

## XYLAZINE/TOLAZOLINE

### Livestock

#### Executive Summary

Xylazine is an alpha-2 adrenergic agonist used as a sedative, analgesic, and muscle relaxant in veterinary medicine. Tolazoline is a mixed alpha-1 and alpha-2 adrenergic receptor antagonist used in both animals and humans, and in animals it is used to reverse the effects of xylazine sedation. Currently, both xylazine and tolazoline are not approved by the FDA for use in food-producing animals, and are not on the National List of approved synthetic materials in organic livestock production. The goal of the xylazine/tolazoline petition is attain approval for these materials for organic livestock use.

#### Summary of TAP Reviewers' Analyses<sup>1</sup>

<i>Synthetic/ Nonsynthetic</i>	<i>Allow without restrictions?</i>	<i>Allow only with restrictions? (See Reviewers' comments for restrictions)</i>
Synthetic (3)	Yes (1) No (2)	No (2)

#### Identification

##### Chemical names:

Xylazine: 2-(2,6-xylidino)-5,6-dihydro-4H-1,3-thiazine (hydrochloride) (IUPAC); N-(2,6-dimethylphenyl)-5,6-dihydro-4H-1,3-thiazine-2-amine (hydrochloride) (CAS); C<sub>12</sub>H<sub>16</sub>N<sub>2</sub>S \*HCl

Tolazoline: 4,5-dihydro-2-(phenylmethyl)-1H-imidazole, C<sub>10</sub>H<sub>12</sub>N<sub>2</sub> \*HCl

##### Other names:

Xylazine: N-(5,6-dihydro-4H-1,3-thiazinyl)-2,6-xylidine (hydrochloride); 2-(2,6-dimethylanilino)-5,6-dihydro-4H-1,3-thiazine (hydrochloride); 2-(2,6-dimethylphenylamino)-4H-5,6-dihydro-1,3-thiazine (hydrochloride); BAY 1470, BAY VA 1470, Rompun hydrochloride, WH 7286, Xilazina [INN-Spanish], Xylazinum [INN-Latin]

Tolazoline: 2-Benzyl-2-imidazoline, 2-Benzyl-4,5-imidazoline, 2-Benzylimidazoline, 2-Benzylimidazoline, 4,5-Dihydro-2-(phenylmethyl)-1H-imidazole, 4,5-Dihydro-2-(phenylmethyl)-1H-imidazole, 5-23-06-00488 (Beilstein Handbook Reference), Artonil, BRN 0128757, Benzazoline, Benzidazol, Benzolin, Benzolin (VAN), Benzolin (vasodilator) (VAN), Benzylimidazoline, Benzylimidazoline, Ciba 3259, Dilatol ASI, Divascol, Imidalin, Kasimid, Lambril, NSC 35110, Olitensol, Peripherine, Phenylmethyylimidazoline, Prefaxil, Priscol, Priscoline, Tolazolin, Tolazolina [INN-Spanish], Tolazolinum [INN-Latin], Vasimid, Vasodil, Vasodilatan

##### CAS Number:

Xylazine: 7361-61-7

Tolazoline: 59-98-3

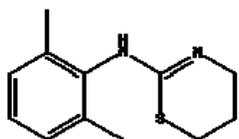
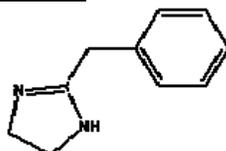
<sup>1</sup> This Technical Advisory Panel (TAP) review is based on the information available as of the date of this review. This review addresses the requirements of the Organic Foods Production Act to the best of the investigator's ability, and has been reviewed by experts on the TAP. The substance is evaluated against the criteria found in section 2119(M) of the OFPA [7 USC 6517(m)]. The information and advice presented to the NOSB is based on the technical evaluation against that criteria, and does not incorporate commercial availability, socio-economic impact, or other factors that the NOSB and the USDA may want to consider in making decisions.

**Other numbers:****Xylazine:**

ACX #X1017277-3  
EINECS #230-902-1

**Tolazoline:**

ACX #X1019515-6  
EINECS #200-448-9  
Generic #55875

**Chemical structures:****Xylazine:****Tolazoline:****Characterization****Composition/Properties:****Xylazine:**

Molecular Weight: 220.3318; 256.79 with HCl

Pure active ingredient: Assay mm. 99%

Appearance: White or almost white crystalline substance

Melting point: 165-168°C

Solubility: Freely soluble in water, very soluble in methanol and chloroform, practically insoluble in hexane and ether

**Tolazoline**

Molecular Weight: 160.2182

Appearance: White to off-white crystalline powder

Melting Pt : 174°C

Solubility: Freely soluble in water and alcohol. Water solubility is 373 mg/L at 25°C. Solutions are slightly acid to litmus.<sup>2</sup>

Vapor Pressure: 1.02E-005 mm Hg at 25°C

pKa Dissociation Constant: 10.3-10.5

Henry's Law Constant: 5.18E-008 atm-m<sup>3</sup>/mole at 25°C

Atmospheric OH Rate Constant: 7.66E-011 cm<sup>3</sup>/molecule-sec at 25°C+<sup>3</sup>

**Specific Uses/Actions:**

Xylazine is an alpha-2 adrenergic agonist and clonidine analogue used as a sedative, analgesic, and muscle relaxant in veterinary medicine. Its sedative and analgesic properties are related to nervous system depression, acting on presynaptic and postsynaptic receptors of the central and peripheral nervous systems. Its effects include bradycardia, hypotension, and inhibition of the effects of postganglionic nerve stimulation. Administration of tolazoline reverses xylazine's effects, resulting in rapid recovery from sedation. The competitive blocking of the

<sup>2</sup> "Tolazoline." USP DI Approved Drug Products and Legal Requirements. Vol. 3. The United States Pharmacopeial Convention, Inc., 2001. pp. 537.

<sup>3</sup> "4,5-dihydro-2-(phenylmethyl)-1H-imidazole." SRC PhysProp Database.

<http://esc.syrres.com/interkow/webprop.exe?CAS=59-98-3&submit=Submit+CAS>

alpha-2 adrenergic receptor by tolazoline displaces xylazine from these sites and thereby rapidly cancels the effect of the xylazine. Onset of arousal is usually apparent within 5 minutes of tolazoline administration, depending on the depth and duration of xylazine-induced sedation.<sup>4,5</sup> Xylazine is not used in humans.

Xylazine can be administered intravenously, intramuscularly, subcutaneously or orally. The commercial product contains 23.32 mg/ml xylazine hydrochloride in water based injectable solution. Xylazine can be obtained also as pure crystalline powder. There is a significant species dependent response to xylazine administration. Intramuscular dose of up to 0.3 mg/kg for cattle has been suggested by the manufacturer. The recommended doses for horses were 0.6 mg/kg and for sheep 1.0 mg/kg (Garcia-Villar et al., 1981). For dogs the dose was higher.<sup>6</sup>

For xylazine, see also FAO/WHO report, p. 12.

Tolazoline is used in both humans and animals. It belongs to the synthetic group of alpha-adrenergic blocking agents known as the imidazoline derivatives. It is a mixed alpha-1 and alpha-2 adrenergic receptor antagonist that competitively inhibits alpha-adrenoceptors. Tolazoline is also a direct peripheral vasodilator. It has direct actions on blood vessels—decreasing the pulmonary arterial pressure and peripheral resistance, and increasing venous capacitance and cardiac output.<sup>7,8</sup> Vasodilation is produced by means of a direct effect on peripheral vascular smooth muscle and indirect effects produced, in part, by release of endogenous histamine. Cardiac effects are sympathomimetic, resulting in cardiac stimulation, both inotropic and chronotropic.<sup>9</sup> Tolazoline also stimulates secretion by gastric, salivary, lacrimal, and sweat glands.<sup>10</sup>

In humans tolazoline is used in treatment of persistent pulmonary hypertension of the newborn (persistent fetal circulation) when systemic arterial oxygenation cannot be maintained by supplemental oxygen and/or mechanical ventilation. It is also used in disorders of the peripheral vascular bed such as gangrene and Raynaud's disease. Its antihistaminic actions can cause gastric stimulation.<sup>11,12</sup> Gastric effects may be parasympathomimetic—involving stimulation of gastrointestinal tract that is blocked by atropine—or involving stimulation of gastric secretion. Tolazoline is no longer considered effective in treating spastic peripheral vascular disorders.<sup>13</sup> However, it may be used as an aid in visualizing distal peripheral vessels during arteriography. Alternatives to tolazoline in the newborn include prostaglandins or nitric oxide.<sup>14</sup> Veterinary applications of tolazoline include use as a reversal of the sedative/analgesic xylazine hydrochloride in animals during surgery. Side effects in animals include CNS excitement, muscle tremors, salivation, increased respiratory rates, and hyperemic mucous membranes.<sup>15</sup>

<sup>4</sup> LLOYD, Inc. Research, 1989-1995. <http://www.lloydinc.com/labs/tolazine.html>

<sup>5</sup> Soback, S. Xylazine monograph. *Residues of some veterinary drugs in animals and foods: FAO Food and Nutrition Paper (41/9)*. 1997. <http://www.fao.org/docrep/W4601E/w4601e0f.htm>

<sup>6</sup> Soback, S. Xylazine monograph. *Residues of some veterinary drugs in animals and foods: FAO Food and Nutrition Paper (41/9)*. 1997. <http://www.fao.org/docrep/W4601E/w4601e0f.htm>

<sup>7</sup> Tranquilli, W.J. and Thurmon, J.C. *Alpha adrenoceptor pharmacology*. JAVMA 1984; 184: pp. 1400-1402. <http://www.lloydinc.com/labs/tolazine.html>

<sup>8</sup> “Drugs and the Nervous System.” <http://chemweb.calpoly.edu/chem/bailey/377/TextPart3.pdf>

<sup>9</sup> “USP DI Professional Information: Tolazoline hydrochloride.” Drugs.com.

<http://www.drugs.com/index.cfm?pageID=0&xml=001658&type=pros&bn=Tolazoline%20Hydrochloride>

<sup>10</sup> “Chapter 10: Catecholamines, Sympathomimetic Drugs, & Adrenergic Receptor Antagonists.” *Goodman and Gilman's The Pharmacological Basis of Therapeutics, Tenth ed.* Ed. J.G. Hardman, L.E. Limbird. New York: McGraw-Hill, 2001. pp. 245.

<sup>11</sup> Tranquilli, W.J. and Thurmon, J.C. *Alpha adrenoceptor pharmacology*. JAVMA 1984; 184: pp. 1400-1402. <http://www.lloydinc.com/labs/tolazine.html>

<sup>12</sup> “Drugs and the Nervous System.” <http://chemweb.calpoly.edu/chem/bailey/377/TextPart3.pdf>

<sup>13</sup> “USP DI Professional Information: Tolazoline hydrochloride.” Drugs.com.

<http://www.drugs.com/index.cfm?pageID=0&xml=001658&type=pros&bn=Tolazoline%20Hydrochloride>

<sup>14</sup> Gouyon, J.B., and Francoise, M. “Vasodilators in persistent pulmonary hypertension of the newborn: a need for optimal appraisal of efficacy,” *Dev. Pharmacol. Ther.*, vol. 19. 1992. pp. 62-68.

<sup>15</sup> Blake, John. “Alpha-2-adrenergic antagonists.” 23 April 2001.

[http://www.uaf.edu/iacuc/training/module\\_2/2\\_alpha\\_2\\_antagonist.html](http://www.uaf.edu/iacuc/training/module_2/2_alpha_2_antagonist.html)

Following are examples of veterinary dosages of both xylazine and tolazoline:

### **Veterinary Dosages:**

#### Xylazine:

Bird	Used in combination with ketamine
Cat	0.5 mg/lb BW IV (Kinsell, 1986) 1 mg/lb BW IM, SC (Kinsell, 1986)
Dog	0.5 mg/lb BW IV (Kinsell, 1986) 1 mg/lb BW IM, SC (Kinsell, 1986)
Goat	0.05-1 mg/kg BW IM (Swindle and Adams, 1988); however this is considered unpredictable when given IM 0.01 mg/kg BW IV (NCSU, 1987)
Guinea pig	3-5 mg/kg BW IM (Harkness and Wagner, 1983)
Hamster	4 mg/kg BW IM (Bauck, 1989)
Mouse	4-8 mg/kg BW IM (Harkness and Wagner, 1983) 10 mg/kg BW IP (Flecknell, 1987)
NHP	1-2 mg/kg BW IM (Green, 1982; CCAC, 1984)
Rat	4-8 mg/kg BW IM (Harkness and Wagner, 1983) 1-3 mg/kg BW IM (Flecknell, 1987)
Rabbit	3-5 mg/kg BW IM (Harkness and Wagner, 1983) 5 mg/kg BW IM (Hughes, 1981) 1-3 mg/kg BW IM (Flecknell, 1987)
Sheep	0.05-1 mg/kg BW IM (Swindle and Adams, 1988) 0.1-0.15 mg/kg BW slow IV (NCSU, 1987)
Swine	10 mg/kg BW IM (Swindle and Adams, 1988) <sup>16</sup>

#### Tolazoline:

Bird	15 mg/kg BW IV (Sinn, 1997)
Bovine	0.2 mg/kg BW IV (Schultz, 1989) 0.5 mg/kg BW SC (Schultz, 1989)
Goat	2-5 mg/kg BW IV slowly over 1 min (NCSU, 1987)
Sheep	2-5 mg/kg BW IV slowly over 1 min (NCSU, 1987) <sup>17</sup>

### **Combinations:**

In veterinary anesthesia, xylazine and ketamine are often used in combination. Ketamine, unlike xylazine, is used in both humans and other animals. It is indicated to provide anesthesia for short diagnostic and surgical procedures that do not require skeletal muscle relaxation, as well as to induce anesthesia prior to administration of other general anesthetics.<sup>18</sup> Xylazine may also be used as a sedative prior to the administration of lidocaine, a local anesthetic.<sup>19</sup>

This petition, however, is in regard to the use of xylazine as the sole anesthetic.

### **Status**

<sup>16</sup> "Xylazine." *Analgesics and Sedatives*. Yale Animal Resources Center, Yale University, 1999. <http://info.med.yale.edu/yarc/vcs/analgesics2.htm#Xylazine> 17 February 2000.

<sup>17</sup> "Tolazoline." *Miscellaneous Drugs*. Yale Animal Resources Center, Yale University, 1999. <http://info.med.yale.edu/yarc/vcs/miscellaneous3.htm#Tolazoline> 17 February 2000.

<sup>18</sup> "Ketamine hydrochloride." Drugs.com. <http://drugs.com/index.cfm?pageID=0&xml=001365&type=pros&bn=Ketamine%20Hydrochloride>

<sup>19</sup> Weary, Daniel M. "Calf Management." *BC Organic Grower*. vol 4, no 3. Summer 2001. [http://www.certifiedorganic.bc.ca/Booksand/BCOrganicGrower/Vol\\_4-3.pdf](http://www.certifiedorganic.bc.ca/Booksand/BCOrganicGrower/Vol_4-3.pdf)

**Historic Use by Organic Farmers:**

Although xylazine is not approved for use in food-producing animals in the United States, xylazine is still one of the most widely used sedatives in non-organic farming, particularly in ruminants. It is approved for use in food animals in Canada, the UK, France, Germany, and Switzerland. Extension of cattle recommendations to goats and sheep is supported by pharmacokinetic data that indicate similar half-lives and volumes of distribution in all species studied.

<sup>20</sup>

Thusfar, both xylazine and tolazoline are unavailable synthetic materials to organic farmers.

**USDA, FDA Final Rule:**

The **FDA** has approved xylazine hydrochloride for use as a veterinary anesthetic, and tolazoline hydrochloride as a reverser of xylazine, but in both cases, use of these medications in “food-producing animals” is specifically unapproved. Tolazoline is limited to use in horses and its safety has not been evaluated for reversing xylazine used as a preanesthetic to a general anesthetic.

Directly following are pertinent excerpts from the **USDA** Federal Organic Foods Production Act of 1990 regarding acceptable practices in the medical treatment of livestock, and Title 21 of the Federal Code of Regulations detailing the **FDA** status of both xylazine and tolazoline.

After are the recommendations by USDA subsidiaries the **NOP** and the **NOSB** with regard to organic livestock production and the use of xylazine and tolazoline in organic livestock.

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**FEDERAL ORGANIC FOODS PRODUCTION ACT OF 1990*****6509 ANIMAL PRODUCTION PRACTICES AND MATERIALS.***

(a) **In General.** Any livestock that is to be slaughtered and sold or labeled as organically produced shall be raised in accordance with this chapter.

(d) **Health Care.**

(1) **Prohibited Practices.** For a farm to be certified under this chapter as an organic farm with respect to the livestock produced by such farm, producers on such farm shall not

(A) use subtherapeutic doses of antibiotics;

(B) use synthetic internal paraciticides on a routine basis; or

(C) administer medication, other than vaccinations, in the absence of illness.

(2) **Standards.** The National Organic Standards Board shall recommend to the Secretary standards in addition to those in paragraph (1) for the care of livestock to ensure that such livestock is organically produced.

(e) **Additional Guidelines.**

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<sup>20</sup> Craigmill, Arthur L., Martha Rangel-Lugo, Paul Damian, Jim E. Riviere. “Extralabel use of tranquilizers and general anesthetics.” *FARAD Digest*. 1998. <http://www.farad.org/vets/digest4.html>

(1) **Poultry.** With the exception of day old poultry, all poultry from which meat or eggs will be sold or labeled as organically produced shall be raised and handled in accordance with this chapter prior to and during the period in which such meat or eggs are sold.

(2) **Dairy Livestock** A dairy animal from which milk or milk products will be sold or labeled as organically produced shall be raised and handled in accordance with this chapter for not less than the 12-month period immediately prior to the sale of such milk and milk products.

**(f) Livestock Identification.**

(1) **In General.** For a farm to be certified under this chapter as an organic farm with respect to the livestock produced by such farm, producers on such farm shall keep adequate records and maintain a detailed, verifiable audit trail so that each animal (or in the case of poultry, each flock) can be traced back to such farm.

(2) **Records.** In order to carry our paragraph (1), each producer shall keep accurate records on each animal (or in the case of poultry, each flock) including

(A) amounts and sources of all medications administered; and

(B) all feeds and feed supplements bought and fed.

(g) **Notice and Public Comment.** The Secretary shall hold public hearings and shall develop detailed regulations, with notice and public comment, to guide the implementation of the standards for livestock products provided under this section.

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**CODE OF FEDERAL REGULATIONS**

**Title 21, Volume 6**

Revised as of April 1, 2001

**21CFR522.2662**

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES--(Continued)

PART 522--IMPLANTATION OR INJECTABLE DOSAGE FORM NEW ANIMAL DRUGS

Sec. 522.2662 Xylazine hydrochloride injection.

(a) Specifications. Xylazine hydrochloride injection is a sterile aqueous solution containing xylazine hydrochloride equivalent to 100 milligrams of xylazine in each milliliter of solution when intended for use in horses, wild deer, and elk, and 20 milligrams of xylazine per milliliter of solution when intended for use in dogs and cats.

(b) Sponsor. See 000856 in Sec. 510.600(c) of this chapter for use in horses, wild deer, and elk. See 000859 and 061651 in Sec. 510.600(c) of this chapter for use in horses, wild deer, elk, dogs, and cats. See 061690 in Sec. 510.600(c) of this chapter for use in horses, wild deer, elk, dogs, and cats. See 000010 in Sec. 510.600(c) of this chapter for use in horses only.

(c) Conditions of use.

(1) The drug is used in horses, wild deer, elk, dogs, and cats to produce sedation, as an analgesic, and a preanesthetic to local anesthesia. It may also be used in horses, dogs, and cats as a preanesthetic to general anesthesia.

(2) It is administered as follows:

(i) To horses from a solution containing 100 milligrams of xylazine per milliliter, intravenously at 0.5 milligram per pound of body weight, or intramuscularly at 1.0 milligram per pound of body weight.

(ii) To dogs and cats from a solution containing 20 milligrams of xylazine per milliliter; intravenously at 0.5 milligram per pound of body weight or intramuscularly or subcutaneously at 1.0 milligram per pound of body weight. In dogs over 50 pounds, a dosage of 0.5 mg. per pound administered intramuscularly may provide sufficient sedation and/or analgesia for most procedures.

(iii) To wild deer and elk from a solution containing 100 milligrams of xylazine (as xylazine hydrochloride) per milliliter, intramuscularly, by hand syringe or syringe dart, in the heavy muscles of the croup or shoulder as follows:

(a) Fallow deer, 2 to 4 milligrams per pound.

(b) Mule deer, sika deer, and whitedeer, 1 to 2 milligrams per pound.

(c) Elk, 0.25 to 0.5 milligram per pound.

(3) Not to be administered to food-producing animals.

(4) Federal law restricts this drug to use by or on the order of a licensed veterinarian.

[40 FR 13858, Mar. 27, 1975, as amended at 41 FR 24884, June 21, 1976; 41 FR 28265, July 9, 1976; 53 FR 4848, Feb. 18, 1988; 53 FR 23608, June 23, 1988; 53 FR 40728, Oct. 18, 1988; 55 FR 18724, May 4, 1990; 55 FR 32616, Aug. 10, 1990; 59 FR 14367, Mar. 28, 1994; 60 FR 33110, June 27, 1995; 60 FR 35122 and 35123, July 6, 1995; 61 FR 46548, Sept. 4, 1996; 62 FR 35077, June 30, 1997]<sup>21</sup>

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**CODE OF FEDERAL REGULATIONS**

**Title 21, Volume 6**

Revised as of April 1, 2001

**21CFR522.2474**

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES--(Continued)

PART 522--IMPLANTATION OR INJECTABLE DOSAGE FORM NEW ANIMAL DRUGS

Sec. 522.2474 Tolazoline hydrochloride injection.

(a) Specifications. Each milliliter of sterile aqueous solution contains tolazoline hydrochloride equivalent to 100 milligrams of base activity.

(b) Sponsor. See No. 061690 in Sec. 510.600(c) of this chapter.

(c) Conditions of use. It is used as follows:

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<sup>21</sup> 21CFR522.2662. Code of Federal Regulations. United States. Revised 1 April 2001.  
<http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/ShowCFR.cfm?FR=522.2662>

(1) Horses—

(i) Amount. Administer slowly by intravenous injection 4 milligrams per kilogram of body weight or 1.8 milligrams per pound (4 milliliters per 100 kilograms or 4 milliliters per 220 pounds).

(ii) Indications for use. For use in horses when it is desirable to reverse the effects of sedation and analgesia caused by xylazine.

(iii) Limitations. The safety of Tolazine (TM) has not been established in pregnant mares, lactating mares, horses intended for breeding, foals, or horses with metabolically unstable conditions. The safety of Tolazine has not been evaluated for reversing xylazine used as a preanesthetic to a general anesthetic. This drug is for use in horses only and not for use in food-producing animals. Users with cardiovascular disease (for example, hypertension or ischemic heart disease) should take special precautions to avoid accidental exposure to this product.

Accidental spillage on the skin should be washed off immediately with soap and water. Federal law restricts this drug to use by or on the order of a licensed veterinarian.

(2) [Reserved]

[61 FR 25785, May 23, 1996]<sup>22</sup>

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### **NOP/NOSB:**

#### **NOP:**

NOP does not name either xylazine or tolazoline on the National List as synthetics allowable in organic livestock production. The excerpts below detail practices permitted and forbidden in organic livestock production, specifically in the medical treatment of organic livestock.

#### DEPARTMENT OF AGRICULTURE

Agricultural Marketing Service

#### **7 CFR Part 205**

[Docket Number: TMD-00-02-FR]

RIN: 0581-AA40

#### **NATIONAL ORGANIC PROGRAM**

AGENCY: Agricultural Marketing Service, USDA.

ACTION: Final Rule with request for comments.

SUMMARY: This final rule establishes the National Organic Program (NOP or program) under the direction of the Agricultural Marketing Service (AMS), an arm of the United States Department of Agriculture (USDA). This national program will facilitate domestic and international marketing of fresh and processed food that is organically produced and assure consumers that such products meet consistent, uniform standards. This program establishes national standards for the production and handling of organically produced products, including a National List of substances approved for and prohibited from use in organic production and handling. This final rule establishes a national-level accreditation program to be administered by AMS for State officials and private persons who want to be accredited as certifying agents. Under the program, certifying agents will certify production and handling operations in compliance with the requirements of this regulation and initiate compliance actions to enforce program requirements. The final rule includes requirements for labeling products as organic and containing organic

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<sup>22</sup> 21CFR522.2474. Code of Federal Regulations. United States. Revised 1 April 2001.  
<http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/ShowCFR.cfm?FR=522.2474>

ingredients. This final rule also provides for importation of organic agricultural products from foreign programs determined to have equivalent organic program requirements. This program is authorized under the Organic Foods Production Act of 1990, as amended.

## **Subpart C - Organic Crop, Wild Crop, Livestock, and Handling Requirements**

### **Description of Regulations**

#### **General Requirements**

This subpart sets forth the requirements with which production and handling operations must comply in order to sell, label, or represent agricultural products as "100 percent organic," "organic," or "made with organic (specified ingredients or food group(s))." The producer or handler of an organic production or handling operation must comply with all applicable provisions of subpart C. Any production practice implemented in accordance with this subpart must maintain or improve the natural resources, including soil and water quality, of the operation. Production and handling operations which sell, label, or represent agricultural products as organic in any manner and which are exempt or excluded from certification must comply with the requirements of this subpart, except for the development of an organic system plan.

#### **Livestock Production**

Any livestock product to be sold, labeled, or represented as organic must be maintained under continuous organic management from the last third of gestation or hatching with three exceptions. Poultry or edible poultry products must be from animals that have been under continuous organic management beginning no later than the second day of life. Milk or milk products must be from animals that have been under continuous organic management beginning no later than 1 year prior to the production of such products, except for the conversion of an entire, distinct herd to organic production. For the first 9 months of the year of conversion, the producer may provide the herd with a minimum of 80-percent feed that is either organic or produced from land included in the organic system plan and managed in compliance with organic crop requirements. During the final 3 months of the year of conversion, the producer must provide the herd feed in compliance with section 205.237. Once the herd has been converted to organic production, all dairy animals shall be under organic management from the last third of gestation. Livestock used as breeder stock may be brought from a nonorganic operation into an organic operation at any time, provided that, if such livestock are gestating and the offspring are to be organically raised from birth, the breeder stock must be brought into the organic operation prior to the last third of gestation.

Should an animal be brought into an organic operation pursuant to this section and subsequently moved to a nonorganic operation, neither the animal nor any products derived from it may be sold, labeled, or represented as organic. Breeder or dairy stock that has not been under continuous organic management from the last third of gestation may not be sold, labeled, or represented as organic slaughter stock. The producer of an organic livestock operation must maintain records sufficient to preserve the identity of all organically managed livestock and all edible and nonedible organic livestock products produced on his or her operation.

Except for nonsynthetic substances and synthetic substances included on the National List that may be used as feed supplements and additives, the total feed ration for livestock managed in an organic operation must be composed of agricultural products, including pasture and forage, that are organically produced. Any portion of the feed ration that is handled must comply with organic handling requirements. The producer must not use animal drugs, including hormones, to promote growth in an animal or provide feed supplements or additives in amounts above those needed for adequate growth and health maintenance for the species at its specific stage of life. The producer must not feed animals under organic management plastic pellets for roughage or formulas containing urea or manure. The feeding of mammalian and poultry slaughter by-products to mammals or poultry is prohibited. The producer must not supply animal feed, feed additives, or feed supplements in violation of the Federal Food, Drug, and Cosmetic Act.

The producer of an organic livestock operation must establish and maintain preventive animal health care practices. The producer must select species and types of livestock with regard to suitability for site-specific conditions and resistance to prevalent diseases and parasites. The producer must provide a feed ration including vitamins, minerals, protein, and/or amino acids, fatty acids, energy sources, and, for ruminants, fiber. The producer must establish

appropriate housing, pasture conditions, and sanitation practices to minimize the occurrence and spread of diseases and parasites. Animals in an organic livestock operation must be maintained under conditions which provide for exercise, freedom of movement, and reduction of stress appropriate to the species. Additionally, all physical alterations performed on animals in an organic livestock operation must be conducted to promote the animals' welfare and in a manner that minimizes stress and pain.

The producer of an organic livestock operation must administer vaccines and other veterinary biologics as needed to protect the well-being of animals in his or her care. When preventive practices and veterinary biologics are inadequate to prevent sickness, the producer may administer medications included on the National List of synthetic substances allowed for use in livestock operations. The producer may not administer synthetic parasiticides to breeder stock during the last third of gestation or during lactation if the progeny is to be sold, labeled, or represented as organically produced. After administering synthetic parasiticides to dairy stock, the producer must observe a 90-day withdrawal period before selling the milk or milk products produced from the treated animal as organically produced. Every use of a synthetic medication or parasiticide must be incorporated into the livestock operation's organic system plan subject to approval by the certifying agent.

The producer of an organic livestock operation must not treat an animal in that operation with antibiotics, any synthetic substance not included on the National List of synthetic substances allowed for use in livestock production, or any substance that contains a nonsynthetic substance included on the National List of nonsynthetic substances prohibited for use in organic livestock production. The producer must not administer any animal drug, other than vaccinations, in the absence of illness. The use of hormones for growth promotion is prohibited in organic livestock production, as is the use of synthetic parasiticides on a routine basis. The producer must not administer synthetic parasiticides to slaughter stock or administer any animal drug in violation of the Federal Food, Drug, and Cosmetic Act. The producer must not withhold medical treatment from a sick animal to maintain its organic status. All appropriate medications and treatments must be used to restore an animal to health when methods acceptable to organic production standards fail. Livestock that are treated with prohibited materials must be clearly identified and shall not be sold, labeled, or represented as organic.

A livestock producer must document in his or her organic system plan the preventative measures he or she has in place to deter illness, the allowed practices he or she will employ if illness occurs, and his or her protocol for determining when a sick animal must receive a prohibited animal drug. These standards will not allow an organic system plan that envisions an acceptable level of chronic illness or proposes to deal with disease by sending infected animals to slaughter. The organic system plan must reflect a proactive approach to health management, drawing upon allowable practices and materials. Animals with conditions that do not respond to this approach must be treated appropriately and diverted to nonorganic markets.

The producer of an organic livestock operation must establish and maintain livestock living conditions for the animals under his or her care which accommodate the health and natural behavior of the livestock. The producer must provide access to the outdoors, shade, shelter, exercise areas, fresh air, and direct sunlight suitable to the species, its stage of production, the climate, and the environment. This requirement includes access to pasture for ruminant animals. The producer must also provide appropriate clean, dry bedding, and, if the bedding is typically consumed by the species, it must comply with applicable organic feed requirements. The producer must provide shelter designed to allow for the natural maintenance, comfort level, and opportunity to exercise appropriate to the species. The shelter must also provide the temperature level, ventilation, and air circulation suitable to the species and reduce the potential for livestock injury. The producer may provide temporary confinement of an animal because of inclement weather; the animal's stage of production; conditions under which the health, safety, or well-being of the animal could be jeopardized; or risk to soil or water quality. The producer of an organic livestock operation is required to manage manure in a manner that does not contribute to contamination of crops, soil, or water by plant nutrients, heavy metals, or pathogenic organisms and optimizes nutrient recycling.

#### **§ 205.238 Livestock health care practice standard.**

(b) When preventive practices and veterinary biologics are inadequate to prevent sickness, a producer may administer synthetic medications: Provided that such medications are allowed under § 205.603. Parasiticides allowed under § 205.603 may be used on

(1) Breeder stock, when used prior to the last third of gestation but not during lactation for progeny that are to be sold, labeled, or represented as organically produced; and

(2) Dairy stock, when used a minimum of 90 days prior to the production of milk or milk products that are to be sold, labeled, or represented as organic.

(c) The producer of an organic livestock operation must not:

(1) Sell, label, or represent as organic any animal or edible product derived from any animal treated with antibiotics, any substance that contains a synthetic substance not allowed under § 205.603, or any substance that contains a nonsynthetic substance prohibited in § 205.604.

(2) Administer any animal drug, other than vaccinations, in the absence of illness;

(3) Administer hormones for growth promotion;

(4) Administer synthetic parasiticides on a routine basis;

(5) Administer synthetic parasiticides to slaughter stock;

(6) Administer animal drugs in violation of the Federal Food, Drug, and Cosmetic Act; or

(7) Withhold medical treatment from a sick animal in an effort to preserve its organic status. All appropriate medications must be used to restore an animal to health when methods acceptable to organic production fail. Livestock treated with a prohibited substance must be clearly identified and shall not be sold, labeled, or represented as organically produced.

#### **§ 205.603 Synthetic substances allowed for use in organic livestock production.**

In accordance with restrictions specified in this section the following synthetic substances may be used in organic livestock production:

[Xylazine and Tolazoline not listed.]<sup>23</sup>

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#### **NOSB:**

The NOSB will be reviewing xylazine/tolazoline for use in livestock medical treatment as a sedative, analgesic, and colic treatment in September 2002.<sup>24</sup>

#### **Regulatory: EPA/Other Sources:**

**OSHA:** No exposure limits set for xylazine or tolazoline.<sup>25</sup>

**ACGIH:** No exposure limits set for xylazine or tolazoline.<sup>26</sup>

**NIOSH:** No exposure limits set for xylazine or tolazoline.<sup>27</sup>

<sup>23</sup> *Final Rule, 2002.* NOP. <http://www.ams.usda.gov/nop/nop2000/nop2/finalrulepages/entirerule2.htm> 2002.

<sup>24</sup> *Materials to Review.* NOSB. <http://www.ams.usda.gov/nop/materialsmay2002.pdf> 2002.

<sup>25</sup> NIOSH/OSHA Exposure Limits. <http://www.inchem.org/documents/ilodb/explimit/niosh.htm>

<sup>26</sup> ACGIH Exposure Limits. <http://www.inchem.org/documents/ilodb/explimit/acgih.htm>

<sup>27</sup> NIOSH/OSHA Exposure Limits. <http://www.inchem.org/documents/ilodb/explimit/niosh.htm>

EPA: No exposure limits set for xylazine or tolazoline.<sup>28</sup>

### **Status Among U.S. Certifiers**

#### **State Organic Certifiers:**

Minnesota: Follows USDA suggested guidelines.

Oregon: Follows USDA suggested guidelines.

Pennsylvania: Follows OMRI suggested guidelines.

### **International**

**IFOAM:** In the Basic Standards for Organic Production and Processing, Final Draft 2002, IFOAM does not list either xylazine or tolazoline as permissible, and therefore they are not allowed.

#### **FAO/WHO/JECFA:**

WHO Technical Report Series, No. 876 (in press).

#### **EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD**

Joint FAO/WHO Expert Committee on Food Additives

Rome, 4-13 June 1996

2. The Committee evaluated two adrenoceptor agonists (clenbuterol and xylazine), two anthelmintic agents (abamectin and moxidectin), seven antimicrobial agents (chlortetracycline, oxytetracycline, tetracycline, neomycin, spiramycin, thiamphenicol and tilmicosin), and two insecticides (cypermethrin and "-cypermethrin). Acceptable Daily Intakes (ADIs) or temporary ADIs were established for all of these substances except xylazine. The Committee recommended Maximum Residue Limits (MRLs) in appropriate tissues (muscle, liver, kidney and fat), milk and/or eggs for all substances except xylazine.<sup>29</sup>

#### **Xylazine**

The Committee was unable to establish an ADI for xylazine because it concluded that a metabolite, 2,6-xylydine, is genotoxic and carcinogenic. The Committee was unable to establish MRLs for xylazine because of the lack of information on metabolism and residue depletion in edible tissues.

The following information would be required for further review:

- Data on xylazine metabolism in target species sufficient to identify a suitable marker residue and target tissues.
- Additional data on residue depletion of xylazine and its metabolites in target species. These data should include evidence to show, in particular, whether 2,6-xylydine is present at the recommended withdrawal times.
- A suitable analytical method for determining the marker residue in target tissues.<sup>30</sup>

The following is the extensive report on xylazine, conducted by WHO, that led to the above ruling.

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XYLAZINE

<sup>28</sup> EPA website. <http://www.epa.gov/>

<sup>29</sup> "Reports of Advisory Bodies and Related Issues," from the 102nd Session. 15 April 1998. [http://www.who.int/gb/EB\\_WHA/PDF/EB102/ee9.pdf](http://www.who.int/gb/EB_WHA/PDF/EB102/ee9.pdf)

<sup>30</sup> Soback, S. Xylazine monograph. *Residues of some veterinary drugs in animals and foods: FAO Food and Nutrition Paper (41/9)*. 1997. <http://www.fao.org/docrep/W4601E/w4601e0f.htm>

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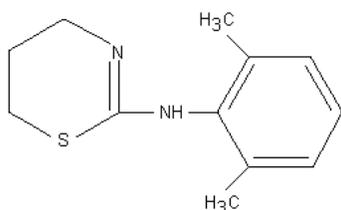
1. Explanation
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## 1. EXPLANATION

Xylazine is a clonidine analogue that acts on presynaptic and postsynaptic receptors of the central and peripheral nervous systems. It is an  $\alpha_2$ -adrenergic agonist used in animals, including cattle, horses, dogs, cats and deer, for its tranquillizing, muscle relaxant and analgesic effects, but it has numerous other pharmacological effects. It inhibits the effects of postganglionic cholinergic nerve stimulation.

Xylazine is administered by the intramuscular, intravenous or subcutaneous (in cats) routes, often in combination with other anaesthetic agents, e.g., barbiturates, chloral hydrate, halothane and ketamine.

Xylazine had not been previously evaluated by the Committee. The molecular structure of xylazine is shown below.



## 2. BIOLOGICAL DATA

- 2.1 Biochemical aspects
  - 2.1.1 Pharmacodynamics

With respect to xylazine's sedative effect, there are marked species differences in the dose rates required to achieve this state. Table 1 illustrates dosages required for various animal species (Gross & Tranquilli, 1989).

Table 1. Dosage of xylazine in various animal species

Species	Xylazine (mg/kg bw)	
	Intravenous	Intramuscular
Horses	0.5 to 1.1	1 to 2
Cattle	0.03 to 0.1 <sup>1</sup>	0.1 to 0.2 <sup>1</sup>
Sheep	0.05 to 0.1 <sup>1</sup>	0.1 to 0.3 <sup>1</sup>
Goats	0.01 to 0.5 <sup>1</sup>	0.05 to 0.5 <sup>1</sup>
Swine		2 to 3
Dogs	0.5 to 1	1 to 2
Cats	0.5 to 1	1 to 2
Birds		5 to 10

<sup>1</sup> Lower end of dose range should be used if sedation without recumbency is desired (Gross & Tranquilli, 1989).

Mydriasis is a feature of xylazine-induced sedation in the cat. The mechanism has been determined as central inhibition of parasympathetic tone in the iris due to xylazine's activation of post-synaptic alpha-2 receptors (Hsu *et al.*, 1981).

Thermoregulatory control is impaired in cats administered xylazine. They become more susceptible to hyper- and hypothermia both during and after recovery from the sedative effects of the drug. Foals have demonstrated a hypothermic response to xylazine. Thermoregulatory effects in cattle have been variable (Ponder & Clark, 1980; Booth, 1988; Robertson *et al.*, 1990).

Cardiovascular effects of xylazine include decreased heart rate and variable effects on blood pressure. Xylazine-induced arrhythmia is common in the horse due to sinoatrial and atrioventricular blocks. Arrhythmias have also been recorded in dogs, but could not be induced in sheep. The induction of cardiovascular effects may be influenced by route of administration, e.g., xylazine administered epidurally to horses produced no cardiovascular changes, whereas cattle injected by this route experienced decreases in heart rate and arterial blood pressure (Sagner *et al.*, 1969; Holmes & Clark, 1977; Freire *et al.*, 1981; Hsu *et al.*, 1981; Wasak, 1983; Singh *et al.*, 1983; Leblanc & Eberhart, 1990; Skarda *et al.*, 1990).

The effects of xylazine on respiration, acid-base balance and blood gas values vary according to species and anaesthetic combination. In cattle, xylazine causes a slowing of the respiratory rate. This is accompanied by an increase in pH and metabolic acidosis. Respiratory rate is also slowed in dogs administered xylazine, but arterial pH, pO<sub>2</sub> or pCO<sub>2</sub> are not significantly affected. The literature contains conflicting reports on the effect of xylazine on the respiratory rate of horses. Tachypnoea is characteristic of the ovine response to xylazine. Hypoxaemia induced by xylazine in sheep can be life-threatening (DeMoor & Desmet, 1971; Klide *et al.*, 1975; Holmes & Clark, 1977; Hsu *et al.*, 1989; Carter *et al.*, 1990; Wagner *et al.*, 1991).

Hyperglycaemia is induced by xylazine in adults of all target species. Increased blood glucose concentrations are accompanied by a decrease in insulin levels. In adult horses, hyperglycaemia is accompanied by increased urine volume without glycosuria. Xylazine administered to neonatal foals did not result in hyperglycaemia. The hyperglycaemic effect of xylazine is thought to be due to its direct effect on alpha-2-adrenoceptors of pancreatic islet beta cells resulting in an inhibition of insulin release (Symonds, 1976; Feldberg & Symonds, 1980; Hsu & Hummel, 1981; Thurmon *et al.*, 1982, 1984; Benson *et al.*, 1984).

Serum chemistry and cerebral spinal fluid alterations were observed in adult female goats administered intramuscularly with 0.2 mg xylazine/kg bw. Significant elevations of urea nitrogen, total protein and total cholesterol were found in serum. Glucose and urea nitrogen levels were significantly increased (P<0.01) and chloride levels were significantly decreased (P<0.05) in the cerebral spinal fluid (Amer & Misk, 1980).

Erythrocyte counts, haematocrit values and haemoglobin concentrations in cattle and dogs have shown significant but reversible decreases following xylazine administration (Eichner *et al.*, 1979; Wasak, 1983).

Gastrointestinal effects in ruminants include decreased gut motility, prolongation of gastrointestinal transit time and inhibition of reticulorumen contractions. Xylazine causes decreased muscle tone of the colon and rectum which facilitates rectal examination. Xylazine inhibition of rumen contractions can lead to tympany, which is a potential cause of death in xylazine-sedated ruminants. Ruminants are fasted prior to sedation and maintained in sternal recumbency during sedation to reduce the risk of xylazine-induced tureen tympany. Because xylazine also impairs deglutition, the head and neck of xylazine-sedated ruminants are lowered to avoid aspiration of saliva or ruminal fluid. Tolazoline (an alpha-2-adrenergic antagonist) has shown effectiveness in reversing recumbency, gastric paresis and loss of voluntary lingual control caused by xylazine in cattle (Swift, 1977; Bolte & Stupariu, 1978; Ruckebusch & Toutain, 1984).

Gastrointestinal effects in dogs and cats include decreased transit time and vomiting. The mechanism for induction of vomiting is thought to involve the effect of xylazine on alpha-2-adrenoceptors in the area postrema (the chemoreceptor trigger zone for vomiting) in the medulla oblongata (Cullen & Jones, 1977; Colby *et al.*, 1981; Hsu & McNeel, 1983; Hikasa *et al.*, 1987, 1989).

## 2.1.2 Absorption, distribution and excretion

### 2.1.2.1 Rats

Male Sprague-Dawley rats (170 g bw) were administered xylazine at dosages of 0.02 to 10 mg/kg bw (i.v.) or 0.02 to 100 mg/kg bw (oral). The drug was labelled with both <sup>35</sup>S and <sup>14</sup>C on the thiazine ring. Following oral administration, absorption was > 95% with a half-life of approximately 5 minutes. After i.v. administration, the drug was distributed within a few minutes to almost all organs but primarily to the kidneys and central nervous system. Relatively high activity concentrations occurred in the pancreas, thyroid glands, liver and cranial glands (e.g., extraorbital, sublingual). Several hours following i.v. administration of 2 mg/kg bw, only small concentrations (< 0.3 µg/g tissue) were present in the musculature. Following oral or i.v. administration, approximately 70% of the administered dose was eliminated in urine and 30% in faeces. Renal elimination following oral or i.v. administration was associated with a half-life of 2 to 3 hours. High oral doses (100 mg/kg bw) were associated with a delay in renal elimination. Faecal elimination was comparable to biliary elimination after oral or i.v. administration. Enterohepatic circulation did not occur to a notable extent (Duhm *et al.*, 1968, 1969).

### 2.1.2.2 Cattle

Three male calves (200-250 kg) and one dairy cow (450 kg) were injected intramuscularly with a 0.33 mg/kg dose of <sup>14</sup>C-xylazine labelled in the thiazine ring. Radioactivity in blood plasma reached its peak in the first 1.5 hours after injection. Total excretion of radioactivity in urine and faeces was 68, 86, 83 and 100% at 10, 24, 48 and 72 hours, respectively (Murphy & Jacobs, 1975).

In another study, five 2-month old calves and four lactating cows were administered a single intramuscular dose (0.3 or 0.6 mg/kg bw) of xylazine hydrochloride. Maximum concentrations of xylazine were achieved in blood 20 minutes after dosing. These were 0.04 mg/litre for the 0.3 mg/kg bw dose and 0.06 mg/litre for the 0.6 mg/kg bw dose. No xylazine was found in blood 8 hours after administration (Takase *et al.*, 1976).

Three lactating cows were administered an i.m. dose of 0.2 mg xylazine/kg bw and two others were administered an i.m. dose of 0.4 mg xylazine/kg bw. Milk was analysed for the presence of xylazine at 5 and 21 hours following administration. No xylazine was found at either time point for either dose. The limit of detection was 0.06 mg/litre (Pütter & Sagner, 1973).

Urinary excretion of xylazine was studied in three cows. Two were administered an i.m. dose of 0.2 mg xylazine/kg bw and one was administered an i.m. dose of 0.5 mg xylazine/kg bw. Less than 1% of the dose was excreted unchanged in the urine. Unchanged xylazine was no longer detectable 6 hours following administration. Metabolites were no longer detected in urine 10 hours after administration. The limit of detection for unchanged xylazine was 1-5 µg/litre (Pütter & Sagner, 1973).

### 2.1.2.3 Comparative pharmacokinetics in dogs, sheep, cattle and horses

The comparative pharmacokinetics of xylazine in dogs, sheep, cattle and horses are summarized in Table 2.

Pharmacokinetic parameters do not vary greatly between species following intravenous administration. The rapid elimination of xylazine is attributed to extensive metabolism, and not to rapid renal excretion of unchanged xylazine. Significant amounts of parent xylazine were not found in the urine of sheep collected at 10-minute intervals after dosing. The pharmacokinetics of xylazine were unmodified when it was administered to rabbits with occluded renal arteries. The lack of correlation between pharmacokinetic parameters and clinical effects of xylazine in cattle suggests that clinical effects in cattle are due to a rapidly produced long-acting metabolite(s) and not due to an increased sensitivity to xylazine (Garcia-Villar *et al.*, 1981).

Table 2. Single-dose pharmacokinetics of xylazine in domestic species (Garcia-Villar *et al.*, 1981)

Species	<u>Dog</u>	<u>Sheep</u>	<u>Cattle</u>	<u>Horse</u>
Body weight range (kg)	14-24	42-65	240-440	415-550
Dose rate (mg/kg bw) <sup>1</sup>	1.4	1.0	0.2	0.6
Number	4	6	4	4
<b>Intravenous<sup>2</sup></b>				
Distribution half-life (min)	2.57	1.89	1.21	5.97
Volume of distribution (l/kg)	2.52	2.74	1.94	2.46
Elimination half-life (min)	30.13	23.11	36.48	49.51
Body clearance (ml/min/kg)	81	83	42	21
<b>Intramuscular<sup>2</sup></b>				
Absorption half-life (min)	3.44	5.45	ND	2.72
Elimination half-life (min)	34.65	22.36	ND	57.7
C <sub>max</sub> (mg/ml)	0.43	0.13	ND	0.17
T <sub>max</sub> (min)	12.7	14.68	ND	12.92
Bioavailability:				
mean (%)	73.9	40.8	ND	44.6
standard deviation (%)	17.89	23.81		4.16
range (%)	52-90	17-73		40-48

<sup>1</sup> Dosage expressed as xylazine-base

<sup>2</sup> Blood sampling times after injection: 1, 2, 4, 8, 16, 30 and 120 minutes. ND = Not determined (assay was not sensitive enough to determine xylazine plasma concentrations lower than 0.01 mg/litre)

### 2.1.3 Biotransformation

#### 2.1.3.1 Rats

Studies were conducted with urine and bile of rats administered 2 mg xylazine (<sup>35</sup>S or <sup>14</sup>C)/kg bw intravenously. Approximately 20 metabolites were detected and quantified as xylazine equivalents. Approximately 8% of the dose was eliminated as unchanged compound in the urine 24 hours after dosing. The major metabolite comprised 35% of the administered dose. Final products of metabolism were inorganic sulfate and carbon dioxide (Duhm *et al.*, 1968).

Specific metabolites of xylazine were identified following incubation of xylazine with rat liver microsomes. Those metabolites were 2-(4'-hydroxy-2',6'-dimethylphenylamino)-5,6-dihydro-4H-1,3-thiazine, 2-(3'-hydroxy-2',6'-dimethylphenylamino)-5,6-dihydro-4H-1,3-thiazine, N-(2,6-dimethylphenyl)thiourea and 2-(2',6'-dimethylphenylamino)-4-oxo-5,6-dihydro-1,3-thiazine. N-(2,6-dimethylphenyl)thiourea was the major metabolite produced *in vitro*. Figure 1 shows the proposed metabolic pathways of xylazine based on these findings (Mutlib *et al.*, 1992).

#### 2.1.3.2 Horses

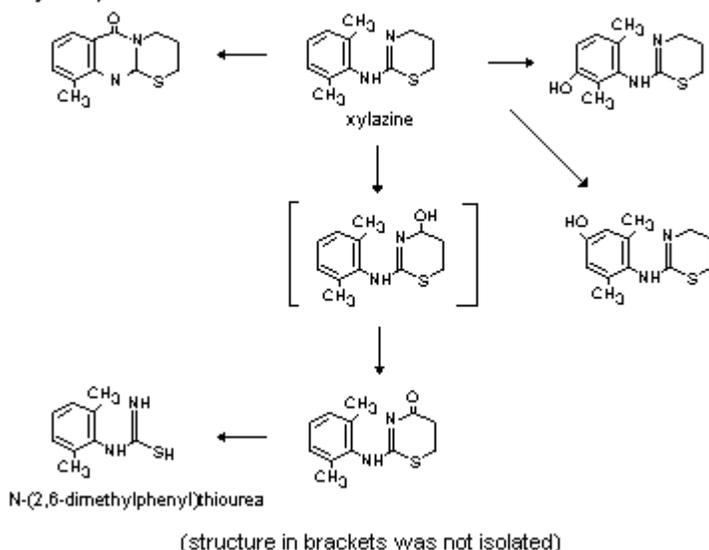
One mare was administered a 1 g dose of xylazine (route not stated) and urine was collected over 24 hours. Metabolites were recovered from horse urine only after the urine was hydrolysed with beta-glucuronidase. The major urinary metabolites detected were the same as those produced by incubating xylazine with rat liver microsomes, described in section 2.1.3.1 (Mutlib *et al.*, 1992).

### 2.1.3.3 Cattle

Urine from three cows administered an i.m. dose of 0.2 mg xylazine/kg bw (two cows) or 0.5 mg xylazine/kg bw (one cow) was examined for metabolites. One urinary metabolite, identified as 2,6-xylidine<sup>1</sup>, was found in both free and conjugated forms. The authors concluded that xylazine was essentially eliminated in cattle by rapid biotransformation. Breakdown of the thiazine ring, resulting in formation of 2,6-xylidine, was proposed as the primary biotransformation pathway (Patter & Sagner, 1973).

<sup>1</sup> 2,6-xylidine is also known as 1-amino-2,6-dimethylbenzene and as 2,6-dimethylaniline

**Figure 1. Proposed metabolic pathways of xylazine (from Mutlib *et al.*, 1992)**



## 2.2 Toxicological studies

Because 2,6-xylidine is also a chemical intermediate used in dyes, a component of tobacco smoke and a degradation product of aniline-based pesticides, its toxicology has been studied extensively. Toxicological studies conducted with this compound were also reviewed and will be presented in addition to the review and results of toxicological studies of xylazine.

### 2.2.1 Acute toxicity studies of xylazine and 2,6-xylidine

The acute systemic toxicity of xylazine has been investigated in both laboratory and domestic species. It is generally recognized that ruminants are much more sensitive than most other species to the pharmacological and toxicological effects of xylazine.

Results of LD<sub>50</sub> studies of xylazine and 2,6-xylidine are summarized in Table 3.

#### 2.2.1.1 Acute toxicity of xylazine in dogs

Adult dogs (four males, four females) and cats (two males, four females) were administered a single i.m. or i.v. dose of 22 mg xylazine/kg bw (10 times the recommended therapeutic dose). One cat out of three receiving the i.v. dose

died, and two dogs out of four receiving the i.m. dose died. All others recovered from convulsions, unconsciousness and respiratory depression with no apparent after-effects. The authors concluded that xylazine was slightly toxic in this study (Crawford *et al.*, 1970a).

Table 3. Results of acute toxicity studies on xylazine and 2,6-xylidine

<u>Species</u>	<u>Sex</u> <sup>1</sup>	<u>Route</u>	<u>LD<sub>50</sub></u> (mg/kg bw)	<u>Reference</u>
<b>Xylazine</b>				
Rat	NA	p.o.	130	Sagner, 1967
Cat	male & female <sup>2</sup>	s.c.	100-110	Bauman & Nelson, 1969
Dog	male & female <sup>3</sup>	i.m.	47	Nelson et al., 1968b
Dog	4 male & 3 female	i.v.	20-25	Nelson et al., 1968b
Horses	NA <sup>4</sup>	i.m.	60-70 <sup>5</sup>	Nelson et al., 1968a
		i.v.	15-28 <sup>5</sup>	
<b>2,6-Xylidine</b>				
Mouse	male	p.o.	710	Vernot et al., 1977
Rat		p.o.	2042	Lindstrom et al., 1969
	male	p.o.	840	Jacobson, 1972
	male	p.o.	630	Short et al., 1983
	male	p.o.	1230	Vernot et al., 1977
	female	p.o.	1160 & 1270	US National Toxicology Program, 1990
	male	p.o.	620-1250 & 1310	

<sup>1</sup> NA = Information not available

<sup>2</sup> Number per sex not stated; 10 animals were used

<sup>3</sup> Number per sex not stated; 17 animals were used

<sup>4</sup> Sex of test animals was not stated; 5 animals were used

<sup>5</sup> Minimum lethal dose

#### 2.2.1.2 Acute toxicity of xylazine in horses

Adult horses were administered 11 mg xylazine/kg bw, i.v. (three mares, one gelding) or 22 mg xylazine/kg bw, i.m. (two mares, two geldings). One mare died following i.v. administration. All other test animals recovered from treatment-related effects 24 hours following i.v. administration and 48 hours following i.m. administration. The authors concluded that the i.v. dose was slightly toxic and that the i.m. dose produced no apparent toxicity in this study (Crawford *et al.*, 1970b).

#### 2.2.2 Short-term toxicity studies

##### 2.2.2.1 Xylazine

###### a) Rats

Xylazine was administered in the diet to Wistar rats (10/sex/ group) for 32 weeks. Dosages administered were 0, 50, 100, 250 or 500 mg/kg diet (equal to 0, 3, 6, 21 or 41 mg/kg bw per day for males and 0, 4, 8, 19 or 45 mg/kg bw per day for females). Haematology, urinalysis and gross and histopathological evaluations were performed.

Decreases in body weight observed in females in the two highest-dose groups (statistically significant ( $p < 0.02$ ) at 500 mg/kg diet) were considered by the author to be treatment-related. Microscopic examination of livers, lungs and kidneys revealed that animals in all groups were diseased but no treatment-related pathology was identified. Based on the dose-related decrease in weight gain observed in females at 250 and 500 mg/kg diet, the NOEL in this

study was 100 mg/kg diet, equal to 6 mg/kg bw per day. The author regarded the dose of 250 mg/kg diet as the non-toxic application rate. The reliability of any NOEL derived from this study should be considered questionable, owing to the presence of infection in all groups (Tettenborn & Hobik, 1968a; Trossmann & Hobik, 1970).

#### b) Dogs

Dogs of undetermined breed or source were given xylazine orally in gelatin capsules for 14-16 weeks at dose levels of 25, 50 or 100 mg/kg bw per day, 5 days/week. The low-dose group consisted of one male and one female, the mid-dose group of two males and the high-dose group of two males and two females. Haematology, clinical chemistry, blood coagulation, urinalysis and postmortem gross and microscopic evaluations were performed.

During week 8 of the test, one animal in the high-dose group died and was replaced with a new animal. Postmortem gross findings in this animal included diffuse reddening of the stomach and intestinal mucous membrane.

Histopathological findings in livers (fatty degeneration and necrosis) and kidneys (tubular epithelial necrosis and fat accumulation) of the high-dose group were considered treatment-related. Fatty deposits were noted in the liver and kidneys of the low-dose female. These findings were attributed to parturition, which occurred 3 weeks before the animal was killed. The author concluded that the NOEL for this study was 50 mg/kg bw per day. The reliability of any NOEL derived from this study should be considered questionable due to the lack of a control group and small numbers of animals in each test group (Tettenborn & Hobik, 1968b).

Beagle dogs (two/sex/group) were administered xylazine orally by dietary admixture for 13 weeks. Dosages administered were 0, 10, 30 or 100 mg/kg diet (equal to 0, 0.3, 0.9 or 3 mg/kg bw per day). At the beginning of the test the animals were approximately 8.5 months old and weighed 7-10 kg. Parameters evaluated included general appearance, ophthalmology, electrocardiography, haematology, clinical chemistry, urinalyses and gross and microscopic pathology.

No treatment-related adverse effects were observed in any of the parameters evaluated. The NOEL for this study was 3 mg/kg bw per day (Tettenborn, 1969; Mawdesley-Thomas, 1970).

#### 2.2.2.2 2,6-Xylidine

##### a) Rats

Three groups (nine or ten/group) of male Fischer-344 rats were given oral (gavage) doses of 160 mg 2,6-xylidine/kg bw per day for 5, 10 or 20 days. The dosage administered was 25% of the estimated LD<sub>50</sub> determined by the investigator. A significant increase in splenic haemosiderosis (indicative of erythrocyte damage) after 20 days was noted as a treatment-related effect in this study. Splenic congestion and evidence for increased erythropoiesis were minimal (Short *et al.*, 1983).

Three groups of Sprague-Dawley rats (five/sex/group for controls, low and mid dose; four/sex/group for high dose) were administered 0, 20, 100 or 500-700 mg 2,6-xylidine/kg bw per day by gavage for 4 weeks. Treatment-related effects included decreased weight gain, decreased haemoglobin levels and hepatomegaly. In this study, the rat appeared to be about 10 times less susceptible to hepatotoxicity of 2,6-xylidine than the dog (see section 2.2.2.2b) (Magnusson *et al.*, 1971; IARC, 1993).

Two groups of 8-week-old Sprague-Dawley rats (5/sex/group) were orally administered (gastric intubation) a dose of 0 or 400 mg 2,6-xylidine per kg bw per day for 1 week immediately followed by a daily dose of 0 or 500 mg/kg bw for 3 weeks. Decreased body weight gain and hepatomegaly (most pronounced in centrilobular regions) were noted as treatment-related effects. Electron microscopy of liver tissue showed proliferation of hepatic smooth endoplasmic reticulum, which was deemed responsible for the observed hepatomegaly in treated rats. An increase in microsomal glucuronyltransferase was observed in males while aniline hydroxylase levels were increased in females. Decreases in liver glycogen and glucose-6-phosphatase activity were also observed in the centrilobular regions of treated animals (Magnusson *et al.*, 1979).

Male Osborne-Mendel rats were administered up to 10 000 mg 2,6-xylidine per kg in the diet for 3-6 months. Treatment-related effects included 25% weight reduction, anaemia, hepatomegaly with no associated microscopic changes, splenic congestion and renal toxicity (Lindstrom *et al.*, 1963).

Groups of F-344/N rats (five/sex/group) were administered doses of 0, 80, 160, 310, 620 or 1250 mg/kg bw of 2,6-xylidine in corn oil by gavage 5 days/week for 2 weeks. Parameters evaluated included clinical observations, body weight, urinalysis, haematology, blood pH and carbon dioxide determinations, and gross postmortem findings.

Treatment-related deaths occurred at and above 620 mg/kg bw. All animals in the highest dose group died before the end of the study. A decrease of more than 10% in body weight was observed in males at and above 310 mg/kg bw and in females at and above 160 mg/kg bw. Generalized leukocytosis and an increase in the number of nucleated red blood cells were observed in male rats administered 310 or 620 mg/kg bw. Slight anisocytosis, poikilocytosis and polychromasia of the red blood cells occurred more frequently in dosed animals than in vehicle control animals. Moderate poikilocytosis occurred at 310 mg/kg bw and moderate polychromasia at 310 and 620 mg/kg bw. Slightly macrocytic erythrocytes were observed at the two highest doses. Slight anisocytosis, poikilocytosis and polychromasia were observed in female rats at 310 and 620 mg/kg bw. The NOEL for this study was 80 mg/kg bw per day (US National Toxicology Program, 1990).

Groups of F-344/N rats (10/sex/group) were given doses of 0, 20, 40, 80, 160 or 310 mg/kg bw of 2,6-xylidine in corn oil by gavage, 5 days/week for 13 weeks. Parameters evaluated included clinical observations, haematology, urinalysis, serum chemistry and enzyme analyses, gross and histopathological postmortem examinations.

A decrease in body weight gain of more than 10% occurred in males and females in the highest dose group and in females at 40 and 160 mg/kg bw per day. In the highest dose group, relative liver weights were significantly ( $P=0.003$ ) increased for males and females. Relative liver weight was also increased for males in the 160 mg/kg bw group. The liver weight to brain weight and kidney weight to brain weight ratios were significantly increased in females at 310 mg/kg bw per day.

Treatment-related effects on haematology included significantly decreased total leukocyte counts in males at doses of 40 mg/kg bw or more. These were accompanied by decreases in the percentage of lymphocytes and increases in the percentage of segmented neutrophils at doses of 80 mg/kg bw or more. In males, haemoglobin levels were significantly decreased at 160 and 310 mg/kg bw and erythrocyte and haematocrit levels were decreased at 310 mg/kg bw. The NOEL for this study was 20 mg/kg bw per day (US National Toxicology Program, 1990).

#### b) Dogs

Four groups of beagle dogs (one/sex/group) were given an oral (gelatin capsule) dose of 0, 2, 10 or 50 mg 2,6-xylidine/kg bw per day for 4 weeks. Treatment-related effects included vomiting (mid- and high-dose groups), poor condition and decreased body weights (high-dose group), hyperbilirubinaemia (mid- and high-dose groups), hypoproteinaemia (mid- and high-dose groups) and fatty degenerative changes in the liver that increased in severity with increasing dose (Magnusson *et al.*, 1971; IARC, 1993).

### 2.2.3 Long-term toxicity/carcinogenicity studies

#### 2.2.3.1 Xylazine

No carcinogenicity studies have been performed with xylazine

#### 2.2.3.2 2,6-Xylidine

Four groups of Charles River CRL:COBS CD (SD) BR rats (56/sex/group) were fed diets containing 2,6-xylidine (99.06% pure) at concentrations of 0, 300, 1000 or 3000 mg/kg diet (equivalent to 0, 15, 50 or 150 mg/kg bw per day) for 102 weeks. The animals assigned to this study were F<sub>1a</sub>, generation weanlings from a multigeneration study in which animals were fed diets containing 0, 300, 1000 or 3000 mg/kg 2,6-xylidine beginning at 5 weeks of age.

Parameters evaluated in the carcinogenicity study included clinical observations, haematology, blood urea nitrogen, glucose, SGOT, alkaline phosphatase and gross and microscopic postmortem examinations.

Treatment-related clinical effects included a decrease in mean body weight gain in high-dose males and females (>10%). Mortality was significantly ( $P < 0.001$ ) increased (relative to controls) in males in the high-dose group. Mortality was also increased for mid-dose males. Survival at 105 weeks was 43/56, 40/56, 33/56 and 14/56 for males in the control, low-, mid- and high-dose groups, respectively. For females, survival was 33/56, 25/56, 32/56 and 24/56 for the controls, low-, mid- and high-dose groups, respectively.

Microscopically, a significant increase in carcinoma of the nasal cavity was observed in high-dose males (26/56;  $P < 0.001$ , life table test). For females, the incidence of carcinomas of the nasal cavity were 0/56, 0/56, 1/56 and 24/56 in the low-, mid- and high-dose groups, respectively ( $P < 0.001$ , life table test). Two adenocarcinomas were diagnosed in high-dose males. The incidence of papillary adenomas in males was 0/56 in controls, 0/56 in low-dose, 2/56 in mid-dose and 10/56 in high-dose rats ( $P = 0.001$ , incidental tumour test). For females, nasal adenomas occurred in 0/56 in controls, 0/56 in low-dose, 1/56 in mid-dose and 6/56 in high-dose rats ( $P = 0.02$ , incidental tumour test). Several unusual neoplasms of the nasal cavity were also considered to be related to treatment. These included one undifferentiated sarcoma identified in one high-dose female, rhabdomyosarcomas which occurred in two high-dose male and two high-dose females and malignant mixed tumours having features associated with both adenocarcinoma and rhabdomyosarcoma were observed in one high-dose male and one high-dose female rat. Non-neoplastic nasal cavity lesions included acute inflammation (rhinitis), epithelial hyperplasia and squamous metaplasia. These occurred at increased incidence (relative to controls) in high-dose male and female rats. The incidence of subcutaneous fibromas and fibrosarcomas combined in males was 0/56, 2/56, 2/56 and 5/56 for the control, low-, mid- and high-dose groups, respectively ( $P = 0.001$ , life table test;  $P < 0.001$  life table trend test). For females, the incidence of these tumours combined was 1/56, 2/56, 2/56 and 6/56 for controls, low-, mid- and high-dose groups, respectively ( $P = 0.01$ , life table trend test). Neoplastic nodules occurred in livers of female rats with a significant positive trend. The incidence was 0/56, 1/56, 2/56 and 4/55 for the controls, low-, mid-, and high-dose groups, respectively ( $P = 0.03$ , incidental test;  $P = 0.012$ , incidental trend test).

Treatment-related effects on haematology included decreases in erythrocyte counts and haemoglobin levels at 18 months in the high-dose males. Decreases in these parameters were also observed in the mid- and high-dose females at 12 months. The author remarked that these changes were not severe enough to be considered indicative of anaemia.

The author concluded that under the conditions of this study, 2,6-xylidine was clearly carcinogenic for male and female Charles River CD rats. This was based on the observed significant increases in the incidence of adenomas and carcinomas of the nasal cavity. Additionally the author stated that the increased incidence of subcutaneous fibromas and fibrosarcomas in male and female rats and increased incidence of neoplastic nodules of the liver in female rats could have been treatment-related (US National Toxicology Program, 1990).

The International Agency for Research on Cancer (IARC) has evaluated the carcinogenic risk of 2,6-xylidine to humans. The Working Group concluded that there was inadequate evidence in humans but sufficient evidence in experimental animals for the carcinogenicity of 2,6-xylidine. The IARC classified 2,6-xylidine as Group 2B (possibly carcinogenic to humans) (IARC, 1993).

#### 2.2.4 Special studies on teratogenicity

Xylazine was administered by gavage to groups of pregnant rats (22 animals/group) on gestation days 6 to 15, then killed on day 20 for examination of uterine contents. Dosages administered were 0, 1, 4 or 16 mg/kg bw per day. The study was conducted in accordance with the principles of Good Laboratory Practice Standards and Guidelines of the OECD, United Kingdom, FDA and Japan.

Treatment-related maternal effects included partial closing of the eyelids, underactivity, ataxia, flat posture and slightly reduced body weight gain in the high-dose group only. Fetal effects included a decrease in mean fetal weight in the high-dose group only. A teratogenic potential of xylazine was not evident at levels up to and including 16 mg/kg bw per day. The NOEL in this study was 4 mg/kg bw per day (Reynolds, 1994).

### 2.2.5 Special studies on genotoxicity

The results of genotoxicity studies with xylazine and 2,6-xylidine are summarized in Table 4.

The results of bacterial mutagenicity testing of xylazine were considered to be negative by the author. Reviewing the data, the Committee concluded that a more than two-fold reproducible increase in revertant colonies in tester strains TA1535 and TA1538 represents weak mutagenic activity, even in the absence of a clear dose-response.

### 2.2.6 Special studies on methaemoglobin and haemoglobin adduct formation with 2,6-xylidine

#### 2.2.6.1 Cats and dogs

Cats and dogs (numbers not specified) were administered an i.v. dose of 30 mg 2,6-xylidine/kg bw or an oral dose of 164 mg N-acetyl 2,6-xylidine/kg bw. 2,6-Xylidine induced a 10% methaemoglobinaemia in cats, and N-acetyl 2,6-xylidine induced a 5% methaemoglobinaemia in cats. Haemoglobin was unaffected in dogs in this study (McLean *et al.*, 1967).

Five adult cats (>24 months old) were administered an i.v. dose of 30 mg 2,6-xylidine/kg bw. Blood samples were drawn at 1, 2, 3, 4 and 5 hours after dosing and analysed for methaemoglobin formation. The mean methaemoglobin concentration determined from these sampling intervals was 7% (range = 4.8% to 8.7%). Prior to treatment the mean methaemoglobin concentration of the 152 cats used in this study was approximately 1% (McLean *et al.*, 1969).

#### 2.2.6.2 Humans

2,6-Xylidine-haemoglobin adduct levels have been found to be elevated in human patients receiving lidocaine treatment for local anaesthesia (1 mg/kg bw) or cardiac arrhythmias (up to 50 mg/kg bw, i.v.). 2,6-Xylidine-haemoglobin adducts are also found in humans with no known exposure to lidocaine. This is attributed to the 120-day lifespan of the erythrocyte and chronic exposure to environmental or iatrogenic sources of aromatic amines, e.g., cigarette smoke. The levels of 2,6-xylidine-haemoglobin adducts found correspond to an estimated daily exposure (from iatrogenic and environmental sources) of 23 µg (IARC, 1993; Bryant *et al.*, 1994).

Methaemoglobinaemia induced by i.v. administration of lidocaine was studied in 40 human cardiac patients. Treatment consisted of a 1 mg/kg bw i.v. bolus followed 15 minutes later with a 0.5 mg/kg bw i.v. bolus. Patients were maintained between and after bolus doses with an infusion rate of 1-4 mg lidocaine/min. Blood samples were drawn before treatment and 1 and 6 hours after treatment. Although the investigators found methaemoglobin levels in these patients to be significantly elevated, the increase was not large enough to be of clinical concern. The highest methaemoglobin level attained was 1.2%. The author did not address the possible role of the 2,6-xylidine metabolite in the observed increases in methaemoglobin levels in treated patients (Weiss *et al.*, 1987).

Table 4. Genotoxicity assays with xylazine and 2,6-xylidine

Test system	Test object	Concentration	Results	Reference
<b>Xylazine</b>				
<i>In vitro</i>				
Reverse mutation <sup>1</sup>	S. typhimurium			
	TA1535,	0.4-12 mg/plate	Weak positive (-S9)	Herbold, 1984
	TA1538	0.4-12 mg/plate	Weak positive (-S9)	Herbold, 1984
	TA100,	0.4-12 mg/plate	Negative	Herbold, 1984
	TA98,	0.4-12 mg/plate	Negative	Herbold, 1984
	TA1537	0.4-12 mg/plate	Negative	Herbold, 1984

Mammalian cell forward mutation <sup>1</sup>	V79/HGPRT	62-1250 µg/ml (-S9) 2-40 µg/ml (+S9)	Negative	Brendler-Schwaab, 1994
<i>In vivo</i> Cytogenetic assay	Mouse bone marrow	50 mg/kg bw, i.p. <sup>2</sup>	Negative	Herbold, 1995

Table 4. Genotoxicity assays with xylazine and 2,6-xylidine (cont'd).

<u>Test system</u>	<u>Test object</u>	<u>Concentration</u>	<u>Results</u>	<u>Reference</u>
<b>2,6-Xylidine</b>				
<i>In vitro</i>				
Reverse mutation <sup>1</sup>	S. typhimurium TA1535	100-9900 µg/plate	Negative	US National Toxicology Program, 1990
		3 µmol/plate	Negative	Florin et al., 1980 <sup>4</sup>
		0.1-10 mg/plate	Negative	Zeiger et al., 1988
	TA100	100-9900 µg/plate	Negative	US National Toxicology Program, 1990
		360 µg/plate	Negative	Florin et al., 1980 <sup>4</sup>
		0.1-10 mg/plate	Neg (-S9), Pos (+S9)	Zeiger et al., 1988 <sup>3</sup>
	480-4000 µg/plate	Negative	Kugler-Steigmeier et al., 1989	

Table 4. Genotoxicity assays with xylazine and 2,6-xylidine (cont'd).

<u>Test system</u>	<u>Test object</u>	<u>Concentration</u>	<u>Results</u>	<u>Reference</u>
Gene mutation <sup>1</sup>	TA1537	100-9900 µg/plate	Negative	US National Toxicology Program, 1990
		360 µg/plate	Negative	Florin et al., 1980 <sup>4</sup>
		0.1-10 mg/plate	Negative	Zeiger et al., 1988
	TA98	100-9900 µg/plate	Negative	US National Toxicology Program, 1990
		360 µg/plate	Negative	Florin et al., 1980 <sup>4</sup>
		0.1 - 10 mg/plate	Negative	Zeiger et al., 1988
	Mouse lymphoma L5178Y cells,	Not given	Positive	Rudd et al., 1983 <sup>7</sup>

	tk locus			
Sister chromatid exchange <sup>1</sup>	Chinese hamster ovary cells	30-1500 µg/ml	Positive	Galloway et al., 1987

Table 4. Genotoxicity assays with xylazine and 2,6-xylidine (cont'd).

<u>Test system</u>	<u>Test object</u>	<u>Concentration</u>	<u>Results</u>	<u>Reference</u>
<i>In vivo</i>				
Cytogenetic assay	ICR mouse bone marrow	350 mg/kg bw, p.o.	Inconclusive <sup>6</sup>	Parton et al., 1988
		375 mg/kg bw, p.o.	Inconclusive <sup>6</sup>	Parton et al., 1990
In vivo-in vitro DNA repair assay	Rat primary hepatocytes	40-850 mg/kg bw, p.o.	Negative	Mirsails et al., 1989
Covalent DNA binding	Rats	87.2 µCi <sup>14</sup> C-labelled 2,6-xylidine/rat, i.p. <sup>5</sup>	Positive	Short et al., 1989

<sup>1</sup> Both with and without rat liver S9 fraction

<sup>2</sup> Cyclophosphamide positive control

<sup>3</sup> Weakly positive in two of three laboratories, negative in the third

<sup>4</sup> Spot tests only

<sup>5</sup> Pretreatment with unlabelled 262.5 mg/kg bw 2,6-xylidine daily for 9 days

<sup>6</sup> Results suggest test article may not have reached target tissue (bone marrow)

<sup>7</sup> Reference was an abstract and doses were not stated in that reference

### 2.3 Observations in humans

A 34-year-old man self-injected 10 ml of a 100 mg/ml solution of xylazine intramuscularly. The estimated dose was 15 mg/kg bw. The individual was discovered (30 minutes after retiring for bed) in a deeply comatose, apnoeic and areflexic state. An empty Rompun (xylazine) bottle (known to have contained 10 cc earlier) lay by his side. He was immediately admitted to the hospital. Upon admission his pupils were of moderate size and responded slowly to light. Other findings included a blood pressure of 120/70 mmHg and heart rate of 60 bpm with stable sinus rhythm. Lactate dehydrogenase (LDH) activity was elevated, with the LDH-1 isoenzyme predominating. Creatine phosphokinase (CPK) activity was also elevated, particularly in the CPK-3 and CPK-2 isoenzymes. These enzyme changes persisted for 5 to 7 days. Plasma glucose level was also elevated. Two days following hospital admission sinus tachycardia developed, interspersed with runs of multifocal premature ventricular contractions which were controlled with lidocaine infusion. Blood pressure remained approximately 120/80 for the duration of hospitalization. Coma and respiratory depression lasted 60 hours. The patient was discharged from the hospital 17 days after admission.

The author noted that the patient might have died if he had not been found shortly after the injection was administered, owing to the marked respiratory depression that occurred. Hypotension, which has been reported as an effect of xylazine in humans, did not occur in this case. According to the author, the enzyme activity increases indicated that myocardial muscle damage had occurred and an intrinsic cardiotoxic effect of xylazine was suspected. Finally, in this case the greatest threat to life was the CNS-depressant effect of xylazine (Carruthers *et al.*, 1979).

A 20-year-old woman ingested 400 mg of xylazine. Approximately 2 hours later she became drowsy, incontinent (urine), difficult to arouse and occasionally unresponsive to verbal commands. She was admitted to the hospital approximately 3 hours after the ingestion. In a similar way to the case of human poisoning described by Carruthers *et al.* (1979), she experienced a relatively low initial cardiac rate, which later gradually increased, significant central nervous system and respiratory depression, transient hyperglycaemia and ventricular arrhythmias. However there was no evidence of myocardial damage. A sample of this patient's urine was analysed using a gas chromatograph-mass spectrometer computer system. Xylazine was found largely unchanged in the urine as shown by a lack of any structurally related compounds in the basic urine extract. No xylazine was found in a blood sample taken at the time of admission. The author concluded that plasma levels were below the limit of detection of the method (100 ng/ml). The patient was discharged ambulatory and without apparent adverse effects two days following admission to the hospital (Gallanos *et al.*, 1981).

A 36-year-old man died following ingestion of alcohol and clorazepate combined with an injection of approximately 40 ml of xylazine (100 mg/ml). Xylazine was found in the decedent's blood, brain, kidney, liver, lung, fat and urine at concentrations of 0.2, 0.4, 0.6, 0.9, 1.1, 0.05 and 7 ppm, respectively (Poklis *et al.*, 1985).

A 29-year-old woman self-injected 40 mg of xylazine intramuscularly. The estimated dose was 0.73 mg/kg bw. Clinical findings included disorientation, miosis, hypotension and bradycardia, but no cardiac arrhythmias were noted. The abnormalities resolved spontaneously (Spoerke *et al.*, 1986).

A 37-year-old woman self-injected 24 ml (2400 mg) xylazine intramuscularly. The estimated dose was 22 mg/kg bw. Twenty minutes after the injection her blood pressure was 166/130 mmHg, heart rate was 76 bpm and respirations 18 per minute. The serum glucose level was 175 mg/dl. Blood pressure later decreased to 130/90 mmHg and she became apnoeic. No cardiac arrhythmias were observed during her 3 days of hospitalization. Hypotension and bradycardia occurred two days after the injection. The patient survived (Spoerke *et al.*, 1986).

A 29-year-old woman self-injected an unknown amount of xylazine intravenously. She became apnoeic and had an initial blood pressure of 130/90 mmHg with a pulse of 60 bpm. Serum glucose levels did not exceed 90 mg/dl. Twenty-four hours after the injection the patient experienced hypotension and bradycardia. Spontaneous respiration resumed 18 hours after hospital admission. The patient recovered fully (Spoerke *et al.*, 1986).

A 19-year-old man accidentally injected himself subcutaneously with 2 ml (100 mg/ml) of xylazine. The dose administered was 3 mg/kg bw. Thirty minutes later he became difficult to rouse and was hospitalized. Clinical findings included miosis, hyporeflexia, hypotension, bradycardia, respiratory and central nervous system depression and hyperglycaemia. He was treated with intravenous fluids and assisted ventilation. Eight hours after hospitalization the patient was alert and responsive. Twenty-four hours later he was released (Samanta *et al.*, 1990).

A 39-year-old woman was admitted to the hospital with symptoms of tiredness, faintness and blurred vision. Clinical findings included sinus bradycardia with a blood pressure of 130/90. Xylazine was found in the urine and serum at concentrations of 1674 µg/litre and 30 µg/litre, respectively (Lewis *et al.*, 1983).

### 3. COMMENTS

The Committee considered toxicological data on xylazine, including the results of acute and short-term toxicity studies as well as studies on pharmacodynamics, pharmacokinetics, reproductive and developmental toxicity, genotoxicity and effects in humans. In addition, toxicological studies on 2,6-xylidine, a metabolite of xylazine, were reviewed; these included studies on acute and short-term toxicity, carcinogenicity and genotoxicity.

Numerous pharmacological side-effects of xylazine have been observed in treated animals, including mydriasis, impairment of thermo-regulatory control, various effects on the cardiovascular system, acid-base balance and respiration, hyperglycaemia, and haematological and gastrointestinal effects. Cattle and sheep are approximately 10 times more sensitive to xylazine than horses, dogs and cats.

Rats were administered radiolabelled xylazine intravenously at doses of 0.02 to 10 mg/kg bw or orally at doses of 0.02 to 100 mg/kg bw. More than 95% of the oral dose was absorbed, with a half-life of approximately 5 minutes. Following oral or intravenous administration, approximately 70% of the administered dose was eliminated in urine and 30% in faeces. Renal excretion following oral or intravenous administration was associated with a half-life of 2 to 3 hours. Enterohepatic circulation did not occur to a notable extent. In cattle administered an intramuscular dose of 0.2 or 0.5 mg xylazine/kg bw, less than 1% of the dose was excreted unchanged in the urine, and the parent compound was detected in the urine up to 6 hours following administration. Metabolites of xylazine were detected in urine from these cattle up to 10 hours following administration.

Pharmacokinetic parameters following intravenous administration showed minor variations between species. Xylazine disappeared rapidly from plasma following intravenous administration, with an elimination half-life of approximately 40 minutes in cattle and approximately 20 minutes in sheep. Xylazine could not be detected in the plasma of cattle following intramuscular administration of a single therapeutic dose.

In rats administered an intravenous dose of 2 mg/kg bw radio-labelled xylazine, approximately 20 metabolites were quantified as xylazine equivalents in urine and bile. The major metabolite comprised 35% of the administered dose. Approximately 8% of the dose was eliminated as unchanged xylazine 24 hours after dosing. In an *in vitro* study, 4 metabolites were identified when xylazine was incubated with rat liver microsomes. The same metabolites were identified in the urine of horses treated with xylazine. The major metabolite in both cases was identified as *N*-(2,6-dimethylphenyl) thiourea. In cattle administered an intramuscular dose of 0.2 mg xylazine/kg bw (two cows) or 0.5 mg xylazine/kg bw (one cow), 2,6-xylidine was identified as a metabolite excreted in urine in both conjugated and unconjugated forms.

The acute oral toxicities of xylazine and 2,6-xylidine were tested in mice and rats. Xylazine was determined to be moderately toxic ( $LD_{50} = 121\text{-}240$  mg/kg bw) and 2,6-xylidine to be slightly toxic ( $LD_{50} = 600\text{-}1000$  mg/kg bw).

Three studies on the short-term toxicity of xylazine were reviewed. A 32-week dietary study in rats and a 16-week oral (capsules) study in dogs were considered inadequate for the determination of the toxicity of xylazine owing to the use of insufficient numbers, poor quality animals and inadequate study design. The third was a 13-week oral study in beagle dogs fed diets containing 0, 10, 30 or 100 mg/kg xylazine in the feed (equal to 0.3, 0.9 or 3 mg/kg bw per day). No treatment-related effects were observed in any of the treated groups.

In a two-week oral (gavage) toxicity study in rats with 2,6-xylidine, rats were dosed with 80, 160, 310, 620 or 1250 mg 2,6-xylidine/kg bw per day, 5 days per week. Treatment-related effects included increased mortality (all animals in the high-dose group died), decreased body weight (males at 310 mg/kg bw per day and above and females at 160 mg/kg bw per day and above) and various effects on haematological parameters as indicated by leukocytosis and changes in red blood cell parameters indicative of increased erythropoiesis (males and females at 310 mg/kg bw per day and above). The NOEL in this study was 80 mg/kg bw per day.

In a 13-week oral (gavage) toxicity study in rats with 2,6-xylidine, rats were dosed with 20, 40, 80, 160 or 310 mg 2,6-xylidine/kg bw per day, 5 days per week for 13 weeks. Treatment-related effects included decreased body weight gain (males at 310 mg/kg bw per day and females at 40 mg/kg bw per day and above), increased absolute and relative liver weights (females at 160 mg/kg bw per day and above; males at 310 mg/kg bw per day), leukopenia (males at 40 mg/kg bw per day and above), haemoglobinaemia (males at 160 mg/kg bw per day and above) and anaemia (males at 310 mg/kg bw per day). The NOEL was 20 mg/kg bw per day.

In a carcinogenicity study, male and female rats were fed diets containing 2,6-xylidine at concentrations of 300, 1000 or 3000 mg/kg food (equivalent to 15, 50 or 150 mg/kg bw per day). Significant increases in the incidences of papillomas and carcinomas of the nasal cavity were observed in high-dose males and females. There was a significant dose-related increase in the incidence of adenomas in the nasal cavity in both males and females. In addition, unusual rhabdo-myosarcomas and malignant mixed tumours of the nasal cavity were observed in the high-dose males and females. There was a dose-related significant increase in the incidence of subcutaneous fibromas and fibrosarcomas in both treated males and females. In females, neoplastic nodules occurred in livers with a significant positive trend and the increase was significant in the high-dose group by the incidental tumour test. The Committee concluded that 2,6-xylidine was carcinogenic in this study.

The International Agency for Research on Cancer has evaluated the carcinogenic risk of 2,6-xylidine and has classified it as Group 2B (possibly carcinogenic to humans).

In a teratogenicity study, xylazine was administered to pregnant rats at doses of 1, 4 or 16 mg xylazine/kg bw per day on gestation days 6 to 15. Treatment-related maternal effects included partial closing of the eyelids, hypoaesthesia, ataxia, flat posture and slightly reduced body weight gain in the high-dose group only. A decrease in mean fetal weight was seen in the high-dose group. No teratogenic effects were noted in this study. The NOEL for maternal and fetal effects was 4 mg/kg bw per day.

Xylazine has been tested in reverse mutation assays in *Salmonella*, a forward mutation assay in cultured mammalian cells and in an *in vivo* cytogenetic assay. In *Salmonella*, weak positive results were obtained. Negative results were observed in a forward mutation assay on cultured mammalian cells and in a mouse bone marrow micronucleus test. The Committee concluded that xylazine is weakly mutagenic.

2,6-Xylidine was tested in a series of *in vitro* and *in vivo* genotoxic assays. It was weakly positive for reverse mutation in *Salmonella*. In mammalian cells, it induced forward mutation and was positive in a sister chromatid exchange test. Inconclusive results were obtained in a mouse bone marrow micronucleus test because there was no assurance that the bone marrow had been adequately exposed. 2,6-Xylidine was found to be inactive in an *in vivo-in vitro* rat hepatocyte unscheduled DNA synthesis assay. Covalent binding of the compound to DNA was observed in rats. The Committee concluded that 2,6-xylidine is genotoxic.

The potential for 2,6-xylidine to induce methaemoglobinaemia was reviewed by the Committee. Single doses of 30 mg 2,6-xylidine/kg bw intravenously or 164 mg/kg bw *N*-acetyl-2,6-xylidine orally have been shown to induce methaemoglobinaemia in cats but not in dogs. 2,6-Xylidine has also been shown to be a product of lidocaine metabolism in humans. Methaemoglobin and 2,6-xylidine-haemoglobin adduct levels have been shown to increase in human cardiac patients receiving lidocaine treatment.

Effects of xylazine on humans poisoned following accidental or intentional self-injection (0.7-15 mg/kg bw) or ingestion (7 mg/kg bw) included symptoms of central nervous system depression, respiratory depression, hypo- and hypertension, bradycardia, tachycardia, ventricular arrhythmias, and transient hyperglycaemia.

#### 4. EVALUATION

The Committee was unable to establish an ADI for xylazine because it concluded that the 2,6-xylidine metabolite was genotoxic and carcinogenic. Annex 4 lists the information that would be required for further review.

#### 5. ACKNOWLEDGMENTS

(Please see website.)

----- [end of WHO report] -----

**IARC:** "The International Agency for Research on Cancer (IARC) has evaluated the carcinogenic risk of 2,6-xylidine to humans. The Working Group concluded that there was inadequate evidence in humans but sufficient evidence in experimental animals for the carcinogenicity of 2,6-xylidine. The IARC classified 2,6-xylidine as Group 2B (possibly carcinogenic to humans) (IARC, 1993)." (From report above, regarding the xylazine metabolite 2,6-xylidine.)

#### **Canadian General Standards:**

Canadian law allows for xylazine use in food-producing animals. Allowable doses are 0.11-0.33 mg/kg intramuscularly. Meat may be taken from the treated livestock after 3 days; milk may be taken after 48 hours.<sup>31</sup>

In Canadian organic standards, xylazine and tolazoline are not listed as permissible, and therefore are not allowed.<sup>32</sup>

<sup>31</sup> "Foreign tranquilizer and anesthetic approvals for cattle (1995-97)." <http://www.farad.org/vets/Fortab.html>

However, in the British Columbia Certified Organic publication “Organic Grower” (Summer 2001), xylazine is suggested as a sedative during the dehorning of cattle.<sup>33</sup> This is contrary to what is decreed by the organic standards.

**EEC:** According to Article 14 of “Council Regulation (EEC) No. 2377/990,” the administration to food-producing animals of veterinary medicinal products containing pharmacologically active substances which are not included in Annex I, II, or III are prohibited. “Xylazine hydrochloride” is listed under Annex II and is thus permissible. However, tolazoline is not listed, and is therefore not permissible.<sup>34</sup>

The UK permits the use of xylazine in food-producing animals (in general). The allowable doses are 0.05-0.3 mg/kg intramuscularly. Meat may be taken from the treated livestock after 14 days; milk may be taken after 48 hours.<sup>35</sup>

**CODEX:** In “Guidelines for the Production, Processing, Labeling, and Marketing of Organically Produced Food: Annex 2: Permitted Substances for the Production of Organic Foods,” xylazine and tolazoline are not listed as permissible, and therefore are not allowed.<sup>36</sup>

**Japan Agricultural Standards** for Organic Agricultural Products and Their Processed Foods: Xylazine and tolazoline are not listed as permissible, and therefore are not allowed.<sup>37</sup>

Japan, in general, does not allow xylazine use in food-producing animals.

#### **Section 2119 OFPA U.S.C. 6518(m)(1-7) Criteria**

1. *The potential of the substance for detrimental interactions with other materials used in organic farming systems.*

There is no evidence that the use of xylazine and tolazoline for the purposes petitioned would have detrimental interactions with other materials used in organic farming systems.

2. *The toxicity and mode of action of the substance and of its break down products or any contaminants, and their persistence and areas of concentration in the environment.*

#### **Xylazine:**

The excerpts that follow are from a study regarding residues of xylazine in the tissues and milk of livestock. No ADI was established due to the incompleteness of the residue studies and insufficient knowledge regarding the metabolism of xylazine. Concerns revolve around the persistence of metabolites in the body, particularly 2,6-xylidine, as mentioned in the FAO/WHO report (p. 12). No information has been found voicing environmental concerns. Also following is an article focusing on the possible negative effects of xylazine in cattle, and abstracts from xylazine studies in animals. See item 4 for abstracts of studies in humans.

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<sup>32</sup> “Certified Organic Management Standards.” Certified Organic Associations of British Columbia. [http://www.certifiedorganic.bc.ca/Standards/Bk2\\_COABC\\_Organic\\_Management\\_Standards\\_Version4-03-02.pdf](http://www.certifiedorganic.bc.ca/Standards/Bk2_COABC_Organic_Management_Standards_Version4-03-02.pdf) March 2002.

<sup>33</sup> Weary, Daniel M. “Calf Management.” *BC Organic Grower*. vol 4, no 3. Summer 2001. [http://www.certifiedorganic.bc.ca/Booksand/BCOrganicGrower/Vol\\_4-3.pdf](http://www.certifiedorganic.bc.ca/Booksand/BCOrganicGrower/Vol_4-3.pdf)

<sup>34</sup> “Status of MRL Procedures.” EEC. <http://www.emea.eu.int/pdfs/vet/srwp/076599en.pdf> 19 March 2002.

<sup>35</sup> Craigmill, Arthur L., Martha Rangel-Lugo, Paul Damian, Jim E. Riviere. “Extralabel use of tranquilizers and general anesthetics.” *FARAD Digest*. 1998. <http://www.farad.org/vets/digest4.html>

<sup>36</sup> “Guidelines for the Production, Processing, Labeling, and Marketing of Organically Produced Food: Proposed Draft Amendments.” Codex Alimentarius Commission. [http://www.hc-sc.gc.ca/food-aliment/friia-raaii/ip-pi/codex/pdf/e\\_cl01\\_48e.pdf](http://www.hc-sc.gc.ca/food-aliment/friia-raaii/ip-pi/codex/pdf/e_cl01_48e.pdf) December 2001.

<sup>37</sup> Japanese Agricultural Standards. <http://www.fas.usda.gov/gainfiles/200004/25647377.pdf>

Tolazoline:

Following is an abstract from a study regarding suspected tolazoline toxicosis in a llama. To date, no studies have been conducted on the carcinogenic potential of tolazoline use in humans. Safety for use during pregnancy has not been established, and it is considered Category C (Use only when clearly needed and when the potential benefits outweigh the potential hazards to the fetus.) It is uncertain whether tolazoline is excreted in breast milk.<sup>38</sup> See item 4 for more information about tolazoline in humans.

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**XYLAZINE**

First draft prepared by Dr. S. Soback  
Ministry of Agriculture  
Kimron Veterinary Institute  
Beit Dagan, Israel

**RESIDUES IN FOOD AND THEIR EVALUATION****...METABOLISM****General**

Investigations of rat urine and bile after administration of radiolabeled xylazine (35S and <sup>14</sup>C, when both markers were on the thiazine ring) by paper electrophoresis and paper chromatography, approximately 20 metabolites were detected but not identified (Duhm et al., 1969). Only 8% of the labeled parent compound was recovered in the urine. The "principal" metabolite in urine represented 35 % of the total radioactivity. The ratio between renal and biliary excretion of the radiolabeled compound was 7:3 but the report did not explicitly indicate if all of the radioactivity was recovered.

Putter and Sagner (1973) showed that less than 1 % of the parent radiolabeled compound administered as xylazine hydrochloride could be recovered in cattle urine. Therefore, xylazine in cattle appears to undergo metabolic clearance only. The major metabolite excreted in cattle urine in free and conjugated form was identified as 1-amino-2,6-dimethylbenzene also known as 2,6-xylidine.

In a study utilizing LC/MS/MS and GC/MS techniques xylazine metabolites were characterized in horses *in vivo* and in rat liver *in vitro* (Mutlib et al., 1992). The major metabolites were identified as 2-(4'-hydroxy-2',6'-dimethylphenylamino)-5,6-dihydro-4H-1,3-thiazine, 2-(3'-hydroxy-2',6'-dimethylphenylamino)-5,6-dihydro-4H-1,3-thiazine, N-(2,6-dimethylphenyl)thiourea, and 2-(2',6'-dimethylphenylamino)-4-oxo-5,6-dihydro-1,3-thiazine. There were no data on xylazine metabolism for other species than rats and horses.

**Pharmacokinetics**

Comparative pharmacokinetics of xylazine in several species was reported by Garcia-Villar et al. (1981). The drug was administered intravenously and intramuscularly at recommended doses. The data was generated by analyzing serum drug concentration in samples obtained at 1, 2, 4, 8, 16, 30 and 120 min after xylazine administration. Compartmental analysis of the data was performed and the data best fitted a two-compartment open model. The major pharmacokinetic parameters are given in Table 1.

**Table 1.** Major pharmacokinetic parameters of xylazine in horse, cattle, sheep and dog after intravenous administration at 0.6, 0.2, 1.0 and 1.4 mg/kg, respectively

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<sup>38</sup> "Tolazoline HCl." *Drug Facts and Comparisons 2002, 56th ed.* St. Louis, MO: Facts and Comparisons, 2001. pp. 502-3.

Parameter	Horse (n = 4)	Cattle (n = 4)	Sheep (n = 6)	Dog (n = 4)
Weight (kg)	415-550	240-440	42-65	14-24
t <sub>1/2</sub> (min)	50	36	25	30
CL <sub>b</sub> (ml/min/kg)	21	42	83	81
V <sub>d(area)</sub> (l/kg)	2.4	1.9	2.7	2.5

The terminal half-life of xylazine in all species was short indicating that xylazine concentration would decrease to undetectable level within a few hours. The total body clearance varied significantly and was fastest in sheep and dog and slowest in horse. Xylazine clearance has been attributed mainly to metabolic clearance. Therefore, there seems to be species variations in the metabolic rate of the drug. The volume of distribution was large in all species apparently because of the lipophilic nature of the compound.

The pharmacokinetic parameters after IM administration are given in Table 2. There were no differences in the half-lives after IM administration when compared to those after IV administration. The T<sub>max</sub> values were reached within 15 minutes from drug administration and the peak concentrations were very low. Because of the low concentrations of the drug in bovine plasma, pharmacokinetic parameters after IM administration could not be determined in cattle.

**Table 2.** Major pharmacokinetic parameters of xylazine in horse, cattle, sheep and dog after intramuscular administration at 0.6, 0.2, 1.0 and 1.4 mg/kg, respectively

Parameter	Horse (n = 4)	Cattle (n = 4)	Sheep (n = 6)	Dog (n = 4)
Weight (kg)	415-550	240-440	42-65	14-24
t <sub>1/2</sub> (min)	58	N.D.	22	35
T <sub>max</sub> (min)	13	N.D.	15	13
C <sub>(max)</sub> (μg/ml)	0.2	N.D.	0.1	0.4

## TISSUE AND MILK RESIDUE DEPLETION STUDIES

### Tissues

Two studies using radiolabeled xylazine were performed (Murphy and Jacobs, 1975 and Murphy et al., 1978). One study in which 4 animals, two steer calves, one bull calf and a dairy cow, were given xylazine intramuscularly utilized <sup>14</sup>C-label in the 4'-position of the thiazine ring of the compound. The other study was conducted on 5 animals, two steer calves, one bull calf and two dairy cows, and were administered xylazine intramuscularly that carried a <sup>14</sup>C-label in 4-position of the aniline ring of the molecule. In both studies a dose of 0.33 mg/kg was used. In both studies recovery of radioactivity from urine and faeces increased as a function of time (Tables 3 and 4). At 10 hours after administration of the two differently labeled compounds 51-68 % of the radioactivity was recovered. Between 24-72 hours post administration 83-100% of the radiolabel was recovered except for one bull calf where a recovery of only 38% was recorded at 72 hours following administration.

**Table 3.** Recovery of radioactivity in urine and faeces of 4 animals (cattle) treated intramuscularly with xylazine at 0.33 mg/kg carrying <sup>14</sup>C-label in the 4'-position of the thiazine ring of the compound

Animal	Steer calf	Steer calf	Bull calf	Dairy cow
Time after administration (h)	10	24	48	74
	% radioactivity recovered			
Urine	65	71	63	77
Faeces	3	15	20	23
<b>Total</b>	<b>68</b>	<b>86</b>	<b>83</b>	<b>100</b>

**Table 4.** Recovery of radioactivity in urine and faeces of 4 animals (cattle) treated intramuscularly with xylazine at 0.33 mg/kg carrying  $^{14}\text{C}$ -label in the 4-position of the aniline ring of the compound

Animal	Steer calf	Steer calf	Bull calf	Dairy cow	Dairy cow
Time after administration (h)	10	48	72	72	72
	<b>% radioactivity recovered</b>				
Urine	48	82	35	73	85
Faeces	3	15	3	10	14
<b>Total</b>	<b>51</b>	<b>97</b>	<b>38</b>	<b>83</b>	<b>99</b>

After administration of xylazine  $^{14}\text{C}$ -labelled in the 4'-position of the thiazine ring at 0.33 mg/kg, radioactivity equivalent to 0.004 mg xylazine/kg or higher was found in all the 12 different analyzed tissues collected from the treated animals. Highest concentrations were measured in the injection site, kidney and liver (0.022-0.406 mg/kg). When xylazine was administered as above but with a  $^{14}\text{C}$ -label in the 4-position of the aniline ring, radioactivity exceeding the detection limit was found in all injection site, kidney and liver samples (0.009-1.152 mg/kg), and in all samples collected from the steer calf sacrificed 10 hours after drug administration (0.009-0.761 mg/kg). The characteristics of these residues were not studied and due to the difference in sensitivity of the radiolabel detection in the two studies it was difficult to predict whether the residues consist of double or single ring structures.

Several other tissue residue depletion studies were conducted (Putter and Sagner, 1969, Dorn and Maasfeld, 1990, Redgrave and Cameron, 1991a, Heukamp, 1991a). The first of these studies showed that the injection site residues declined to less 1/1000 in 20 hours after xylazine administration at 1.0 mg/kg to sheep. In the same study peripheral muscle concentrations were between 0.09 and 0.21 mg/kg during the same period. None of the other studies were able to detect xylazine residues in tissues when detection level was 0.01 mg/kg in muscle and 0.05 mg/kg in liver and kidney tissues. These studies were conducted in bovine after single IM dose of 0.3 mg/kg. It should be emphasized that the analytical procedures used in the different studies were essentially different and apparently contributed significantly to the discrepancies between the studies.

### Milk

Detectable radioactivity in milk was found up to 72 hours after administration of the  $^{14}\text{C}$ -xylazine labeled in the 4'-position of the thiazine ring and up to 24 hours after administration of the  $^{14}\text{C}$ -xylazine labeled in the 4-position of the aniline ring. The chemical nature of these residues was not investigated.

Xylazine residues in bovine milk were investigated (Dorn and Maasfeld, 1990b, Redgrave and Cameron, 1991b and Heukamp, 1991b). A single IM dose of 0.3 mg/kg was used. In the first study xylazine concentrations exceeding the 0.01 ppm detection level were not observed when milk samples were collected after each milking for 7 days. In the second study, in 3 samples out of 6, concentrations ranging from 0.012 to 0.019 were detected 5-8 hours after xylazine administration at 0.3 mg/kg IM to lactating cows.

In an earlier study xylazine milk concentrations in 2 cows after IM administration at 0.2 mg/kg were determined (Putter and Sagner, 1973). In this study concentrations ranging from 0.03 to 0.08 mg/ml were found at 5 and 21 hour after administration.

It should be emphasized that the analytical procedures used in the different studies were essentially different and apparently contributed significantly to the discrepancies between the studies.

### METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The early reports concerning xylazine residues in tissues were analyzed with a method based liquid-liquid extraction from alkaline solution with hexane, cleaned by passage through basic aluminum oxide column and filtered (Putter and Sagner, 1969; Putter and Sagner, 1973). The hexane fraction was then concentrated and xylazine was extracted

to phosphate buffer pH 5.0. A spectrophotometer adjusted at 240 nm was then used for detection. Muscle, milk and urine samples could be analyzed by practically similar procedures. These papers describe also a thin layer chromatography method based on silica gel stationary phase and ethanol: water or ethanol: benzene: chloroform mobile phases. The spots were made visible by  $\text{AgNO}_3$  and fluorescein.

Xylazine analysis based on paper, liquid and gas chromatography procedures have been described (Duhm et al., 1969, Maasfeld, 1991, Mutlib et al., 1992). Two multiresidue methods for tissue based on reversed phase liquid chromatography using either phenyl or C18 columns and UV and/or fluorescence detection were published (Etter et al., 1984 and Keukens and Aerts, 1989). The first method used a mixture of dichloromethane and petroleum ether for extraction of the compound from alkaline muscle or kidney tissue homogenate. In the second method swine kidneys were homogenized with acetonitrile and sodium chloride was added before solid phase extraction by use of C18 cartridge. After elution with acidic acetonitrile and hexane extraction of the eluate the aqueous phase was used for chromatography. Both methods claim a 5-10 mg/kg detection limit but the reported recovery for xylazine was low (45-70% depending on the tissue and concentration) by use of either method. The performance characteristics of the method of Maasfeld (1991), which was used in the subsequent tissue residue depletion studies, were insufficiently described.

### APPRAISAL

Depletion studies with thiazine ring radiolabeled  $^{14}\text{C}$ -xylazine administered orally indicated that in rats 2 % of radioactivity was still present 48 hours after administration. The ratio for recovery of the radiolabeled compound in urine and faeces was 7:3.

Pharmacokinetic data concerning the parent compound were reported in studies including cattle, horses, sheep, dogs and laboratory animals. Xylazine had a very short plasma half-life which in most species was approximately 0.5 hours and in horses 0.9 hours. The compound underwent a rapid clearance. Species differences in clearance indicate different metabolic activity and/or different metabolic pathways. The apparent volume of distribution was large 1.9-2.5 l/kg due to the lipophilic nature of the drug. Plasma depletion of unlabeled compound in cattle was more rapid than depletion of total radioactivity in a similar study using  $^{14}\text{C}$ -xylazine. Therefore, clarification of xylazine metabolism is required in order to better understand its pharmacokinetics.

The excretion of thiazine ring radiolabeled  $^{14}\text{C}$ -xylazine administered intramuscularly to cattle (3 calves and one milking cow) and slaughtered at different time intervals was complete at 74 hours. The ratio of the radioactivity between urine and faeces was 3:1. In a related study using intramuscular administration of  $^{14}\text{C}$ -xylazine labelled in the aniline ring, the excretion of the radioactivity was variable, ranging from 38-99%. In a second study, the respective ratios of the radioactivity for urine and faeces ranged from 12:1 to 6:1.

Studies on xylazine in rat and horse urine indicated extensive metabolism. However, no data concerning xylazine metabolism in other animals were available. Due to the lack of these data the possibility that metabolism causes the discrepancy between the depletion studies using radiolabeled compound and the unlabeled compound cannot be evaluated.

Two  $^{14}\text{C}$ -radiolabel depletion studies using intramuscular administration of xylazine in cattle were submitted. The first study with three calves and one lactating cow used xylazine, labeled in the thiazine ring, and the second study used four calves and two lactating cows administered xylazine, labeled in the aniline ring. The radiolabeled tissue residue depletion studies showed that total residues in mg/kg xylazine equivalents in kidney, liver, and injection site were 0.009-0.020, 0.022-0.050 and 0.030-1.152, respectively, at 72 hours after administration. Results were similar in the study using a thiazine ring labeled  $^{14}\text{C}$ -xylazine. In milk the radioactivity as xylazine equivalents had declined to 0.01 mg/l after treatment with the drug labeled in the thiazine ring or in the aniline ring by 60 and 12 hours, respectively.

The data generated in tissues of cattle and milk residue depletion studies in which only the concentration of the parent compound, xylazine, was determined were in clear contrast with the radiolabel studies. The studies with unlabeled compound failed to detect xylazine at 0.01 mg/kg in muscle, kidney, liver and fat. Similarly, xylazine

concentrations in milk exceeded the 0.01 mg/l detection level only occasionally. Thus the majority of the residues were not parent drug, but were unidentified metabolites.

A number of analytical methods, mainly for parent compound, such as photometry, liquid chromatography, gas chromatography, and mass spectrometry, were described. Performance characteristics were poorly determined but a limit of detection of 0.01 mg/kg was claimed. No method validation data were available for evaluation.

#### Maximum Residue Limits

The following factors were considered by the Committee with respect to the assignment of MRLs:

No ADI was established;

Lack of adequate data on metabolism of the compound;

No marker residue could be assigned; and

There were insufficient residue depletion studies available.

The Committee did not recommend MRLs.

The following information would be required before a further review:

Data on xylazine metabolism in target species sufficient to identify a suitable marker residue and target tissues;

Additional data on residue depletion of xylazine and its metabolites in target species. These data should include evidence to show, in particular, whether 2,6-xylidine is present at the recommended withdrawal period; and

A suitable analytical method for determining the marker residue in target tissues.<sup>39</sup>

----- [end of xylazine residue report] -----

#### **Xylazine Sedation Antagonized With Tolazoline**

Tolazoline, a mixed alpha-1, alpha-2 adrenoceptor antagonist, may work better than yohimbine, which is a more selective alpha-2 antagonist, when reversing the mixed action of xylazine.

#### **DIAGNOSTIC**

Xylazine is an alpha-2 adrenoceptor agonist that has profound sedative-analgesic-muscle relaxant effects in ruminants mediated by receptors in the central nervous system. It is approved by the FDA for horses, dogs, cats, deer, and elk only, and meat and milk withdrawals for ruminants have not been established.

Adverse effects of xylazine mediated by receptors in peripheral tissues include rumen hypomotility with potentially life threatening bloat (fast for 24 hours in advance to avoid), decreased heart rate characterized by sinus bradycardia and/or atrioventricular blockade, drooling (from depression of the swallowing reflex), and low bellowing. Xylazine also causes a major increase in urine output and can lead to rupture of the bladder in animals undergoing examination or surgery for urinary obstruction. Peak plasma concentrations in cattle and sheep occur within 12 to 14 minutes after administration of xylazine. The systemic half-life is approximately 23 minutes in sheep and 36 minutes in cattle. Analgesia does not extend to the end of the xylazine-induced sedation, and painful procedures should be restricted to the initial 15 to 30 minutes after drug administration or else supplemental local or regional analgesia should be used.

Tolazoline has mixed alpha-1 and alpha-2 adrenoceptor antagonistic activity. It can be used to antagonize xylazine-induced sedation and initiate arousal. Tolazoline also antagonizes the less desirable side effects. The recommended dosage for cattle, given slowly IV, is 1.1 mg/kg while 2.2 mg/kg can be given in cases of accidental overdose or after administration of the higher dose recommended for horses or cattle.

Tolazoline antagonizes the sedative effects of epidural xylazine anesthesia while anesthesia caudal to the injection site of the xylazine persists. When xylazine and butorphanol have been used together, the postoperative analgesia provided by the butorphanol persists after antagonism of the xylazine with tolazoline.

<sup>39</sup> Soback, S. Xylazine monograph. *Residues of some veterinary drugs in animals and foods: FAO Food and Nutrition Paper (41/9)*. 1997. <http://www.fao.org/docrep/W4601E/w4601e0f.htm>

When used to reverse xylazine sedation in llamas, a nasotracheal tube should be left in place until the llama can stand and walk. Airway obstruction and death has occurred in llamas which have regained consciousness, but remained recumbent after nasotracheal tube removal.

*J. C. Thurmon et al, Utah State Univ. Extension  
Veterinary Newsletter, March 2000.*<sup>40</sup>

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#### XYLAZINE AND TOLAZOLINE STUDY ABSTRACTS:

##### **Toxicity and blood concentrations of xylazine and its metabolite, 2,6-dimethylaniline, in rats after single or continuous oral administrations.**

**Yasuhara K, Kobayashi H, Shimamura Y, Koujitani T, Onodera H, Takagi H, Hirose M, Mitsumori K.**

Division of Pathology, National Institute of Health Sciences, Tokyo, Japan.

To cast light on whether the carcinogenic risk of 2,6-dimethylaniline (DMA), a metabolite of xylazine, may increase by ingestion of edible tissues from domestic animals treated with xylazine, the following studies of xylazine and DMA were performed. In Experiment I, male F344 rats received a single oral administration of 150 mg/kg of xylazine hydrochloride. Rats showed symptoms suggesting loss of sensation and pain immediately after the treatment. These signs had disappeared after 3 hr, but the animals died of hydrothorax and pulmonary edema by 9 hr. The plasma concentration of xylazine was 2.88 +/- 0.95 micrograms/ml at 15 min, and then decreased to 0.10 +/- 0.01 microgram/ml at 6 hr. The plasma level of DMA remained at 0.03 to 0.04 microgram/ml during the measurement period. In Experiment II, male F344 rats were fed a diet containing 1000 ppm of xylazine hydrochloride, regarded as the maximum tolerated dose, for 4 weeks. No clear clinical signs were evident and the plasma levels of xylazine and DMA were at the detection limit (0.02 microgram/ml) or less, although follicular cell hypertrophy of the thyroid was observed in all the treated animals. In Experiment III, male F344 rats were fed a diet containing 3000 ppm or 300 ppm of DMA for 4 weeks. Histological changes, such as atrophy of Bowman's gland and irregular arrangement of olfactory epithelial cells, were only observed in the olfactory epithelium of the 3000 ppm group. The plasma levels of DMA were 0.20 to 0.36 microgram/ml in the 3000 ppm group, but under the detection limit in the 300 ppm group. These results suggest that the probability of nasal carcinogenic effects of DMA on consumers via ingestion of edible tissues from food-producing animals treated with xylazine is extremely low, since DMA levels in the blood of rats subjected to continuous administration of high doses of xylazine remained under the detection limit.<sup>41</sup>

##### **Suspected tolazoline toxicosis in a llama.**

**Read MR, Duke T, Toews AR.**

Department of Veterinary Anesthesiology, Radiology, and Surgery, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada.

Clinical signs of tolazoline toxicosis developed in a 4-year-old llama that received 2 doses of tolazoline hydrochloride to reverse xylazine-induced sedation. The full first dose (4.3 mg/kg [2.0 mg/lb] of body weight) was erroneously injected i.v., and the second dose was administered half i.v., half i.m. 45 minutes later, because the llama became weak and recumbent. Signs of anxiety, hyperesthesia, profuse salivation, and tachypnea were the first detectable clinical signs of tolazoline toxicosis. Convulsions, hypotension, gastrointestinal tract hypermotility, and

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<sup>40</sup> "Xylazine Sedation Antagonized By Tolazoline." *Veterinary News*. Cooperative Extension, College of Agricultural Sciences, The Pennsylvania State University. May 2000. pp. 2.

<http://www.vetsci.psu.edu/Ext/Newsletters/vn/vn0005.pdf>

<sup>41</sup> *J Toxicol Sci* 2000 May;25(2):105-13 PMID: 10845188 [PubMed - indexed for MEDLINE]

diarrhea also developed. The llama was treated successfully with i.v. administration of diazepam, phenylephrine, and lactated Ringer's solution supplemented with potassium chloride and oxygen administered via nasal insufflation. We suggest that the maximum dose of tolazoline administered at any one time to llamas not exceed 2 mg/kg (0.91 mg/lb). Furthermore, tolazoline should be administered slowly i.v. or i.m. to reduce the risk of adverse reactions.<sup>42</sup>

3. *The probability of environmental contamination during manufacture, use, misuse, or disposal of the substance.*

Xylazine hydrochloride injection is combustible and may emit noxious and toxic fumes when burned/decomposed.<sup>43</sup> There is a single possible documented case of xylazine toxicosis from inhalation in a human. (See second abstract in item 4.) Xylazine metabolite 2,6-xylidine is categorized as 2B (possibly carcinogenic to humans), and both it and unchanged xylazine is excreted in animal urine and feces.

No information was found on possible environmental contamination from tolazoline or its metabolites, although some tolazoline is also excreted unchanged.

4. *The effects of the substance on human health.*

(See also item 2.)

Following are two abstracts from studies of xylazine toxicosis in humans and potential adverse effects of tolazoline in humans.

Xylazine:

**Severe intoxication with the veterinary tranquilizer xylazine in humans.**

**Hoffmann U, Meister CM, Golle K, Zschiesche M.**

Department of Pharmacology, Ernst-Moritz-Arndt University Greifswald, Germany. jaki@mail.uni-greifswald.de

Xylazine (Rompun, Proxylaz) is a veterinary tranquilizing agent. A case of self-injection of 1.5 g xylazine by a 27-year-old farmer is reported. He subsequently became comatose, hypotensive, bradycardic, and mildly glycaemic. An intensive supportive therapy including intubation and ventilation was required. The patient made a full recovery over the next 30 h. The largest concentrations measured were 4.6 mg/L in plasma, 446 mg/L in gastric fluid, and 194 mg/L in urine. The calculated plasma half-life was 4.9 h. Kinetic data correlated with clinical symptoms. Qualitative and quantitative analyses of xylazine were done by thin-layer chromatography, gas chromatography-mass spectrometry, and high-performance liquid chromatography. These methods allow the detection of small amounts substance in stomach, plasma, and urine. Liquid-liquid extraction was used for the isolation of drug. The sensitivity is high, and with these methods, a rapid analysis is possible. Xylazine intoxications in humans are rare. We describe the management of acute poisoning and present a review of xylazine toxicity in humans.<sup>44</sup>

**Severe intoxication from xylazine inhalation.**

**Capraro AJ, Wiley JF 2nd, Tucker JR.**

Department of Pediatrics, University of Connecticut School of Medicine, and Connecticut Children's Medical Center, Hartford, Connecticut, USA.

<sup>42</sup> J Am Vet Med Assoc 2000 Jan 15;216(2):227-9, 194 PMID: 10649759 [PubMed - indexed for MEDLINE]

<sup>43</sup> "Xylazine hydrochloride MSDS." <http://www.bi-vetmedica.com/MSDS/Xylazine%20HCl%20Injection.pdf>

<sup>44</sup> J Anal Toxicol 2001 May-Jun;25(4):245-9 PMID: 11386637 [PubMed - indexed for MEDLINE]

We present the first documented case of overdose from xylazine inhalation. The patient developed findings consistent with alpha 2 adrenergic agonist toxicity, eg coma, miosis, apnea, bradycardia, hypothermia, and dry mouth 2 hours after exposure. Standard dose naloxone did not reverse these effects. The patient fully recovered after appropriate supportive measures. A review of prior reports of xylazine exposure is provided.<sup>45</sup>

#### Tolazoline:

#### **Warnings:**

Stress ulcers: Gastric secretion is stimulated by tolazoline, which may activate stress ulcers. Through this mechanism, it can produce significant hypochloremic acidosis. Pretreatment of infants with antacids may prevent GI bleeding.

Hypotension: Observe closely for signs of systemic hypotension. Hypotension is common in neonates and may occur suddenly. Institute supportive therapy if needed.

Mitral stenosis: Parenteral tolazoline may cause an increase or decrease in pulmonary arterial pressure and total pulmonary resistance. Use with caution in suspected mitral stenosis.

Pregnancy: Category C. Safety for use during pregnancy has not been established. Use only when absolutely needed and when the potential benefits outweigh the potential hazards to the fetus.

Lactation: It is not known whether tolazoline is excreted in breast milk safety for use in the nursing mother has not been established. Exercise caution when administering to a nursing woman.

Geriatrics: No information is available on the relationship of age to the effects of tolazoline in geriatric patients.

#### **Precautions:**

Monitoring: Use tolazoline in a highly supervised environment where vital signs, oxygenation, acid-base status, and fluid and electrolytes can be monitored and maintained.

Acidosis: The effects of tolazoline on pulmonary vessels may be pH-dependent. Acidosis increases pulmonary vasoconstriction and may decrease the effect of tolazoline.

Renal function impairment, reduced urine flow: Decreases tolazoline elimination; may require dosage reduction.

#### **Drug interactions:**

Alcohol: Tolazoline may cause accumulation of aldehyde following alcohol ingestion and theoretically may produce a disulfiram-like reaction.

Dopamine: Tolazoline antagonizes the peripheral vasoconstriction produced by high doses of dopamine.

Ephedrine: Alpha-adrenergic blocking agents such as tolazoline may decrease the pressor response to ephedrine)

Epinephrine or Norepinephrine: Concurrent use with large doses of tolazoline may cause a paradoxical reduction in blood pressure followed by an exaggerated rebound increase; these medications are not recommended for treatment of tolazoline overdose.

Metaraminol: Concurrent use with tolazoline usually decreases, but does not reverse or completely block, the pressor effect of metaraminol.

Methoxamine or Phenylephrine: Prior administration of tolazoline may block the pressor response to methoxamine or phenylephrine, possibly resulting in severe hypotension)

#### **Other, less frequent side effects:**

Diarrhea, nausea and vomiting, Increased pilomotor activity (goose flesh), peripheral vasodilation (flushing), tachycardia (a reflex response to vasodilation and a result of direct cardiac stimulation), mydriasis (rare).<sup>46, 47</sup>

<sup>45</sup> *Pediatr Emerg Care* 2001 Dec;17(6):447-8 PMID: 11753193 [PubMed - indexed for MEDLINE]

<sup>46</sup> "Tolazoline HCl." *Drug Facts and Comparisons* 2002, 56th ed. St. Louis, MO: Facts and Comparisons, 2001. pp. 502-3.

<sup>47</sup> "USP DI Professional Information: Tolazoline hydrochloride." Drugs.com.

<http://www.drugs.com/index.cfm?pageID=0&xml=001658&type=pros&bn=Tolazoline%20Hydrochloride>

5. *The effects of the substance on biological and chemical interactions in the agroecosystem, including the physiological effects of the substance on soil organisms (including the salt index and solubility of the soil), crops, and livestock.*

There is no evidence that xylazine and tolazoline excreted in urine or feces would negatively impact the agroecosystem. The metabolites would not be of sufficient concentration to impact the agroecosystem when these materials are used in the manner petitioned.

6. *The alternatives to using the substance in terms of practices or other available materials.*

Alternatives to xylazine and tolazoline are also synthetic. Yohimbine is another common, synthetic xylazine antagonist, and is similarly not approved in the U.S. for use in food-producing animals. There are no natural alternatives to xylazine and tolazoline for the purposes petitioned.

7. *Its compatibility with a system of sustainable agriculture.*

There is no clear evidence that the use of xylazine and tolazoline would be incompatible with a system of sustainable agriculture. However tissue and fluid residue studies are inconclusive as to whether the presence of xylazine and its metabolites therein, in possible exposure concentrations, are unsafe for human consumption.

## **TAP Reviewers' Discussion**

**Reviewer 1** [*PhD. Reproductive Physiology. Research, consulting, professor of animal sciences with activities related to animal production. Southeast.*]

### **Comments on Database**

No additional information is required.

### **Observations/OFPA Criteria**

*Includes excerpts from report, followed by additional laboratory information.*

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Xylazine is not used in humans. Tolazoline is used in both humans and animals. Xylazine is not approved for use in food-producing animals in the United States. The **FDA** has approved xylazine hydrochloride for use as a veterinary anesthetic, and tolazoline hydrochloride as a reverser of xylazine, but in both cases, use of these medications in "food-producing animals" is specifically unapproved. Tolazoline is limited to use in horses and its safety has not been evaluated for reversing xylazine used as a preanesthetic to a general anesthetic.

## **Subpart C - Organic Crop, Wild Crop, Livestock, and Handling Requirements**

### **Description of Regulations**

#### **General Requirements**

This subpart sets forth the requirements with which production and handling operations must comply in order to sell, label, or represent agricultural products as "100 percent organic," "organic," or "made with organic (specified ingredients or food group(s))." The producer or handler of an organic production or handling operation must comply with all applicable provisions of subpart C....

..... The producer must not withhold medical treatment from a sick animal to maintain its organic status. All appropriate medications and treatments must be used to restore an animal to health when methods acceptable to

organic production standards fail. Livestock that are treated with prohibited materials must be clearly identified and shall not be sold, labeled, or represented as organic.....

..... (c) The producer of an organic livestock operation must not:

(1) Sell, label, or represent as organic any animal or edible product derived from any animal treated with antibiotics, any substance that contains a synthetic substance not allowed under § 205.603, or any substance that contains a nonsynthetic substance prohibited in § 205.604.

(2) Administer any animal drug, other than vaccinations, in the absence of illness;

(3) Administer hormones for growth promotion;

(4) Administer synthetic parasiticides on a routine basis;

(5) Administer synthetic parasiticides to slaughter stock;

(6) Administer animal drugs in violation of the Federal Food, Drug, and Cosmetic Act; or

(7) Withhold medical treatment from a sick animal in an effort to preserve its organic status. All appropriate medications must be used to restore an animal to health when methods acceptable to organic production fail.

Livestock treated with a prohibited substance must be clearly identified and shall not be sold, labeled, or represented as organically produced.....

### **International**

**IFOAM:** In the Basic Standards for Organic Production and Processing, Final Draft 2002, IFOAM does not list either xylazine or tolazoline as permissible, and therefore they are not allowed.

### **FAO/WHO/JECFA:**

WHO Technical Report Series, No. 876 (in press).

### **EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD**

Joint FAO/WHO Expert Committee on Food Additives

Rome, 4-13 June 19961

2. The Committee evaluated two adrenoceptor agonists (clenbuterol and xylazine), two anthelmintic agents (abamectin and moxidectin), seven antimicrobial agents (chlortetracycline, oxytetracycline, tetracycline, neomycin, spiramycin, thiamphenicol and tilmicosin), and two insecticides (cypermethrin and "-cypermethrin).

Acceptable Daily Intakes (ADIs) or temporary ADIs were established for all of these substances **except** xylazine.

The Committee recommended Maximum Residue Limits (MRLs) in appropriate tissues (muscle, liver, kidney and fat), milk and/or eggs for all substances **except** xylazine.

### **Xylazine**

The Committee was unable to establish an ADI for xylazine because it concluded that a metabolite, 2,6-xylidine, is genotoxic and carcinogenic.....

**Pharmacokinetic studies have only been completely conducted on sheep and horses. Partial studies have been conducted on cattle. Data on goats and swine are not presented. As major livestock species, cattle, goats and swine need to have more complete information presented to determine what adverse reactions could occur in humans from the consumption of organic food products that contain xylazine, tolazoline, and/or their metabolites.**

Additionally, numerous pharmacological **side-effects** of xylazine have been observed in treated animals, including mydriasis, impairment of thermo-regulatory control, various effects on the cardiovascular system, acid-base balance and respiration, hyperglycaemia, and haematological and gastrointestinal effects. Cattle and sheep are approximately 10 times **more sensitive** to xylazine than horses, dogs and cats.

The International Agency for Research on Cancer has evaluated the carcinogenic risk of 2,6-xylidine and has classified it as Group 2B (possibly carcinogenic to humans). The Committee concluded that xylazine is weakly mutagenic. The Committee concluded that 2,6-xylidine is **genotoxic**.

In Canadian organic standards, xylazine and tolazoline are not listed as permissible, and therefore are **not allowed**.

**EEC:** According to Article 14 of "Council Regulation (EEC) No. 2377/990," the administration to food-producing animals of veterinary medicinal products containing pharmacologically active substances which are not included in Annex I, II, or III are prohibited. Tolazoline is not listed, and is therefore **not permissible**.

**CODEX:** In "Guidelines for the Production, Processing, Labeling, and Marketing of Organically Produced Food: Annex 2: Permitted Substances for the Production of Organic Foods," xylazine and tolazoline are not listed as permissible, and therefore are **not allowed**.

**Japan Agricultural Standards** for Organic Agricultural Products and Their Processed Foods: Xylazine and tolazoline are not listed as permissible, and therefore are **not allowed**.

Japan, in general, does not allow xylazine use in food-producing animals.

## RESIDUES IN FOOD AND THEIR EVALUATION

### ...METABOLISM

#### General

Investigations of rat urine and bile after administration of radiolabeled xylazine (35S and <sup>14</sup>C, when both markers were on the thiazine ring) by paper electrophoresis and paper chromatography, approximately 20 metabolites were **detected but not identified** (Duhm et al., 1969).

Putter and Sagner (1973) showed that less than 1 % of the parent radiolabeled compound administered as xylazine hydrochloride could be recovered in cattle urine. Therefore, xylazine in cattle appears to undergo metabolic clearance only. The **major metabolite** excreted in cattle urine in free and conjugated form was identified as 1-amino-2,6-dimethylbenzene also known as **2,6-xylidine**.

Clarification of xylazine metabolism is required in order to better understand its pharmacokinetics. Studies on xylazine in rat and horse urine indicated extensive metabolism. However, **no data concerning xylazine metabolism in other animals** were available. Due to the lack of these data the possibility that metabolism causes the discrepancy between the depletion studies using radiolabeled compound and the unlabeled compound cannot be evaluated.

8. *The probability of environmental contamination during manufacture, use, misuse, or disposal of the substance.*

Xylazine hydrochloride injection is combustible and **may emit noxious and toxic fumes** when burned/decomposed. There is a single possible documented case of xylazine toxicosis from inhalation in a human. (See second abstract in item 4.) Xylazine metabolite **2,6-xylidine** is categorized as 2B (possibly **carcinogenic** to humans), and both it and unchanged xylazine is excreted in animal urine and feces.

No information was found on possible environmental contamination from tolazoline or its metabolites, although some tolazoline is also excreted unchanged.

9. *Its compatibility with a system of sustainable agriculture.*

There is no clear evidence that the use of xylazine and tolazoline would be incompatible with a system of sustainable agriculture. However tissue and fluid residue studies are **inconclusive** as to whether the presence of xylazine and its metabolites therein, in possible exposure concentrations, are unsafe for human consumption.

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**The following (additional) reports raise concerns with the toxicity of the compounds in question.**

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This study examined the effects of feeding monensin, lasalocid or salinomycin (at 1x and 2x therapeutic levels for 20 d) on the duration of xylazine+ketamine sleep time and the hepatic cytochrome P-450 content in broiler chickens of different age groups (3- and 6-w-old). The 3-w-old birds fed 120 ppm salinomycin had a reduced xylazine+ketamine sleep time as compared to control (no drug). The 6-w birds had a sleep time similar to the controls. Associated with ionophore feeding was a trend toward P-450 induction when compared to controls. Increased P-450 content was statistically significant in the 3-w birds fed 90 ppm lasalocid and in the 6-w birds fed 200 ppm monensin. Ionophore administration above recommended levels gave conflicting results for duration of xylazine+ketamine sleep time and hepatic cytochrome P-450 levels. The isozymes of cytochrome P-450 responsible for xylazine and ketamine metabolism may be different from those induced by the ionophores<sup>c</sup>.

The domestic pig was used to develop a new model for evaluating the emetogenic potential of anticancer drugs and determining the antiemetic activity of drugs. In each animal, the number of vomits after infusion of the emetogenic drug (infusion in ketamine and xylazine anesthesia) was recorded in 1-hr periods during the first 4 hr and then in a 4- and a 16-hr period. Intravenous infusion of cisplatin caused a concentration-dependent emetic response. Anti-cancer drugs other than cisplatin such as carboplatin, dactinomycin, cyclophosphamide, and ifosfamide, also induced emesis<sup>d</sup>.

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**This study shows the adverse effects of xylazine in swine.**

The arrhythmogenic dose of epinephrine (ADE) was determined in 6 pigs during steady-state anesthesia (1.5% halothane in O<sub>2</sub>) and steady-state anesthesia plus xylazine (1.1 mg X kg<sup>-1</sup> X hr<sup>-1</sup>; IV infusion) and after either prazosin (alpha 1) or metoprolol (beta 1) adrenergic blockade during halothane-xylazine (H-X) anesthesia. A constant infusion (1, 2, 3, 5, and 10 micrograms X kg<sup>-1</sup> X min<sup>-1</sup>) of freshly mixed epinephrine (100 micrograms X ml<sup>-1</sup> in saline solution) was used to determine ADE. The ADE was defined as the total dose of epinephrine which produced 4 or more continuous or intermittent, premature, ventricular contractions within a 15-s period. The mean epinephrine total dose values during 1.5% halothane anesthesia, H-X anesthesia alone, or H-X anesthesia after either prazosin (0.1 mg X kg<sup>-1</sup>) or metoprolol (0.5 mg X kg<sup>-1</sup>) adrenergic blockade were 3.60 +/- 0.844, 2.68 +/- 0.402, 11.85 +/- 3.804, and 5.17 +/- 0.587 micrograms X kg<sup>-1</sup>, respectively. Xylazine administration did not significantly decrease ADE, although mean arterial pressure significantly increased<sup>e</sup>.

To assess the effects of intravenous tolazoline on hemodynamics and regional blood flow distribution, 12 anesthetized newborn piglets were studied. Six piglets received two bolus doses of tolazoline (1 and 2 mg/kg). Mean arterial blood pressure decreased from control levels of 69.4 +/- 5.6 to 54.6 +/- 7.0 and 47.0 +/- 5.6 mm Hg, respectively, after 1 and 2 mg/kg of tolazoline, and heart rate increased from 220 +/- 9 to 270 +/- 13 and 282 +/- 8 beats/min, respectively. Bronchial blood flow was also significantly increased. After a dose of 2 but not 1 mg/kg, the renal blood flow was markedly decreased from 139.8 +/- 17.8 to 104.4 +/- 24.5 ml/min/100 g. We conclude that tolazoline is a potent coronary vasodilator during the neonatal period. In addition we speculate that the decrease in renal blood flow might play a role in the renal toxicity of tolazoline<sup>f</sup>.

**These concerns should be addressed by clear guidelines for uses that restrict applications in 'organic animal production'.**

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### **Reviewer 1 Conclusions**

Xylazine and Tolazoline should not be allowed in organic animal production systems. The therapeutic use of this product in organic livestock production can not be allowed in order to satisfy requirements for 'organic animal' production.

**Reviewer 1 Recommendations Advised to the NOSB**

The substance is Synthetic.

For Livestock, the substance should be Excluded from the National List.

**References**

<sup>c</sup>Roder JD, Sangiah S, Lish JL, Qualls CW Jr. 1996. The influence of ionophores on the duration of xylazine-ketamine anesthesia and the hepatic cytochrome P-450 content of broiler chickens. *Vet Hum Toxicol* 38:353-5.

<sup>d</sup>Szelenyi I, Herold H, Gothert M. 1994. Emesis induced in domestic pigs: a new experimental tool for detection of antiemetic drugs and for evaluation of emetogenic potential of new anticancer agents. *J Pharmacol Toxicol Methods* 32:109-16.

<sup>e</sup>Tranquilli WJ, Thurmon JC, Benson GJ. 1986. Halothane-catecholamine arrhythmias in swine (*Sus scrofa*). *Am J Vet Res* 47:2134-7.

<sup>f</sup>Clozel M, Hascoet JM, Monin P, Vert P. 1987. Effects of tolazoline on regional blood flows in the newborn piglet. *Dev Pharmacol Ther* 10:265-77.

**Reviewer 2** [*Ph.D, Mammalian/Avian Physiology. Associate Professor, Food Science. Currently conducting Nutrition/Physiology research for USDA-ARS, full time research on alleviating micronutrient deficiency. Northeast U.S.*]

**OFPA Criteria Evaluation**

*(1) The potential of such substances for detrimental chemical interactions with other materials used in organic farming systems;*

These substances have been used on a broad array of mammalian species for a number of years, with no significant evidence of harmful effect when used in the manner petitioned. They are rapidly metabolized or eliminated from the body.

*(2) The toxicity and mode of action of the substance and of its breakdown products or any contaminants, and their persistence and areas of concentration in the environment;*

No data to suggest that the mode of action or breakdown products should exist to any significant amount after use in the intended fashion.

*(3) the probability of environmental contamination during manufacture, use, misuse or disposal of such substance;*

No evidence or extremely low probability that these compounds can be manufactured or disposed of in such amounts as to become harmful to humans and other livestock via environmental contamination. These are synthetic pharmaceuticals, manufactured in controlled amounts, thus not a likely environmental hazard.

*(4) the effect of the substance on human health;*

When used in the manner petitioned, there should be no effect on human health.

*(5) the effects of the substance on biological and chemical interactions in the agroecosystem, including the physiological effects of the substance on soil organisms (including the salt index and solubility of the soil), crops and livestock;*

No significant hazard likely from xylazine and tolazoline excreted in urine or feces. The metabolites would not be of sufficient concentration to impact the agroecosystem when these materials are used in the manner petitioned.

*(6) the alternatives to using the substance in terms of practices or other available materials; and*

No alternatives available for xylazine. Yohimbine is a potential alternative to tolazoline but has some drawbacks under certain conditions.

*(7) its compatibility with a system of sustainable agriculture.*

Compatible.

### **Reviewer 2 Conclusion**

These compounds are routinely used in veterinary medicine. When used in the petitioned manner, no harmful effects are likely to be possible.

### **Reviewer 2 Recommendation Advised to the NOSB**

The substance is Synthetic.

For Livestock, the substance should be Added to the National List.

**Reviewer 3** [Ph.D, Animal Science. Professor of Animal Sciences; Teaching, Research and Extension related to animal growth and development. Midwest U.S.]

### **Comments on TAP Report**

Reasonably complete and technically accurate. Two specific points should be improved for clarity. Under the heading Historic Use by Organic Farmers:

*"..Although xylazine is not approved for use in food-producing animals in the United States, xylazine is still one of the most widely used sedatives in non-organic farming, particularly in ruminants."*

The statement is absolutely true. However, one of the reasons illegal use of this drug in food animals has remained common for over 20 years is a lack of direct, straight forward statements in official documents. Consequently, ignorance of the problem is almost as common as overt disregard of the rules. Almost all cattle and a major portion of all other ruminants end up as a food product. Therefore, almost all use of xylazine in cattle and most use in other ruminants violates the Code of Federal Regulations in regard to use of drugs in food animals. This should be clearly stated in the TAP Report.

### **OFPA Criteria**

It is unclear from the TAP Report whether the Organic Foods Production Act of 1990 has any provisions for superceding other Federal regulations applying to animal drugs. If OFPA does not, then any request or actual approval would be moot in this case.

I disagree with the report on OFPA criteria 6. *The alternatives to using the substance in terms of practices or other available materials.*

Xylazine is an anesthetic commonly used for minor procedures in livestock; in many cases it is used to make the animal handler's and/ or veterinarian's job easier or safer, a consideration not mentioned in OFPA. There are in fact, many alternative *practices* available for many uses of xylazine. The report correctly states there are no organic anesthetic compounds available.

### **Reviewer 3 Conclusions**

Xylazine and Tolazoline are synthetic. They are not approved for use in food producing animals. OFPA concerns animals raised to produce food. Therefore, Xylazine and Tolazoline should not be approved for any use in organic farming.

**Reviewer 3 Recommendations Advised to the NOSB**

The substance is Synthetic.

For Livestock, the substance should be Excluded from the National List.

**TAP Conclusion**

Of the three reviewers, one reviewer supported the use of xylazine and tolazoline without restriction and their addition to the National List, while the other two supported their exclusion. All considered them synthetic materials. Concerns included possible toxicity of the compounds or their metabolites, and the fact that both compounds are not allowed for use in food-producing animals by the FDA.