



CENTER FOR
FOOD SAFETY

May 6, 2025

U.S. Environmental Protection Agency
EPA Docket Center
New Active Ingredient Cyclobutrifluram, Mail Code 28221T
1200 Pennsylvania Ave, NW, Washington, DC 20460

RE: Docket ID No.: EPA-HQ-OPP-2022-0003
Comments on Proposed Decision to Register Cyclobutrifluram

On behalf of itself and its 970,000 members and supporters, Center for Food Safety appreciates the opportunity to comment on EPA's proposed decision to register the new active ingredient, cyclobutrifluram. 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.

Introduction

Cyclobutraflurim is a pyridine carboxamide fungicide and nematicide whose pesticidal mode of action is inhibition of complex II succinate dehydrogenase. Succinate dehydrogenase (SDH) is an enzyme that is highly conserved across a broad range of living organisms, including human beings, which raises the possibility that it could adversely affect human health via the same mechanism by which it kills target pests (Bouillaud 2023). Indeed, *in vitro* investigations show eight SDH-inhibiting fungicides inhibit the SDH enzymes of humans as well as those of earthworms and honeybees (Benit et al. 2019). Moreover, SDH is the only enzyme complex involved in both the tricarboxylic acid cycle and the electron transport chain, meaning that inhibitors of SDH potentially interfere with one or both of two major energy-producing cellular processes (Rasheed and Tarjan 2018). Finally, over the past quarter-century there has been an explosion of research findings regarding the role of SDH inhibition and succinate accumulation in carcinogenesis (Ibid, Selak et al. 2005, Zhao et al. 2017, Moreno et al. 2020). Despite these facts, EPA provides no discussion of the potential for cyclobutrifluram to participate in carcinogenesis via its inhibition of SDH, either in its general human health assessment (EPA 4/17/25) or its cancer-specific evaluation of the chemical (EPA 4/15/25).

San Francisco, CA - 600 California Street - Suite 12-013 San Francisco, CA 94108

Washington, DC - 110 Maryland Avenue, NE - Suite 307, Washington DC, 20002

Portland, OR - 2009 NE Alberta St - Suite 207, Portland, OR 97211

centerforfoodsafety.org

office@centerforfoodsafety.org

(415) 826-2770

Cyclobutrifluram Induces Thyroid and Liver Tumors

Rodent carcinogenicity studies

EPA evaluated two unpublished registrant rodent (rat and mouse) studies to evaluate cyclobutraflurim's carcinogenic potential, and gave cursory treatment to an open literature study published by registrant scientists (EPA 4/15/25).

In the rat study, there were statistically significant trends of increasing numbers of thyroid follicular cell tumors with rising dose in both males and females, as follows:

- Carcinomas in male rats ($p = 0.0433$)
- Adenomas and combined adenomas/carcinomas in female rats ($p = 0.01495$ and $p = 0.01874$, respectively)

These results are supported by the elevated number of adenomas, carcinomas and combined tumors in the mid-dose male group vs. controls (all $p = 0.0632$), which barely exceeded the arbitrary statistical cutoff of $p = 0.05$. Preneoplastic thyroid follicular cell hyperplasia was also observed in male rats – in greater total numbers, and with greater severity – in high-dose animals versus controls.¹ Both male and female concurrent control groups exhibited no thyroid tumors, consistent with the historical control range.

In the mouse study, there was a highly significant trend of increasing hepatocellular carcinomas ($p = 0.0139$) in male mice, with the incidence in the high-dose group exceeding the historical control range incidence. Likewise, preneoplastic liver lesions (focus of cellular alteration) also increased monotonically from control to high-dose groups.

The open-literature study by the registrant Syngenta's scientists likewise recorded a dose-responsive increase in hepatocellular carcinomas in male CD-1 mice, like the regulatory mouse study (EPA 4/15/25, Appendix A, p. 39).

Support from other studies

As EPA notes, the 28-day and 90-day rat studies, as well as the two-generation rat study, provide support for cyclobutrafluram's thyroid impacts. These three studies all identified hypertrophy of thyroid follicular cells – the same tissue in which tumors were induced in the rat study discussed above – as the compound's major toxic thyroid effect (in the reproduction study, absolute thyroid weight was also elevated). Hypertrophy can be an adaptive response, but when a compound is found to induce not only hypertrophy, but also, as in the case of cyclobutrifluram, increased absolute thyroid weight (reproduction study), hyperplasia, and both benign and especially malignant thyroid follicular cell tumors (rat carcinogenicity study discussed above), it should be regarded as a stage on the road to cancer. Cyclobutraflurim meets the criteria for chemicals that adversely affect thyroid follicular cells via a combined mode of action model, with effects

¹ EPA's Table 3 miscounts thyroid follicular cell hyperplasia in female rats: while the mid-dose (150 ppm) group is listed as having 5 hyperplasias, only three are accounted for in the breakout of severity: namely, 1 is minimal, 2 are mild, and 0 are moderate, leaving 2 unclassified. EPA should correct this error (EPA 4/15/25, p. 11).

that include hypertrophy, hyperplasia, increased thyroid weight and thyroid follicular cell tumors (Nielsen et al. 2012, pp. 263-264).

EPA concedes that the supporting studies described above demonstrate adverse effects on the thyroid; and that the thyroid is the most sensitive endpoint in the cyclobutrifluram toxicity database (EPA 4/17/25, p. 19). EPA accordingly based the chronic reference dose on the lowest observed **adverse** effect level (LOAEL) for thyroid follicular cell hypertrophy and increased thyroid weights in the two-generation reproduction study (Ibid., p. 23). Yet despite acknowledging these adverse thyroid effects, EPA denies cyclobutrifluram's induction of tumors in the very same thyroid tissue. Thus, EPA illegitimately denies cyclobutrifluram's carcinogenic hazard, classifying it as "not likely to be carcinogenic to humans" (Ibid., p. 22).

Cyclobutrifluram's carcinogenicity would have been still clearer with adequate dosing

Animal carcinogenicity studies must be conducted with a "maximum tolerated dose" or MTD that is sufficiently high to elicit signs of toxicity, but that does not compromise the survival of the animals due to causes other than carcinogenicity (Gad 2024). The rationale behind use of the MTD as the high dose is "to provide the maximum ability to detect treatment-related carcinogenic effects..." which is EPA policy for carcinogen risk assessment and standard practice in laboratories around the world (EPA 2005, p. 2-15). Without use of an MTD, the study simply does not provide an adequately stringent test of a compound's carcinogenic potential.

EPA concedes that neither the rat nor the mouse studies employed high enough doses: "the rats of both sexes could have tolerated higher doses" and "the [mouse] study could have tested higher doses" (EPA 4/15/25, p. 6). Nevertheless, EPA tries to argue that the studies were adequate anyway based on effects that EPA elsewhere deems non-adverse. With regard to the rat study, EPA states "no adverse toxicological effects were observed up to the highest dose tested (23/30 mg/kg/day [M/F] (500 ppm)" (Ibid., p. 11). For the mouse study: "there were no treatment-related adverse effects on survival observed for either male or female mice. No treatment-related adverse effects to body weight, body weight gain, or hematology were observed up to and including the highest doses tested (48/54 mg/kg/day). Based on the aforementioned results, the carcinogenicity study in mice could have tested higher doses" (Ibid., p. 16).

The failure to employ a sufficiently high dose takes on more importance when one considers that the statistically significant tumor results in the carcinogenicity trials were for **increasing trends of more tumors at higher doses**, both in rats and mice (in each study, groups of animals received one of three different doses). This makes it quite likely that a higher dose than was tested in these rodent studies would have elicited more tumors, in line with the statistically significant trends identified at the doses that were tested. With proper dosing, then, the elevation in number of tumors in the high-dose group versus the control group (so-called pairwise comparison) would have also achieved statistical significance, making cyclobutrifluram's carcinogenicity still clearer. However, we emphasize that EPA's Carcinogen Risk Assessment Guidelines find statistically significant

results in **either** trend **or** pairwise comparison tests to be sufficient to reject chance as accounting for the result, and hence to infer carcinogenic hazard (EPA 2005, p. 2-19).

EPA has no valid thyroid hormone data

One important sign of adverse thyroid effects with potential relevance to cancer-causing potential would be disruption of thyroid hormone levels and regulation. Yet EPA has obtained no valid data on this critical endpoint. EPA reports thyroid hormone levels for only one study – the prenatal developmental rat toxicity study² – and notes that no adverse changes were observed in T3, T4 or TSH (thyroid-stimulating hormone) levels in the maternal animals dosed with cyclobutylfluram in amounts up to the highest tested dose of 250 mg/kg/day (EPA 4/17/25, p. 19). EPA at one and the same time “concluded that the data for the rat and rabbit developmental toxicity studies are considered adequate,” and that additional studies were not needed, and also that “the existing TK [toxicokinetic] data are insufficient to conclude that 250 mg/kg/day is a reasonable top dose for the developmental toxicity study” (Ibid., compare pp. 20 and 100). EPA cannot have it both ways, and cannot rely on thyroid hormone data from a study made invalid by failure to employ a high enough dose.

This lack of valid hormone data is made still more egregious by two other considerations. First, EPA failed to require a comparative thyroid assay to provide fuller information on the adverse impact of most concern in the rat study. Second, EPA has failed to subject cyclobutylfluram to its Endocrine Disruptor Screening Program (EDSP), as required by the Food Quality Protection Act, passed in 1996. The EDSP comprises a set of assays specifically designed to detect disruption of estrogen, androgen and thyroid hormone functioning. Yet over the past three decades, EPA has refused to carry out this Congressional mandate, repeatedly defying court orders and court-imposed deadlines. EPA’s latest position is that it can rely entirely on reproduction studies to judge a compounds endocrine disruption potential (EPA 4/17/25, Appendix F, p. 117).

EPA ignores cyclobutylfluram’s mammalian (and human) mode of action

As mentioned above, cyclobutylfluram is an inhibitor of the succinate dehydrogenase enzyme (SDH), and it is by inhibition of this enzyme that it kills both fungi and nematodes. Unfortunately, SDH is also present in nearly all living organisms, including human beings. Defects in the enzyme are associated with various cancers, including thyroid tumors (Rasheed and Tarjan 2018, Mu et al. 2021, Zhao et al. 2017). There is also concern that environmental toxins, and SDH-inhibiting fungicides in particular, could also induce cancer by inhibiting SDH (Bouillaud 2023, Benit et al. 2019). Mechanisms responsible for SDH inhibitor-induced tumorigenesis include succinate, the substrate of SDH that accumulates when the enzyme is inhibited, and transmits a tumor-inducing signal from the mitochondria to the cytosol, where it inhibits hypoxia-inducible factor - α 1 (HIF- α) prolyl hydrolases, leading to stabilization and activation of HIF-1 α (HIF-alpha). In this way, succinate can increase the expression of genes that facilitate angiogenesis, metastasis, and glycolysis, which ultimately leads to tumor

² In prenatal developmental studies such as this one, pregnant animals are dosed with the test compound during all or part of gestation, and both maternal animals and fetuses are examined for adverse effects.

progression (Selak et al. 2005). Zhao et al. (2017) detail other mechanisms by which SDH inhibition and resulting elevated levels of succinate can initiate and promote tumors.

Rather than explore this literature, EPA both admits that cyclobutrifluram adversely affects the mammalian thyroid, but denies any knowledge of its mammalian mode of action (EPA 4/17, 25, p. 19).

Conclusion

EPA's conclusion that cyclobutrifluram is "not likely to be carcinogenic to humans" is entirely unfounded. The Agency has misevaluated the two unpublished registrant studies, and dismissed clear evidence of cancer-causing potential. The studies did not employ adequately high doses to test for carcinogenicity, which if they had been used would have likely provided still stronger evidence of the fungicide's carcinogenic potential. EPA likewise collected no valid data on cyclobutrifluram's effects on thyroid hormone homeostasis, and waived or failed to collect studies needed to better elucidate those effects (comparative thyroid assay, endocrine disruption screening). Finally, EPA ignores the well established mammalian relevance of cyclobutrifluram's mode of action – inhibition of succinate dehydrogenase – which a growing mountain of evidence links to carcinogenesis.

EPA is urged to reject the proposed registration decision for this novel fungicide, and postpone any decision unless or until adequate data demonstrates its safety for humans and other organisms.

Regards,
Bill Freese, Science Director
Center for Food Safety

References

Bénit P, Kahn A, Chretien D, Bortoli S, Huc L, Schiff M, et al. (2019). Evolutionarily conserved susceptibility of the mitochondrial respiratory chain to SDHI pesticides and its consequence on the impact of SDHIs on human cultured cells. *PLoS ONE* 14(11): e0224132. <https://doi.org/10.1371/journal.pone.0224132>.

Bouillaud F (2023). Inhibition of Succinate Dehydrogenase by Pesticides (SDHIs) and Energy Metabolism. *Int J Mol Sci.* 24(4): 4045. doi: 10.3390/ijms24044045.

EPA (4/17/25). Cyclobutrifluram. Human Health Risk Assessment to Support the Registration of a New Active Ingredient for Proposed Uses on Cotton Seed; Soybean Seed; Romaine Lettuce; Turf; Ornamentals; and Non-Bearing (Juvenile) Fruit and Nut Trees, Vines and Berries, EPA, EPA-HQ-OPP-2022-0003-0025, April 17, 2025.

EPA (4/15/25). Cyclobutrifluram: Report of the Cancer Assessment Review Committee. EPA, EPA-HQ-OPP-2022-0003-0018. April 15, 2025.

EPA (2005). Guidelines for Carcinogen Risk Assessment, Risk Assessment Forum, U.S. Environmental Protection Agency, March 2005.

Gad SH (2024). Maximum tolerated dose. *Encyclopedia of Toxicology* (4th edition), Vol. 6: 43-44. <https://www.sciencedirect.com/science/article/abs/pii/B9780128243152005327>.

Hurley PM (1998). Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. *Environ Health Perspectives* 106(8): 437-45. doi: 10.1289/ehp.98106437.

Moreno C, Santos RM, Burns R, Zhang WC (2020). Succinate Dehydrogenase and Ribonucleic Acid Networks in Cancer and Other Diseases. *Cancers (Basel)* 12(11): 3237. doi: 10.3390/cancers12113237.

Mu R, Ma Z, Lu C, Wang H, Cheng X, Tuo B, Fan Y, Liu X, Li T (2021). Role of succinylation modification in thyroid cancer and breast cancer. *Am J Cancer Res.* 11(10): 4683-4699.

Nielsen E et al. (2012). Identification of Cumulative Assessment Groups of Pesticides. External Scientific Report submitted to EFSA [European Food Safety Authority]. EFSA Supporting Publications, Vol. 9, Issue 4, April 2012. <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2012.EN-269>.

Rasheed MRHA and Tarjan G (2018). Succinate Dehydrogenase Complex: An Updated Review. *Arch Pathol Lab Med.* 142(12): 1564-1570. doi: 10.5858/arpa.2017-0285-RS.

Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Simon MC, Thompson CB, Gottlieb E (2005). Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. *Cancer Cell* 7(1): 77-85. doi: 10.1016/j.ccr.2004.11.022.

Zhao T, Mu X, You Q (2017). Succinate: An initiator in tumorigenesis and progression. *Oncotarget* 8(32): 53819-53828. doi: 10.18632/oncotarget.17734.