United States Department of Agriculture
Agricultural Marketing Service | National Organic Program
Document Cover Sheet
https://www.ams.usda.gov/rules-regulations/organic/national-list/petitioned

Document Type:

☐ **National List Petition or Petition Update**

A petition is a request to amend the USDA National Organic Program's National List of Allowed and Prohibited Substances (National List).

Any person may submit a petition to have a substance evaluated by the National Organic Standards Board (7 CFR 205.607(a)).

Guidelines for submitting a petition are available in the NOP Handbook as NOP 3011, National List Petition Guidelines.

Petitions are posted for the public on the NOP website for Petitioned Substances.

☒ **Technical Report**

A technical report is developed in response to a petition to amend the National List. Reports are also developed to assist in the review of substances that are already on the National List.

Technical reports are completed by third-party contractors and are available to the public on the NOP website for Petitioned Substances.

Contractor names and dates completed are available in the report.

Xylazine/Tolazoline

Livestock

Identification of Petitioned Substance

Chemical Names:
Xylazine
Xylazine
Xylazine Hydrochloride
Xylazine Monohydrochloride
N-(2,6-Diphenylmethyl)-5,6-dihydro-4H-1,3-thiazin-2-amine
N-(2,6-Diphenylmethyl)-5,6-dihydro-4H-1,3-thiazin-2-amine hydrochloride

Tolazoline
Tolazoline
Tolazoline hydrochloride
2-Benzylimidazoline
2-Benzyl-4,5-dihydro-1H-imidazole
2-Benzyl-4,5-dihydro-1H-imidazole hydrochloride

Other Names:
Xylazine
Xylazine HCl
Xylaxine
Xylazin
Xilazina
Xylazinium
Xylazine Chloride

Tolazoline
Tolazoline HCl
Tolazoline Chloride
Imidaline Hydrochloride
Benzidazol
Benzalolin
Benzazoline Hydrochloride
Pridazole
Phenylmethylimidazoline
Tolazolinum
Tolazolina
Peripherin

Trade Names:
Xylazine
AnaSed® LA
Rompun®
TranquiVed Injection
X-Ject SA
XylaMed™Celactal

Tolazoline
Tolazil™
Tolazoline® Injection
Priscoline
Priscoline Hydrochloride
Divascol
Olitensol
Lambril
Arterodyl
Priscol

CAS Numbers:
7361-61-7 (Xylazine)
23076-35-9 (Xylazine Hydrochloride)
59-98-3 (Tolazoline)
59-97-2 (Tolazoline Hydrochloride)

Other Codes:
EC No. 230-902-1 (Xylazine)
NSC No. 758142 (Xylazine)
UNII No. 2KF9TP5V8 (Xylazine)
EC No. 245-417-0 (Xylazine Hydrochloride)
UNII No. NGC3S0882S (Xylazine Hydrochloride)
EC No. 200-448-9 (Tolazoline)
NSC No. 35110 (Tolazoline)
UNII No. CHH9H12AQ3 (Tolazoline)
EC No. 200-447-3 (Tolazoline Hydrochloride)
NSC No. 757353 (Tolazoline Hydrochloride)
UNII No. E669Z6S1JG (Tolazoline Hydrochloride)

Summary of Petitioned Use

The United States Department of Agriculture (USDA)'s National Organic Program (NOP) has approved xylazine and tolazoline for medicinal applications in organic livestock production. Both xylazine and tolazoline are restricted to "use by or on the lawful written or oral order of a licensed veterinarian," and must be followed by "a meat withdrawal period of at least 8 days after administering to livestock intended for slaughter; and a milk discard period of at least 4 days after administering to dairy animals," at Title 7 of the Code of Federal Regulations (CFR) Section 205.603. Tolazoline is further restricted for "use only to reverse the effects of sedation

and analgesia caused by Xylazine," at §205.603. This technical report outlines the veterinary applications of xylazine for organic livestock production and serves to update a previous technical report from 2002 (USDA 2002b).

Characterization of Petitioned Substance

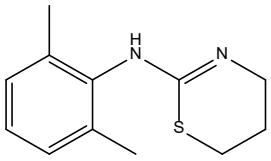
Composition of the Substance:

Xylazine

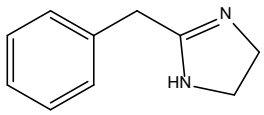
Xylazine (Figure 1) is a synthetic α2-adrenergic agonist, which Farbenfabriken Bayer developed in 1962 to treat hypertension (Kreeger et al. 1986a, Kreeger et al. 1986b, Greene and Thurmon 1988, EMEA 1999, Lester et al. 2012, Thies et al. 2017). Xylazine is a molecule in the clonidine family, and the basic sites on the molecule (nitrogen and sulfur atoms with electron pairs) provide a basic structure important for the biological absorption and distribution of the substance (Garcia-Villar et al. 1981, Greene and Thurmon 1988). Xylazine's neurological activity has resulted in veterinary medicinal uses. The substance is commonly administered as both the neutral compound (Figure 1) and its hydrochloride salt (PubChem 5707, PubChem 68554, Kreeger et al. 1986a, Kreeger et al. 1986b, Lester et al. 2012, Sigma-Aldrich 2014b, Flecknell 2016, Sigma-Aldrich 2017).

Tolazoline

Tolazoline (Figure 1) is a synthetic α2-adrenergic antagonist, which also interacts with histamine and cholinergic receptors (Goetzman and Milstein 1979, McIntosh and Waters 1979, Pawson 2008, Greenough et al. 2012, Ebert 2013). Like xylazine, tolazoline is in the clonidine family and shares the basic attributes (nitrogen atoms with electron pairs) that enable its rapid biological absorption and distribution (Garcia-Villar et al. 1981, Greene and Thurmon 1988). Structural similarities with xylazine allow tolazoline to compete with xylazine for biological binding sites. This provides the mode of action for its approved use in organic livestock production as a reversal agent for xylazine (Levy et al. 1977, Goetzman and Milstein 1979, Kreeger et al. 1986a, Kreeger 1986b, Samanta et al. 1990, JECFA 1998a, JECFA 1998b, Pawson 2008). Tolazoline's neurological activity has resulted in its use in veterinary medicine and is commonly administered as both the neutral compound (Figure 1) and its hydrochloride salt (PubChem 5504, PubChem 6048, Kreeger et al. 1986a, Kreeger et al. 1986b, Sigma-Aldrich 2006, Pawson 2008, Rotta et al. 2011, Ebert 2013, Coleman and Cox 2014, Sigma-Aldrich 2014a).



Xylazine



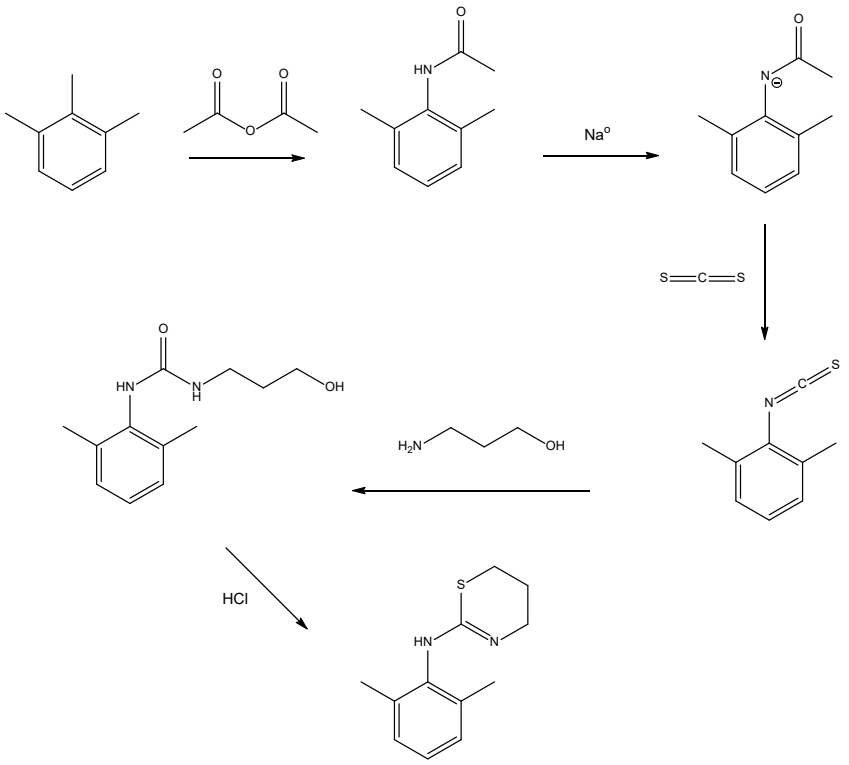
Tolazoline

Figure 1

Source or Origin of the Substance:

Xylazine

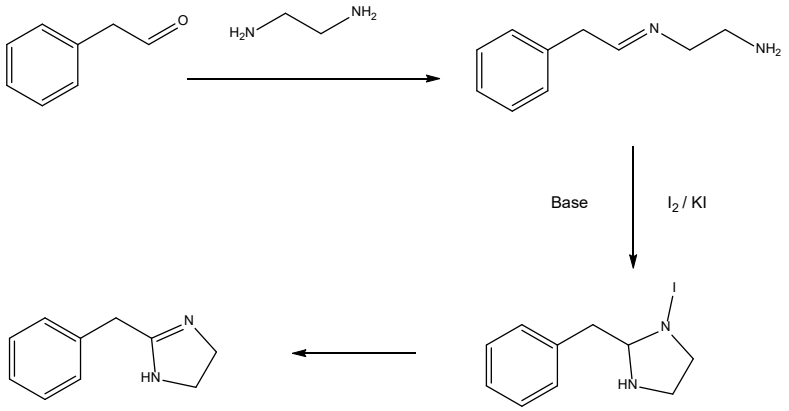
Xylazine is a synthetic substance produced by the multi-step reaction of 2,6-dimethylaniline, as shown in Scheme 1 on the next page (Elliot and Ruehle 1985).



Scheme 1

Tolazoline

Tolazoline is a synthetic substance that is produced by a one-pot process (i.e., no intermediates are isolated) via the reaction of phenylacetaldehyde with ethylene diamine, with the incorporation of an iodine-based oxidation process, as shown in Scheme 2 (Gogoi and Konwar 2006).



Scheme 2

Properties of the Substance:

The properties xylazine, xylazine hydrochloride, tolazoline, and tolazoline hydrochloride are summarized in Table 1.

Table 1. Properties of xylazine, xylazine hydrochloride, tolazoline, and tolazoline hydrochloride

Compound	Xylazine	Xylazine Hydrochloride	Tolazoline	Tolazoline Hydrochloride
CAS No.	7361-61-7	23076-35-9	59-98-3	59-97-2
Molecular Weight	220.33 g/mol	256.79 g/mol	160.22 g/mol	196.68 g/mol
Appearance	White powder	Solid	White powder	Solid
Melting Point	141 °C	N/A	66-69 °C	174-176 °C
Solubility	0.5 g/L (methanol)	N/A	373 mg/L (water)	N/A

Sources: PubChem 5504, PubChem 5707, PubChem 6048, PubChem 68554, Sigma-Aldrich 2006, Sigma-Aldrich 2014a, Sigma-Aldrich 2014b, Sigma-Aldrich 2017.

Specific Uses of the Substance:

Xylazine

The synthetic substance xylazine was originally developed to treat hypertension in humans (Kreeger et al. 1986a, Kreeger et al. 1986b, Greene and Thurmon 1988, EMEA 1999, Lester et al. 2012, Thies et al. 2017). However, the prevalence of its side effects, including the onset of significant cardiac arrythmias, has prohibited its use in human medicine (Green et al. 1981, EMEA 1999, Reyes et al. 2012).

Despite negative side effects in humans, xylazine is widely used in veterinary medicine, where the frequency and danger of side effects are reduced. Xylazine, due to depression of the central nervous system and cardiac outputs, is commonly used as a sedative and analgesic in veterinary medicine (EMEA 1999, Lorenz et al. 2010, Lester et al. 2012, Otto and von Thaden 2012, Ruiz-Colon et al. 2014, Silva-Torres et al. 2014, Thies et al. 2017). Xylazine is the most common co-treatment for ketamine anesthetic applications (Green et al. 1981, Kreeger et al. 1986a, Kreeger et al. 1986b, Saha et al. 2005, Reyes et al. 2012).

The United States Food and Drug Administration (FDA) has restricted the veterinary application of xylazine with the designation "do not use in domestic food-producing animals," at 21 CFR 522.2662. Xylazine is also used as an emetic for cats, as an alternative to surgery to recover foreign objects (Thies et al. 2017).

Tolazoline

Tolazoline is used in both human and veterinary medical applications. In human medicine, tolazoline is used to treat hypotension, hypertension, newborn respiratory distress, and congenital heart disease through interactions with α2-adrenergic and histamine receptors (Grover et al. 1961, Cotton 1965, Korones and Eyal 1975, Yellin et al. 1975, Levy et al. 1977, Goetzman and Milstein 1979, McIntosh and Walters 1979, Kreeger et al. 1986a, Greenough et al. 2012). These treatments rely on the ability of the substance to induce vasodilation, increasing arterial oxygenation (Levy et al. 1977, Kreeger et al. 1986a, Kreeger et al. 1986b).

Tolazoline is commonly used in veterinary medicine as a reversal agent for xylazine sedation (Levy et al. 1977, Goetzman and Milstein 1979, Kreeger et al. 1986a, Kreeger 1986b, Samanta et al. 1990, JECFA 1998a, JECFA 1998b, Pawson 2008). Tolazoline competes with xylazine for α2-adrenergic binding sites; once bound, it blocks xylazine from interacting with the receptor (Goetzman and Milstein 1979, McIntosh and Waters 1979, Pawson 2008, Greenough et al. 2012, Ebert 2013). Tolazoline's side effects, such as increased cardiac and respiratory responses (e.g., tachycardia), work to reverse xylazine's common side effects (e.g., bradycardia, respiratory depression) (Yellin et al. 1975, Levy et al. 1977, Goetzman and Milstein 1979, Kreeger et al. 1986a, Kreeger et al. 1986b).

Approved Legal Uses of the Substance:

Xylazine

USDA allows xylazine for veterinary applications within organic livestock production at 7 CFR 205.603, restricting it for use "by or on the lawful written or oral order of a licensed veterinarian," and must be followed by "a meat withdrawal period of at least 8 days after administering to livestock intended for slaughter; and a milk discard period of at least 4 days after administering to dairy animals."

The FDA has approved the use of xylazine as an animal drug administered through implantation or injection "to produce sedation, as an analgesic, and as a preanesthetic to local or general anesthesia," the application of which is restricted "to use by or on the order of a licensed veterinarian," at 21 CFR 522.2662.

The FDA also gives the following species-specific conditions for xylazine's use, at §522.2662:

> Dogs and cats – amount 0.5 mg/pound (lb) intravenously or 1.0 mg/lb subcutaneously; horses – 0.5 mg/lb intravenously or 1.0 mg/lb intramuscularly; Elk and deer – administer intramuscularly, by hand syringe, or by syringe dart, in the heavy muscles of the croup or shoulder as follows: Elk (*Cervus canadensis*) 0.25 to 0.5 mg/lb; mule deer (*Odocoileus hemionus*), sika deer (*Cervus nippon*), and white-tailed deer (*Odocoileus virginianus*): 1 to 2 mg/lb; Fallow deer (*Dama dama*): 2 to 4 mg/lb.

For the application of xylazine in horses, the regulation stipulates that it must not be used "in horses intended for human consumption." The limits on xylazine's use in agricultural settings are extended provided that xylazine is not for "use in domestic food-producing animals," at §522.2662.

Tolazoline

USDA allows tolazoline for veterinary applications within organic livestock production at 7 CFR 205.603, with the restriction to "use only to reverse the effects of sedation and analgesia caused by xylazine, by or on the lawful written or oral order of a licensed veterinarian," and must be followed by "a meat withdrawal period of at least 8 days after administering to livestock intended for slaughter; and a milk discard period of at least 4 days after administering to dairy animals."

The FDA has approved tolazoline as an animal drug administered to horses through implantation or injection "when it is desirable to reverse the effects of sedation and analgesia caused by xylazine," at 21 CFR 522.2474. The FDA has stipulated that tolazoline be administered to horses "slowly by intravenous injection 4 mg per kilogram of body weight or 1.8 mg per pound (4 milliliters (mL) per 100 kilograms or 4 mL per 220 pounds)." Like xylazine, tolazoline may be administered, provided it not be used in "horses intended for human consumption. Federal law restricts this drug to use by or on the order of a licensed veterinarian," at §522.2474.

Action of the Substance:

Xylazine

Xylazine stimulates the α2-adrenergic receptors in the presynaptic space, which inhibits the release of the neurotransmitters norepinephrine and dopamine (Starke 1977, Hsu 1981, Kreeger et al. 1986a, Ruiz-Colon et al. 2014, Silva-Torres et al. 2014). The inhibited release of these neurotransmitters produces a range of physiological responses, including muscle relaxation, pain relief (analgesia), neural transmission inhibition (giving a general depression of activity to the central nervous system), respiratory system depression (transient hypotension and hypertension, bradycardia, cardiac arrythmias) (Garcia-Villar et al. 1981, Green et al. 1981, Kreeger et al. 1986a, Kreeger et al. 1986b, JECFA 1998a, Delehant et al. 2003, Lester et al. 2012, Otto and von Thaden 2012, Ruiz-Colon et al. 2014, Thies et al. 2017). Xylazine has also been documented to produce transient hyperglycemia due to reduction of insulin levels (Greene and Thurmon 1988, Samanta et al. 1990, JECFA 1998a, JECFA 1998b, Saha et al. 2005).

Xylazine is administered through several methods including intravenously, intramuscularly, or subcutaneously (Garcia-Villar et al. 1981, Reyes et al. 2012). Upon administration, xylazine is rapidly absorbed and distributed throughout the body (Garcia-Villar et al. 1981, JECFA 1998a, JECFA 1998b). The substance is also readily metabolized to approximately 20 metabolites, depending on the species in question (JECFA 1998a, EMEA 1999). Xylazine is also rapidly removed from the body—mostly through metabolism but also through excretion—with biological half-lives of 1-58 (Samanta et al. 1990, JECFA 1998a, EMEA 1999).

Due to the rapid distribution and metabolism of xylazine, the physiological effects of xylazine begin to subside shortly after administration of the drug (15-90 minutes), although the effects (e.g., sedation, bradycardia, respiratory depression) are not completely reversed until the bulk of the substance has been metabolized (2-36 hours) (Garcia-Villar et al. 1981, Green et al. 1981, Samanta et al. 1990, EMEA 1999). The substance and its metabolites are excreted in urine and feces (EMEA 1999). The effective dosage and resulting physiological effects are species-dependent, with ruminants (cattle, sheep) exhibiting elevated sensitivity to xylazine; therefore, their veterinary doses are adjusted to approximately $1/10^{th}$ that of other species (Garcia-Villar et al. 1981, Greene and Thurmon 1988, JECFA 1998a, EMEA 1999).

Tolazoline

Tolazoline is most commonly used as a reversal agent for sedatives, including xylazine, by competing for α2-adrenergic receptors, blocking binding events for xylazine (Levy et al. 1977, Goetzman and Milstein 1979, Kreeger et al. 1986a, Kreeger 1986b, Samanta et al. 1990, JECFA 1998a, JECFA 1998b, Pawson 2008). Tolazoline is a broad-spectrum α2-adrenergic antagonist with cholinergic and histamine interactions that are not well-defined (Grover 1961, Goetzman and Milstein 1979, McIntosh and Walters 1979, Pawson 2008, Rotta et al. 2011, Greenough et al. 2012, Ebert 2013, Coleman and Cox 2014). Therefore, the physiological responses resulting from tolazoline administration do not have well-defined mechanistic biochemical explanations, with some effects being attributed to α2-adrenergic and histamine receptor interactions (Grover 1961, Yellin et al. 1975, McIntosh and Walters 1979, Kreeger et al. 1986a, Pawson 2008, Ebert 2013, Coleman and Cox 2014).

Tolazoline affords several physiological effects, including vasodilation (increasing arterial oxygenation), transient hypotension, and histaminic gastrointestinal effects (Cotton 1965, Korones and Eyal 1975, Yellin et al. 1975, Levy et al. 1977, McIntosh and Walters 1979, Greenough et al. 2012). The α2-adrenergic antagonistic effects of tolazoline result in increased central nervous system and respiratory activity, directly reversing xylazine-induced responses (Yellin et al. 1975, Pieter et al. 1982, Kreeger et al. 1986a, Kreeger et al. 1986b, JECFA 1998a, Pawson 2008, Coleman and Cox 2014). However, the effective dosage for xylazine reversal is dependent on species and the applied xylazine dosage (Kreeger et al. 1986a, Kreeger et al. 1986b).

Tolazoline is administered intravenously, via inhalation (Greenough et al. 2012, Ebert 2013, Coleman and Cox 2014). Tolazoline is rapidly absorbed and distributed throughout the body following administration (Ebert 2013). Tolazoline metabolism is species-dependent; however, in many species, the administered tolazoline is excreted intact or with only minor metabolism (Ebert 2013). Tolazoline has a biological half-life of 313 hours and is largely excreted in urine 2-4 weeks following its administration (Ebert 2013).

Combinations of the Substance:

Xylazine

Xylazine is used as an anesthetic and analgesic in veterinary medicine, both as an individual substance, and as a co-treatment in combination with ketamine (Green et al. 1981, Kreeger et al. 1986a, Kreeger et al. 1986b, JECFA 1998a, Saha et al. 2005, Lester et al. 2012, Otto and von Thaden 2012, Reyes et al. 2012, Flecknell 2016). Studies have shown that, when the substance is used in combination with ketamine, the resulting sedation/analgesia is more effective than applications of ketamine alone (Green et al. 1981, Kreeger et al. 1986a, Otto and von Thaden 2012). Moreover, the incorporation of xylazine as a co-treatment

for ketamine-induced sedation and analgesia has been shown to reduce the ketamine's side effects (Green et al. 1981, Saha et al. 2005, Otto and von Thaden 2012).

Tolazoline

Tolazoline is not commonly used in combination with other substances (USDA 2002b). Studies have shown that tolazoline is effective for the reversal of xylazine and xylazine/ketamine–combined induced sedations (Kreeger et al. 1986a).

Status

Historic Use:

Xylazine

Xylazine has been historically used in veterinary surgical applications as an anesthetic and analgesic (Green et al. 1981, Kreeger et al. 1986a, Kreeger et al. 1986b, JECFA 1998a, JECFA 1998b, Saha et al. 2005, Lester et al. 2012, Otto and von Thaden 2012, Reyes et al. 2012, Flecknell 2016). The interaction of the substance with α2-adreneceptors results in the inhibition of norepinephrine, producing a physiological response including depression activity of the central nervous system (CNS) and resulting in sedation (EMEA 1999, Lorenz et al. 2010, Lester et al. 2012, Otto and von Thaden 2012, Ruiz-Colon et al. 2014, Silva-Torres et al. 2014, Thies et al. 2017). As previously mentioned, the substance is also used as an emetic for cats as a veterinary alternative to surgical procedures to remove foreign objects from the intestinal tract (Thies et al. 2017).

Tolazoline

Tolazoline has been historically used in veterinary medicine as a reversal agent for xylazine sedation in post-surgery applications (Levy et al. 1977, Goetzman and Milstein 1979, Kreeger et al. 1986a, Kreeger 1986b, Samanta et al. 1990, JECFA 1998a, JECFA 1998b, Pawson 2008). Tolazoline competes with xylazine for α2-adrenergic binding sites; once bound, it blocks xylazine from interacting with the receptor (Goetzman and Milstein 1979, McIntosh and Waters 1979, Pawson 2008, Greenough et al. 2012, Ebert 2013).

Organic Foods Production Act, USDA Final Rule:

Neither xylazine nor tolazoline are listed in the Organic Foods Production Act of 1990 (OFPA). Both xylazine and tolazoline are restricted to "use by or on the lawful written or oral order of a licensed veterinarian," and must be followed by "a meat withdrawal period of at least 8 days after administering to livestock intended for slaughter; and a milk discard period of at least 4 days after administering to dairy animals" at 7 CFR 205.603. Tolazoline has the additional restriction for "use only to reverse the effects of sedation and analgesia caused by Xylazine," at §205.603.

International

Canadian General Standards Board Permitted Substances List

Xylazine is listed in the CAN/CGSB-32.311-2015 — Organic production systems - permitted substances list in Table 5.3 "health care products and production aids," as a "sedative."

Tolazoline is not listed in the CAN/CGSB-32.311-2015 — Organic production systems - permitted substances list.

CODEX Alimentarius Commission, Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (GL 32-1999)

Neither xylazine nor tolazoline are listed in the CODEX.

European Economic Community (EEC) Council Regulation, EC No. 834/2007 and 889/2008

Neither xylazine nor tolazoline are listed in the EEC EC No. 834/2007 or 889/2008.

Japan Agricultural Standard (JAS) for Organic Production
Neither xylazine nor tolazoline are listed in the JAS for Organic Production.

International Federation of Organic Agriculture Movements (IFOAM)
Neither xylazine nor tolazoline are listed in IFOAM.

Evaluation Questions for Substances to Be Used in Organic Crop or Livestock Production

Evaluation Question #1: Indicate which category in OFPA that the substance falls under: (A) Does the substance contain an active ingredient in any of the following categories: copper and sulfur compounds, toxins derived from bacteria; pheromones, soaps, horticultural oils, fish emulsions, treated seed, vitamins and minerals; livestock parasiticides and medicines and production aids including netting, tree wraps and seals, insect traps, sticky barriers, row covers, and equipment cleansers? (B) Is the substance a synthetic inert ingredient that is not classified by the EPA as inerts of toxicological concern (i.e., EPA List 4 inerts) (7 U.S.C. § 6517(c)(1)(B)(ii))? Is the synthetic substance an inert ingredient which is not on EPA List 4, but is exempt from a requirement of a tolerance, per 40 CFR part 180?

(A) Xylazine and tolazoline are active ingredients for veterinary medicines. Xylazine is primarily used as a sedative, tranquilizer, and an analgesic (Garcia-Villar et al. 1981, Kreeger et al. 1986a, Kreeger et al. 1986b, JECFA 1998a, JECFA 1998b, EMEA 1999, Saha et al. 2005, Lorenz et al. 2010, Lester et al. 2012, Otto and von Thaden 2012, Flecknell 2016, Thies et al. 2017). The interactions between the substance and α2-adrenceptors results in the depression of the central nervous and respiratory systems, inducing sedation and analgesia (EMEA 1999, Lorenz et al. 2010, Lester et al. 2012, Otto and von Thaden 2012, Ruiz-Colon et al. 2014, Silva-Torres et al. 2014, Thies et al. 2017).

Tolazoline is used in veterinary medicine as a reversal agent for xylazine (Levy et al. 1977, Goetzman and Milstein 1979, Kreeger et al. 1986a, Kreeger 1986b, Samanta et al. 1990, JECFA 1998a, JECFA 1998b, Pawson 2008).

(B) Neither xylazine nor tolazoline are listed by the EPA as an inert ingredient of toxicological concern.

Evaluation Question #2: Describe the most prevalent processes used to manufacture or formulate the petitioned substance. Further, describe any chemical change that may occur during manufacture or formulation of the petitioned substance when this substance is extracted from naturally occurring plant, animal, or mineral sources (7 U.S.C. § 6502 (21)).

Xylazine is a synthetic substance produced from the multi-step process outlined in Scheme 1 (Source or Origin of the Substance). 2,6-dimethylanaline is initially employed as a nucleophile, reacting with the electrophilic acetic anhydride to form the resulting amide. The amide is reduced in the presence of sodium metal (Na^o), to form a reactive intermediate that readily reacts with carbon disulfide to yield 2,6-dimethylisothiocyanate. The electrophilic nature of the central carbon on the isothiocyanate moiety undergoes nucleophilic attack to yield *N*-(2,6-Dimethylphenyl)-5,6-dihydro-4H-1,3-thiazin-2amine; this undergoes subsequent intramolecular ring-closure under the acidic reaction conditions to give the final xylazine product, which is collected in solid form via filtration (Elliot and Ruehle 1985).

Tolazoline is a synthetic substance that is produced by a one-pot process (i.e., no intermediates are isolated) by the reaction of phenylacetaldehyde with ethylene diamine, with the incorporation of an iodine-based oxidation process as shown in Scheme 2 (Source or Origin of the Substance) (Gogoi and Konwar 2006).

In the synthesis of tolazoline phenylacetaldehyde undergoes nucleophilic attack by ethylene diamine to produce a Schiff base. The Schiff base undergoes an intramolecular ring-closure via electrophilic attack of the remaining amine functionality at the electrophilic imine carbon; this in turn undergoes subsequent oxidation with an iodine/potassium iodide mixture (I_2/KI). The final product is formed by the elimination

of the iodide to form the imidazolidine ring, which is isolated as an oil via extraction and subsequent evaporation (Gogoi and Konwar 2006).

Evaluation Question #3: Discuss whether the petitioned substance is formulated or manufactured by a chemical process, or created by naturally occurring biological processes (7 U.S.C. § 6502 (21)).

Xylazine is a synthetic substance that is produced through the multi-step reaction of 2,6-dimethylanaline as shown in Scheme 1 on the previous page (Elliot and Ruehle 1985). Tolazoline is a synthetic substance that is produced by a one-pot process (i.e., no intermediates are isolated) by the reaction of phenylacetaldehyde with ethylene diamine, with the incorporation of an iodine-based oxidation process as shown in Scheme 2 on the previous page (Gogoi and Konwar 2006).

Evaluation Question #4: Describe the persistence or concentration of the petitioned substance and/or its by-products in the environment (7 U.S.C. § 6518 (m) (2)).

Xylazine was first identified as a potential aquatic contaminant in rivers on the Iberian Peninsula in concentrations of 50–100 ng/L (Fabrega et al. 2013, Pugajeva et al. 2017). Fabrega and his colleagues identified xylazine as "one of the chemicals with the highest contribution to the total IRCAP value in the different river basins" (Fabrega et al. 2013). However, these results may not be indicative of typical use in veterinary medicine, as JSC Grindeks, a global producer of the substance, is also located on the Iberian Peninsula (Pugajeva et al. 2017).

Studies on the persistence and activity of xylazine in the soil also support the conclusion that xylazine may contribute to water pollution, due to the mobility within soils and the leaching potential of the substance (Choi et al. 2014). Choi and coworkers have reported that xylazine has a slow rate of dissipation and degradation within soil systems that may result in its environmental accumulation, a trait that may be linked to its relatively small size (Choi et al. 2014, Pugajeva et al. 2017). Choi et al. (2014) were limited to artificial soil environments (laboratory tests rather than actual environmental conditions), which are likely to impede dissipation and degradation of the substances examined.

The by-products of xylazine were not discussed in the above studies by Fabrega et al., Pugajeva et al., and Choi et al.

Evaluation Question #5: Describe the toxicity and mode of action of the substance and of its breakdown products and any contaminants. Describe the persistence and areas of concentration in the environment of the substance and its breakdown products (7 U.S.C. § 6518 (m) (2)).

Environmental studies on xylazine are discussed in Question #4 and highlight the possible persistence of the substance and its accumulation in soil systems as well as its role as an aquatic pollutant (Fabrega et al. 2013, Choi et al. 2014, Pugajeva et al. 2017).

There are no reported studies on the environmental toxicity, persistence, or concentration of tolazoline.

As described in the Characterization of Petitioned Substance section, xylazine is a neurologically active compound that interacts with α2-adreneceptors, histamine, and cholinergic receptors, resulting in sedation and analgesia (Garcia-Villar et al. 1981, Kreeger et al. 1986a, Kreeger et al. 1986b, JECFA 1998a, JECFA 1998b, EMEA 1999, Saha et al. 2005, Lorenz et al. 2010, Lester et al. 2012, Otto and von Thaden 2012, Flecknell 2016, Thies et al. 2017). However, the neurological activity of xylazine also results in undesired side effects, including inhibition of neural transmissions, giving a general depression of activity to the central nervous system; depression of the respiratory system (transient hypotension and hypertension, bradycardia, cardiac arrythmias); and transient hyperglycemia due to reduction of insulin levels (Garcia-Villar et al. 1981, Green et al. 1981, Kreeger et al. 1986a, Kreeger et al. 1986b, Greene and Thurmon 1988, Samanta et al. 1990, JECFA 1998a, Delehant et al. 2003, Saha et al. 2005, Lester et al. 2012, Otto and von Thaden 2012, Ruiz-Colon et al. 2014, Thies et al. 2017).

Signs of xylazine toxicity are typically the result of continued application, with re-administration before the previous doses have been removed from the system (Veilleux-Lemieux et al. 2013). Xylazine has been identified as a substance with moderate toxicity, giving an LD_{50} of 121–240 mg/kg in mice and rat studies (JECFA 1998a, JECFA 1998b). Across species, studies indicate that rats and mice are less sensitive to xylazine than other, larger species (JECFA 1998a). For instance, studies with cats rendered an LD_{50} of 100 – 110 mg/kg, while dogs and horses have still higher sensitivities with LD_{50}s of 20 -47 mg/kg and 15 – 70 mg/kg, respectively (JECFA 1998a).

Xylazine is readily metabolized to approximately 20 metabolites, depending on the species in question (JECFA 1998a, EMEA 1999). Among the metabolites, 2,6-xylidine has been investigated for toxicological effects (JECFA 1998a). Xylidine is present in tobacco smoke and is a degradation product of aniline derived-pesticides; therefore, the toxicological effects of the substance have been studied (JECFA 1998a). Xylidine has been identified as a substance with slight toxicity, having an LD_{50} of 600 – 1000 mg/kg in mice and rat studies (JECFA 1998a). The species dependency of xylidine is not been well-developed, with toxicity studies performed on laboratory mice and rat subjects.

Carcinogenicity studies have been negative or inconclusive, with no significant findings from single-dose exposures, although the JECFA noted that 2,6-xylidine has genotoxic and carcinogenic character (NTP 1990, JECFA 1998a, JECFA 1998b). When multiple doses of xylidine were administered to Charles River rats (10 doses per day), xylidine began accumulating within the body, resulting in significant incidences of nasal cavity adenomas and carcinomas (NTP 1990). These results show that multiple doses and bioaccumulation of xylidine reveal a carcinogenic nature; as a result of such multiple-dose studies, xylidine has been classified as a 2B substance, which may be carcinogenic to humans (IARC 1993).

Tolazoline is a synthetic α2-adrenergic antagonist that also interacts with histamine and cholinergic receptors in a temporary and reversible manner (Goetzman and Milstein 1979, McIntosh and Waters 1979, Kreeger et al. 1986a, Kreeger et al. 1986b, Samanta et al. 1990, Pawson 2008, Greenough et al. 2012, Ebert 2013). Tolazoline affords several physiological effects, including vasodilation (increasing arterial oxygenation), transient hypotension, histaminic gastrointestinal effects (Cotton 1965, Korones and Eyal 1975, Yellin et al. 1975, Levy et al. 1977, McIntosh and Walters 1979, Greenough et al. 2012). There are no published toxicity or carcinogenicity studies on the toxicity or lethal dosages of tolazoline.

Evaluation Question #6: Describe any environmental contamination that could result from the petitioned substance's manufacture, use, misuse, or disposal (7 U.S.C. § 6518 (m) (3)).

Environmental studies on xylazine are discussed in Question #4 and highlight the possible persistence of the substance and its accumulation in soil systems as well as its role as an aquatic pollutant (Fabrega et al. 2013, Choi et al. 2014, Pugajeva et al. 2017). Reports of xylazine environmental contamination on the Iberian Peninsula may be linked with xylazine manufacturing, resulting in high contributions to water pollution in Iberian river systems (Fabrega et al. 2013, Pugajeva et al. 2017). The leaching ability of xylazine and its reported slow degradation in aquatic systems make wastewater pollution a concern in cases of improper use or disposal (Fabrega et al. 2013, Choi et al. 2014, Pugajeva et al. 2017).

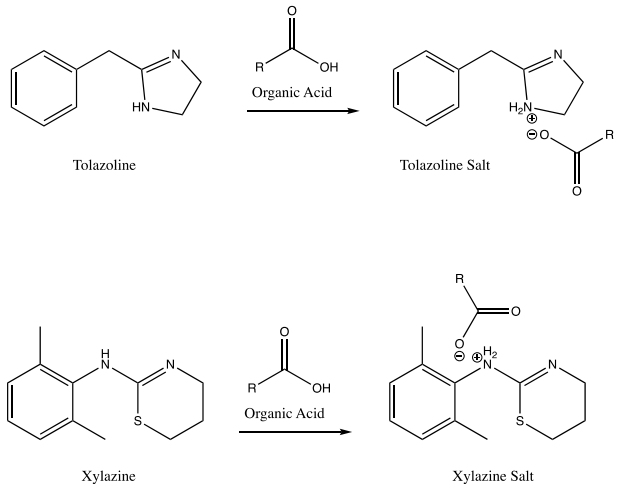
There are no reported studies on the environmental toxicity, persistence, or concentration of tolazoline.

Evaluation Question #7: Describe any known chemical interactions between the petitioned substance and other substances used in organic crop or livestock production or handling. Describe any environmental or human health effects from these chemical interactions (7 U.S.C. § 6518 (m) (1)).

Xylazine and tolazoline are synthetic substances with documented reactivity towards α2-adrenergic, histamine, and cholinergic receptors (Goetzman and Milstein 1979, McIntosh and Waters 1979, EMEA 1999, Pawson 2008, Lorenz et al. 2010, Greenough et al. 2012, Lester et al. 2012, Otto and von Thaden 2012, Ebert 2013, Ruiz-Colon et al. 2014, Silva-Torres et al. 2014, Thies et al. 2017). Due to their neurological activities, specifically their interactions with cholinergic receptors, they may cause interactions with atropine, a cholinergic antagonist (USDA 2002a). Atropine has been approved for use in organic livestock for the

treatment of organophosphate poisoning and acts via competition with acetylcholine for binding interactions with choline receptors (USDA 2002a). Moreover, reports of atropine interactions with xylazine, as with tolazoline, show some ability to reverse the physiological effects of xylazine sedation (Greene and Thurmon 1988, Ruiz-Colon et al. 2014).

As Figure 1 depicts in the Characterization of the Petitioned Substances section, the structures of xylazine and tolazoline both feature nitrogen atoms with localized electron pairs; this structure renders them efficient bases. As such, the substances will react with acids (Scheme 3), resulting in xylazine and tolazoline salts, with the cation being supplied by the acid used in the reaction. Due to the basic nature of the substance, it is likely to undergo neutralization reactions with allowed organic acids such as peracetic acid (7 CFR 205.601(a)), ammonium carbonate (7 CFR 205.601(e)), boric acid (7 CFR 205.601(e)), humic acids (7 CFR 205.601(j)), sulfurous acid (7 CFR 205.601(j)), phosphoric acid (7 CFR 205.603(a)), and formic acid (7 CFR 205.603(b)).



Scheme 3

Due to the ionic nature of the product (xylazine or tolazoline salt), with its identity defined by the acid used in the reaction (identity of R for acids shown in Scheme 3), the effects of potential salts are difficult to predict. Xylazine and tolazoline salts (hydrochlorides) are used for medicinal purposes, and the substances are likely to maintain their medicinal activity in salt forms (PubChem 5504, PubChem 5707, PubChem 6048, PubChem 68554, Sigma-Aldrich 2006, Sigma-Aldrich 2014a, Sigma-Aldrich 2014b, Sigma-Aldrich 2017). However, due to the charged nature of the salt, the ionic form of the substance may be absorbed differently from the neutral form, which could influence the biological delivery mechanisms.

As discussed throughout this technical report, xylazine and tolazoline have both been approved for veterinary applications within organic livestock production. These substances interact with one another by competition for neurological activity by interactions with α2-adreneceptors, cholinergic, and histamine receptors (Levy et al. 1977, Goetzman and Milstein 1979, Kreeger et al. 1986a, Kreeger 1986b, Samanta et al. 1990, JECFA 1998a, JECFA 1998b, Pawson 2008).

Due to the veterinary applications of the substances for approved organic use, they are unlikely to be combined with any of the above acids. Undesirable chemical reactions are unlikely to occur when used as approved, making environmental and human health concerns unlikely.

<u>Evaluation Question #8:</u> Describe any effects of the petitioned substance on biological or chemical interactions in the agro-ecosystem, including physiological effects on soil organisms (including the salt index and solubility of the soil), crops, and livestock (7 U.S.C. § 6518 (m) (5)).

Environmental studies on xylazine are discussed in Question #4 and highlight the possible persistence of the substance and its accumulation in soil systems as well as its role as an aquatic pollutant (Fabrega et al.

2013, Choi et al. 2014, Pugajeva et al. 2017). However, these studies do not discuss the physiological impacts of the substance on the environment.

There are no reported studies on the environmental toxicity, persistence, or concentration of tolazoline.

As discussed in Evaluation Question #5, xylazine and tolazoline are substances with demonstrated neurological activity used in veterinary medicine; therefore, they will likely elicit physiological responses if absorbed by livestock. These responses include inhibition of neural transmissions, giving a general depression of activity to the central nervous system, depression of the respiratory system (transient hypotension and hypertension, bradycardia, cardiac arrythmias), and transient hyperglycemia due to reduction of insulin levels vasodilation (increasing arterial oxygenation), and histaminic gastrointestinal effects (Cotton 1965, Korones and Eyal 1975, Yellin et al. 1975, Levy et al. 1977, McIntosh and Walters 1979, Garcia-Villar et al. 1981, Green et al. 1981, Kreeger et al. 1986a, Kreeger et al. 1986b, Greene and Thurmon 1988, Samanta et al. 1990, JECFA 1998a, Delehant et al. 2003, Saha et al. 2005, Greenough et al. 2012, Lester et al. 2012, Otto and von Thaden 2012, Ruiz-Colon et al. 2014, Thies et al. 2017). The degree of expression induced by the substance is species-dependent, with ruminants showing increased sensitivity (Garcia-Villar et al. 1981, Greene and Thurmon 1988, JECFA 1998a, EMEA 1999).

Evaluation Question #9: Discuss and summarize findings on whether the use of the petitioned substance may be harmful to the environment (7 U.S.C. § 6517 (c) (1) (A) (i) and 7 U.S.C. § 6517 (c) (2) (A) (i)).

Environmental studies on xylazine are discussed in Question #4 and highlight the possible persistence of the substance and its accumulation in soil systems as well as its role as an aquatic pollutant (Fabrega et al. 2013, Choi et al. 2014, Pugajeva et al. 2017). While these studies report the possibility of xylazine environmental contamination, they do not discuss the effects of the substance beyond its persistence in soil and aquatic systems.

There are no reported studies on the environmental toxicity, persistence, or concentration of tolazoline.

Evaluation Question #10: Describe and summarize any reported effects upon human health from use of the petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (i), 7 U.S.C. § 6517 (c) (2) (A) (i)) and 7 U.S.C. § 6518 (m) (4)).

Farbenfabriken Bayer developed xylazine, a synthetic α2-adrenergic agonist, in 1962 to treat human hypertension (Kreeger et al. 1986a Kreeger et al. 1986b, Greene and Thurmon 1988, EMEA 1999, Lester et al. 2012, Thies et al. 2017). Xylazine is a substance with potent hypnotic and muscle-relaxation properties. The side effects of xylazine include significant cardiac arrythmias, which has resulted in its lack of approval for human medical applications (Green et al. 1981, EMEA 1999, Reyes et al. 2012). Due to the lack of approval for use in human medical applications, information on the mode of action and toxicity of xylazine is limited.

Reported cases of xylazine in humans have shown physiological effects like those seen in veterinary applications (Samanta et al. 1990, JECFA 1998a). Upon absorption of xylazine, patients were difficult to rouse and showed signs of confusion (indicative of central nervous system and neuropathic depression) and expressed symptoms of bradycardia, hypotension (respiratory depression), and hyperglycemia (Gallanosa et al. 1981, Spoerke et al. 1986, Samanta et al. 1990). These symptoms indicate that xylazine operates through similar biochemical mechanisms in humans as in veterinary species. This is likely due to interactions with α2-adreneceptors and histamine and cholinergic receptors (JECFA 1998a). With regard to human carcinogenicity, no studies of direct effects have been published; however, the IARC has designated the xylazine metabolite xylidine as potentially carcinogenic to humans based on studies with laboratory animals (NTP 1990, IARC 1993, JECFA 1998a).

The lethal dosage of xylazine in humans is not well known and appears to vary dramatically between individuals (Spoerke et al. 1986, Ruiz-Colon et al. 2014). Fatal doses of xylazine recorded have been as low

as 40 mg, while other individuals have survived exposure to levels as high as 2400 mg (Spoerke et al. 1986, Ruiz-Colon et al. 2014).

Tolazoline is a synthetic substance that offers broad-spectrum α2-adreneergic antagonism and interacts with histamine and cholinergic receptors (Goetzman and Milstein 1979, McIntosh and Waters 1979, Pawson 2008, Greenough et al. 2012, Ebert 2013). Tolazoline is used in both human and veterinary medical applications. In human medicine, tolazoline is used to treat hypotension, hypertension, newborn respiratory distress, and congenital heart disease through interactions with α2-adrenergic and histamine receptors (Grover et al. 1961, Cotton 1965, Korones and Eyal 1975, Yellin et al. 1975, Levy et al. 1977, Goetzman and Milstein 1979, McIntosh and Walters 1979, Kreeger et al. 1986a, Greenough et al. 2012). These treatments rely on the ability of the substance to induce vasodilation, increasing arterial oxygenation (Levy et al. 1977, Kreeger et al. 1986a, Kreeger et al. 1986b). However, no studies on the toxicity, carcinogenicity, or lethal dosages of tolazoline have been published.

Evaluation Question #11: Describe all natural (non-synthetic) substances or products which may be used in place of a petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (ii)). Provide a list of allowed substances that may be used in place of the petitioned substance (7 U.S.C. § 6518 (m) (6)).

Xylazine and tolazoline are active ingredients for veterinary medicines. Xylazine is primarily used as a sedative, tranquilizer, and an analgesic (Garcia-Villar et al. 1981, Kreeger et al. 1986a, Kreeger et al. 1986b, JECFA 1998a, JECFA 1998b, EMEA 1999, Saha et al. 2005, Lorenz et al. 2010, Lester et al. 2012, Otto and von Thaden 2012, Flecknell 2016, Thies et al. 2017). No natural alternatives are common for either substance (i.e., a sedative alternative for xylazine or a xylazine-reversal agent as a tolazoline alternative). Moreover, while there are several synthetic alternatives for both substances, no other synthetic alternatives have been approved by the USDA for use in organic agricultural production.

Evaluation Question #12: Describe any alternative practices that would make the use of the petitioned substance unnecessary (7 U.S.C. § 6518 (m) (6)).

As described in Evaluation Question #11, the substances are used only for veterinary applications, with no natural alternatives or USDA-approved synthetic alternatives. No alternative practices that would make the anesthetic agent unnecessary exist.

Tolazoline may be made unnecessary by allowing the veterinary subject to recover from the effects of xylazine by natural metabolism of the substance, rather than its active reversal. However, the rate of xylazine metabolism is species-dependent; therefore, this may prove problematic in species with slower metabolic rates (e.g., cattle) (Garcia-Villar et al. 1981, Green et al. 1981, Samanta et al. 1990, EMEA 1999). The active reversal of xylazine sedation also reverses xylazine side effects (e.g., bradycardia, hyperglycemia), which may be necessary to treat unexpected medical conditions and responses to xylazine administration (Yellin et al. 1975, Pieter et al. 1982, Kreeger et al. 1986a, Kreeger et al. 1986b, JECFA 1998a, Pawson 2008, Coleman and Cox 2014).

Report Authorship

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All individuals are in compliance with Federal Acquisition Regulations (FAR) Subpart 3.11—Preventing Personal Conflicts of Interest for Contractor Employees Performing Acquisition Functions.

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