect public health and the environment. A few suggestions, far from exhaustive, follow.

Kara et al. (2015) administered via gavage 0.5, 2 or 8 mg/kg/day imidacloprid to infant and adult Wistar rats for 3 months. Learning activities were diminished significantly at 2 and 8 mg/kg/day doses in infant rats, but only at 8 mg/kg/day in adult rats. Unlike the meaningless ENEL from Sheets (2001), this study not only has a solid NOAEL for infant rats of 0.5 mg/kg/day, but it is closely spaced to the LOEL dose, reducing uncertainty as to where the true effect threshold lies. The greater susceptibility of infant vs. adult rats calls for application of a safety factor, similar to the one prescribed by the Food Quality Protection Act, in addition to the usual 100x for interspecies and interindividual differences. Thus, the reference dose would be 0.5 mg/1,000 = 0.5 ug/kg/day.
Burke et al. (2018) infused 0.5 mg/kg/day imidacloprid into pregnant CD-1 mice via an implanted osmotic minipump from gestation day (GD) 4 to post-natal (PN) day 21. Imidacloprid accumulated in livers and brains of maternal mice, and was found in trace levels in offspring. Offspring exhibited a number of neurobehavioral impacts: elevated motor activity, enhanced social dominance, reduced depressive behavior, and a diminution in social aggression compared to controls. Adult male offspring had reduced weight. Maternal animals had significantly reduced fecundity (roughly 8 vs. 13 pups per mother for treatment vs. control groups). Thus, transient exposure to imidacloprid over the developmental period induced long-lasting changes in behavior and brain function in mice. Based on Burke et al. (2018), the LOAEL for imidacloprid is 0.5 mg/kg/day. Although the delivery method, bypassing the gut, may complicate use of this study for the oral POD, “imidacloprid is quickly absorbed by the oral route and rapidly distributed in nearly all organs and tissues” and “[i]n rats, the oral absorption was estimated at 92-99%” (CA DPR 2006, pp. viii, 14-16). Thus, the internal dose delivered by the minipump may not be so different from that ensuing from oral administration. This study would support a reference dose of 0.5 to 5 ug/kg/day, depending on whether an additional safety factor for the young is applied.

The Sun et al. (2016) study described above could also serve as a POD, with 0.06 mg/kg/day serving as the LOEL for potentiating the obesity-inducing effect of a high-fat diet, supporting a reference dose of 0.6 ug/kg/day. High-fat diets are of course common in the U.S., obesity is reaching epidemic proportions, and we must begin to reduce exposure to compounds that exacerbate this health threat.

CFS urges CA DPR to consult Pisa et al. (2021) for a comprehensive review of additional recent animal studies on the adverse effects of imidacloprid and other systemic insecticides for additional POD candidates.

Bill Freese, Scientific Director
Center for Food Safety
References


Burke AP et al. (2018). Mammalian Susceptibility to a Neonicotinoid Insecticide after Fetal and Early Postnatal Exposure. Scientific Reports 8: 16639.


