Ferric Phosphate

Crops

Identification of Petitioned Substance

Chemical Names:
Ferric Phosphate
Iron (III) phosphate
$FePO_4$

CAS Number:
10045-86-0

Trade Names:
Neu1165M Slug and Snail Bait
Ferramol®
Sluggo®

Supplemental Technical Report

Background:

Ferric phosphate is currently included on the National List as a slug or snail bait (7 CFR 205.601 (h)). In 2009, this substance was petitioned to be removed from the National List by the law firm Steptoe & Johnson LLP based on the following claims:

- Ferric phosphate alone is not active or effective as a molluscicide.
- Ferric phosphate only has molluscicidal activity when formulated with the chelating agent ethylenediaminetetraacetic acid (EDTA) or similar chelating agents.
- Based on the results of a recent study (Edwards et al., 2009), there are possible adverse effects on earthworms from the use of iron phosphate baits containing EDTA.
- In 2007, the NOSB Crops Committee voted to reject the petition to include sodium ferric hydroxy EDTA on the National List as a slug or snail bait in part because of the potential for EDTA to be harmful to the environment (NOSB, 2007).
- Therefore, ferric phosphate should be removed the National List because all ferric phosphate molluscicides necessarily contain EDTA or related compounds which the NOSB considers potentially harmful to the environment.

(Steptoe and Johnson, 2009)

Requested Information:
In order to make a recommendation on the petition to remove ferric phosphate from the National List, the National Organic Standards Board (NOSB) Crops Subcommittee has requested this supplemental technical report to evaluate the following questions:

1. Is ferric phosphate alone an effective molluscicide? Can it be combined with other ingredients besides EDTA and still work, or are EDTA and related compounds the only ones that contribute to efficacy?
2. Are there reasons for concern about EDTA beyond what information goes into a tolerance exemption, such as effects on soil organisms or contamination in groundwater?
3. Does the EDTA as used with ferric phosphate pose the same concerns as the EDTA that was reviewed as part of the Sodium Ferric Hydroxyl EDTA?
4. Are there any unbiased studies that back up the findings of Edwards et al. (2009) as cited in the TR or with contrasting results? Does the Edwards et al. (2009) study seem biased?

Responses to the Questions:

1. Is ferric phosphate alone an effective molluscicide? Can it be combined with other ingredients besides EDTA and still work, or are EDTA and related compounds the only ones that contribute to efficacy?

Is ferric phosphate alone an effective molluscicide?

There is limited information available to determine if ferric phosphate alone (without EDTA or another chelating agent) is an effective molluscicide. Ferric phosphate is a simple iron salt. Some simple metallic compounds, including iron salts, have long been recognized as contact and stomach poisons to slugs and snails (Henderson et al., 1989; Young and Armstrong, 2001). According to the reference book, *Molluscs as Crop Pests,* the use of simple metallic compounds to control slugs and snails in agriculture was unsuccessful at first because these compounds were quickly dispersed when applied by broadcast spray and were unappealing to gastropods when incorporated into baits (Henderson and Triebskorn, 2002). However, these authors report that effective bait formulations have been made by combining a metal with "an appropriate organic ligand" to form a metal chelate,[1] for example aluminum and iron chelates (Henderson and Triebskorn, 2002). The compound EDTA is one example of a chelating agent, and it appears that all of the ferric phosphate slug and snail baits currently marketed in the U.S. contain EDTA in their formulations.

The German company W. Neudorff GmbH KG (Neudorff) is the only registrant with the U.S. Environmental Protection Agency (EPA) of a ferric phosphate formulation, which is referred to as NEU1165M (NPIRS, 2012). The active ingredient in this formulation is 1% iron (ferric) phosphate, and the inert ingredients are reported to be EDTA, flour and sugar (April 2010 NOSB Meeting transcripts, Cam Wilson, CTO for Neudorff North America). Neudorff's patented formula is also known as Ferramol® and Sluggo®. The Organic Materials Review Institute (OMRI) Products List includes 13 slug and snail bait products containing ferric phosphate (OMRI, 2012). According to product labels, all of these products are either manufactured in Germany by Neudorff or are made with Ferramol® Slug and Snail bait under a license of W. Neudorff GmbH KG, Germany. That means they all contain the compound EDTA. Indeed, the IFOAM (International Federation of Organic Agriculture Movements) Evaluation of Iron Phosphates as Molluscicide states that iron phosphate is "usually combined with chelating agents, such as ethylene diamine tetracetic acid (EDTA)" (Steptoe and Johnson, 2009, Appendix 2). Furthermore, in a letter to the NOSB, the technical director for OMRI commented, "Based on the evidence compiled by OMRI, ferric phosphate as currently listed at 205.601(h) is not effective as an active ingredient without an additional chelating agent, such as EDTA," and, "chelating agents such as EDTA facilitate the absorption of the metal into the body" (OMRI, 2010). The only evidence provided by OMRI in support of this claim is the content of a U.S. patent issued to Neudorff in 1995 titled, "Ingestible Mollusc Poisons" (Puritch et al., 1995). If any additional primary sources were used by OMRI to make this conclusion, those are not known.

Puritch et al. (1995) claimed that an effective mollusc bait would be composed of both a simple iron compound and a second component, such as edetic acid (EDTA), hydroxyethyl derivative of edetic acid, or a salt of these acids. It also stated that individually neither component is toxic to terrestrial molluscs, but the composition becomes toxic once it is ingested. Therefore, this patent suggests that a chelating agent such as EDTA is necessary for ferric phosphate to be an effective molluscicide. In a recent opinion paper submitted to the NOSB, Neudorff asserted that this patent was submitted at a very early stage in the development of a product before the basic physiological principles of the product had been researched and confirmed (Neudorff, 2010). In addition, the company stated that ferric phosphate alone is toxic to terrestrial molluscs regardless of the presence of EDTA, which is supported by the results of Henderson et al. (1989) and Whaley (2007). Finally, Neudorff claimed that EDTA is added to its slug and snail baits only to encourage ingestion and digestion of ferric phosphate.

[1] A chelate is a compound consisting of a central metal atom attached to a larger molecule (the ligand) in a ring structure (Encyclopaedia Britannica, 2012).

The following information summarizes the available scientific studies that can be used to determine if ferric phosphate alone is an effective molluscicide (Henderson et al., 1989; Whaley, 2007; Zheng et al., 2008).

Henderson et al. (1989)

Henderson et al. (1989) demonstrated the toxicity of various metal-containing compounds to grey field slugs (*Deroceras reticulatum*). Although these researchers did not use ferric phosphate in their experiments, they did test similar iron salts. Iron (II) sulfate was shown to be a contact poison to slugs because it killed a significant proportion of slugs that crawled on a glass plate coated with the compound. Iron (III) sulfate was injected into the gut of slugs and the median lethal dose was found to be 66 µg/slug. A laboratory test was conducted using iron (III) sulfate baits made with milled wheat. Ten slugs were confined with the baits and mortality was observed seven days later. Baits containing iron (III) sulfate were compared with baits containing a chelated form of the metal (iron (III) acetylacetonate). It was reported that the baits containing the chelated compound killed a greater proportion of the slugs than the baits with the simple iron salt, but quantitative results and tests of statistical significance were not provided.

Whaley (2007)

Whaley (2007) compared the molluscicidal activity of various slug and snail baits in outdoor field arenas. In one experiment, slugs (*Deroceras reticulatum*) were released into field arenas with oilseed rape seedlings. In another experiment, snails (*Helix aspersa*) were released into separate outdoor field arenas with organic lettuces. Six different pellet baits were tested with each type of mollusc and included: an untreated control, three different experimental iron phosphate pellets (containing 2.1%, 1.7%, or 1.1% $FePO_4$ without a chelating agent), a European commercial Ferramol® product [1% $FePO_4$, ~3.6% SS-ethylenediaminedisuccinic acid (EDDS)], and one additional product that is assumed by Neudorff (2010) to be a metaldehyde pellet. The pellets were applied to the fields at relevant rates and the number of dead or severely affected slugs and snails were counted and removed at regular intervals. The results showed higher mortality rates for the Ferramol® and metaldehyde pellets when compared to the experimental iron phosphate pellets. Ferramol® and metaldehyde both had a mortality rate of 90% of slugs by day 10 while the mortality rate for the most effective iron phosphate pellets was only 40% by day 10, which was not significantly different than the untreated control. However, by day 14 the mortality rate for the most effective iron phosphate pellets had reached 67.5% of slugs, which was significantly greater than the untreated control (Neudorff Comments on the Whaley Study, 2010). In the snail experiment, the experimental iron phosphate pellets failed to kill a significant percentage of the snails by day 11, while the Ferramol pellets had a mortality rate of 57%.

The study author concluded that the pure iron phosphate baits failed to kill either slugs or snails when compared to the untreated control. However, this conclusion was based on the data for 10 days with slugs and 11 days with snails. If the data for day 14 with snails are included, it can be concluded that experimental iron phosphate baits (1.1% $FePO_4$) did effectively kill slugs in this experiment but the mortality rate was not as high as the Ferramol® pellets. This study was financially sponsored by Lonza Ltd. (Lonza), a direct competitor of Neudorff which manufactures a slug and snail bait containing metaldehyde. Lonza also supplied the experimental iron phosphate pellets used in this study. The full formulation of those pellets was not described in the study report. Furthermore, the report did not include information on the amount of pellets that were ingested by the slugs and snails, which has an impact on the mortality rates. If the iron phosphate baits were unappealing to the slugs and snails that could at least partly explain the lower mortality rates.

Zheng et al. (2008)

In order to explore the effect of EDTA on iron absorption in snails, Zheng et al. (2008) compared the total iron content of selected tissues from snails (*Helix aspersa*) that were fed commercial Ferramol® pellets (1% $FePO_4$ with EDTA), experimental iron phosphate pellets (1% $FePO_4$ without EDTA), or no pellets (control snails). Five snails were used for each treatment and pellets were manually fed to the snails in aquariums.

The report states that snails fed on the Ferramol® pellets were fed 4-5 pellets and died three days afterwards. The snails fed on the iron phosphate pellets were fed 3-4 pellets on three separate occasions over 3 days and "suffered no apparent ill-effects," although it was not reported how much of the iron phosphate pellets were actually consumed by the snails. The authors surmised that snails fed the iron phosphate pellets could be maintained on this diet indefinitely and that it appears the presence of EDTA is necessary for the absorption of toxic levels of iron into the snails. The iron contents of the hearts, kidneys, and dart sacs of treated snails were compared and much higher levels of iron were found in the tissues of snails fed Ferramol® pellets compared to the tissues of snails fed iron phosphate pellets without EDTA (~100× higher in the heart and dart sac, ~300× higher in the kidney). This suggests that much less iron was absorbed into the snails fed iron phosphate pellets without EDTA. However, during dissection the researchers observed that debris from the sticky pellets adhered to the body and shells of the snails despite being rinsed with water. This made is hard to eliminate the possibility that iron or FeEDTA may have been absorbed via the skin or contaminated the organs and also the instruments during dissection. If that were the case, it would have led to measurements that were higher than the actual iron levels in the tissues.

This study was also financially sponsored by Lonza, which supplied the experimental iron phosphate pellets used in this study. The full formulation of those pellets was not described in the study report. The usefulness of this study is limited because it is unknown how much of the experimental iron phosphate pellets were actually consumed by the snails. It is also unclear why the researchers used a different dosing schedule for the two treatments (4-5 pellets of Ferramol® and 3-4 pellets of the experimental iron phosphate pellets). This may have contributed to the differences observed in efficacy. The experimental iron phosphate pellets were ineffective at killing snails after 3 days of feeding in this study, but the reasons for this cannot be determined.

Based on the available studies (summarized in Table 1), there is not enough evidence to definitively conclude that ferric phosphate alone is an effective molluscicide when incorporated into ingestible baits. The limited evidence does support the conclusion that iron baits that contain a chelating agent such as EDTA are typically more effective at killing snails and slugs than iron baits that lack a chelating agent (Henderson et al., 1989; Zheng et al., 2008; Whaley, 2007). However, the Whaley (2007) study demonstrated that ferric phosphate alone can have at least some molluscicidal activity against slugs.

Can ferric phosphate be combined with other ingredients besides EDTA and still work, or are EDTA and related compounds the only ones that contribute to efficacy?

Besides EDTA, at least one other chelating agent has been used in combination with ferric phosphate in order to increase its efficacy as a molluscicide. That compound is (S,S)-ethylenediaminedisuccinic acid (EDDS), a structural isomer of EDTA that is biodegradable (Tandy et al., 2006). Neudorff uses (S,S)-EDDS as an alternative to EDTA in ferric phosphate molluscicides sold outside of the U.S. (April 2010 NOSB Meeting transcripts, Cam Wilson, CTO for Neudorff NA). In 2009, Neudorff petitioned for (S,S)-EDDS to be added to the National List as an inert ingredient for use in pesticide formulations functioning as a chelating agent (Neudorff, 2009). The Crops Committee voted to reject (S,S)-EDDS (NOSB Crops Committee, 2010); however, the petition was withdrawn at the request of the petitioner before a recommendation was made by the NOSB.

EPA's "Metaldehyde Alternatives Assessment" states that, "iron-based molluscicide formulations may contain ethylene diamine tetracetic acid (EDTA), butan, octan, or some other chelating agent to make the iron more biologically active" (EPA, 2006). No further information was found on possible alternative ingredients being used in combination with ferric phosphate to increase its efficacy as a molluscicide.

Table 1. Summary of Available Studies to Determine the Efficacy of Ferric Phosphate Alone

Study	Sponsorship	Study Design	Authors' Conclusions	Critical Review Comments
Henderson et al. (1989)	British Technology Group	Slugs crawled along a glass plate with 20 or 200 µg/cm^2 of iron (II) sulfate; Slugs (n = 10/treatment) were confined with baits made with wheat and iron (III) sulfate or iron (III) acetylacetonate (a chelated form or iron) and mortality was observed 7 days later	A simple iron salt was toxic when absorbed through the foot of crawling slugs; Baits containing a chelated compound killed a greater proportion of slugs than baits with a simple iron salt	This appears to be the only study available that is independent of Lonza Ltd. and Neudorff; Iron (III) phosphate itself was not tested in this study, however the results for similar iron salts are applicable to iron (III) phosphate; Quantitative results for the bait test were not provided in the study report so we do not know how many slugs were killed by the simple iron salt bait
Whaley (2007)	Lonza Ltd.	Slugs (n = 8/treatment) and snails (n = 6/treatment) were released into outdoor field arenas (0.24 m^2) containing seedlings and one of six different pelleted baits (European Ferramol®, three experimental $FePO_4$ baits without chelating agents, metaldehyde, or untreated control); Mortality was assessed at regular intervals	Pure iron phosphate baits failed to kill slugs or snails when compared to untreated controls	The authors did not consider the results in slugs past day 10, which did show a significant effect for one of the $FePO_4$ baits (day 14 results in slugs - available in an appendix); The usefulness of this study is limited because the study report lacked the full formulations of the $FePO_4$ baits, and the amount of pellets actually ingested by slugs and snails was not reported
Zheng et al. (2008)	Lonza Ltd.	Snails (n = 5/treatment) were fed either Ferramol® pellets, an experimental $FePO_4$ bait, or no pellets and then dissected to determine the iron content of selected tissues (heart, kidney, and dart sac)	Pure iron phosphate baits were not toxic to snails and it appears that EDTA is necessary for the absorption of toxic levels of iron into the snails	The usefulness of this study is limited because the study report lacked the full formulation of the $FePO_4$ bait, the amount of the experimental pellets actually ingested by the snails was not reported, different doses were used for the different treatments, and contamination may have occurred during the dissection making the results less reliable

2. Are there reasons for concern about EDTA beyond what information goes into a tolerance exemption, such as effects on soil organisms or contamination in groundwater?

There are some potential concerns about EDTA that were not addressed in the tolerance reassessment decision (EPA, 2004). These concerns are toxicity to soil microorganisms, earthworms, plants, and the potential for groundwater contamination.

Ferric phosphate is exempt from the requirement of a tolerance for residues in or on all food commodities (40 CFR 180.1191). A tolerance reassessment decision was completed by EPA in 2004 when EDTA and its salts became classified as List 4B inert ingredients (EPA, 2004). The tolerance reassessment decision included a review of the data on EDTA and its salts in regards to mammalian toxicity, environmental fate and degradation, and ecotoxicity (aquatic organisms, terrestrial wild animals and birds, monitoring in surface water, and potential for bioconcentration). The review did not include information on potential effects to the agro-ecosystem, such as effects on soil organisms, terrestrial plants, or contamination of groundwater.

EDTA and other chelating agents have been researched for their ability to increase the solubility of heavy metals in soil. This characteristic is utilized by researchers to assist the plant-based phytoextraction technique, which is the use of plants to remove pollutants from the environment (Evangelou et al., 2007). Plants can be used for phytoextraction because they have a natural tendency to take up metals through their roots into their shoots. Many researchers have studied EDTA and other chelating agents for their ability to assist in metal phytoextraction (Evangelou et al., 2007). Application of EDTA to soil has been shown to be effective at enhancing phytoextraction of heavy metals into the roots and shoots of plants. Some of these studies have also shown that EDTA and heavy metal complexes with EDTA can be toxic to soil microorganisms and plants (Grčman et al., 2003; Evangelou et al., 2007; Epelde et al., 2008).

> Grčman et al. (2003) found that addition of 10 mmol EDTA/kg soil (2920 mg/kg) decreased the structure of the fungal community in heavy metal polluted soil compared to a control treatment on days 1 and 56 after application. The structure of bacterial and actinomycetes groups did not appear to be affected by EDTA addition. Results of a different trial showed that EDTA caused stress to soil microorganisms, as indicated by a significant increase in the *trans* to *cis* phospholipid fatty acid ratio (Grčman et al., 2003).
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> In contrast to Grčman et al. (2003), Chander and Joergensen (2011) did not observe an effect on fungal biomass when EDTA (500-2000 mg/kg soil) was added to heavy metal polluted soil.
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> Epelde et al. (2008) studied the effects of EDTA (1000 mg/kg soil) on soil enzyme activities, potentially mineralizable nitrogen, soil basal microbial respiration, and substrate induced respiration (a measure of potentially active microbial biomass). In control non-polluted soils, EDTA caused a significantly negative effect on the soil microbial community activity (evidenced by a decrease in dehydrogenase activity and basal respiration). Potentially mineralizable nitrogen, potentially active microbial biomass, and three soil enzymes (arylsulphatase, β-glucosidase, acid phosphatase) were not significantly affected by EDTA in non-polluted soils.
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> Examples of phytotoxicity observed in studies following the addition of EDTA to soil (1000-2920 mg EDTA/kg soil) include necrotic lesions on cabbage leaves/lowered yield of cabbage biomass, decrease of corn growth to 60% of control, signs of chlorosis and necrosis in white bean, and decreased biomass of cardoon plants (Grčman et al., 2003; Evangelou et al., 2007; Epelde et al., 2008).

The studies demonstrating toxic effects of EDTA on soil microorganisms and plants involved EDTA soil concentrations that are over 10,000 × greater than the EDTA soil concentration expected from the use of Neu1165M/Ferramol®/ Sluggo® slug and snail baits. Those baits contain approximately 1% EDTA (Neudorff, 2010). The recommended application rate for the baits is 5 g/m^2, which is equivalent to 54 mg EDTA/m^2 (Neudorff, 2010). This would result in an approximate final soil concentration of only 0.09 mg EDTA/kg soil (Neudorff, 2010). In comparison, the soil concentration at which microbial effects were observed by Epelde et al. (2008) is 11,000 times greater. It is not known if toxic effects on soil microorganisms and plants would occur from the use of slug and snail baits containing EDTA because no studies were found that tested relevant concentrations of EDTA in soil.

There is a potential concern about toxicity to earthworms from the use of slug and snail baits containing EDTA (Langan and Shaw, 2006; Edwards et al., 2009). This potential concern is discussed in the response to Question #4. Based on the available studies (summarized in Table 2), there is not enough evidence to definitively conclude whether ferric phosphate molluscicides containing EDTA are toxic to earthworms following typical rates of application.

Chelating agents such as EDTA have the potential to influence the mobilization of metals from sediments and aquifers, thereby posing a risk of groundwater pollution (Nowack and VanBriesen, 2005). Leaching of metals after addition of EDTA to soil has been demonstrated in laboratory studies (Evangelou et al., 2007). Those studies observed metal leaching with EDTA soil concentrations much higher than the EDTA soil concentration expected from the use of a slug and snail bait containing 1% EDTA (e.g., Neu1165M/Ferramol®/ Sluggo®). No information was found linking the specific use of EDTA in pesticide formulations to groundwater pollution.

Table 2. Summary of Available Studies to Evaluate the Potential Toxicity to Earthworms of Ferric Phosphate Molluscicides Containing EDTA

Study	Sponsorship	Study Design	Authors' Conclusions	Critical Review Comments
Edwards et al. (2009)	Lonza Ltd.	OECD artificial soil test: Earthworms (n = 10/treatment) were released into soil mixed with a treatment chemical and the median lethal dose (LD_{50}) was estimated Microcosm test: Earthworms (n = 4/treatment) were confined in small containers of soil for 14 days and exposed to molluscicide pellets to determine feeding, body weight, and mortality	The combinations of $FePO_4$ with EDTA or EDDS were the most toxic to earthworms in the OECD artificial soil test, followed by EDDS and EDTA alone; $FePO_4$ alone did not appear to have toxic effects up to 10,000 mg/kg in soil; Data from the microcosm test provided evidence that $FePO_4$ combined with either EDDS or EDTA can have adverse effects on earthworm activity or growth	No significant effects were observed at relevant soil concentrations; The median lethal doses for $FePO_4$ combined with EDTA or EDDS were around 1000 times greater than expected soil concentrations based on the recommended application rate; The effects on body weight observed by the study authors in the microcosm test were not statistically significant
Langan and Shaw (2006)	Unknown; however Lonza employees provided experimental treatments and advice	The survival and burrowing activity of earthworms (n = 20/treatment) were compared following confinement with Sluggo®, metaldehyde, or control pellets	Sluggo® caused negative effects on earthworm survival and growth compared to metaldehyde and control pellets	Although there was an increase in mortality in the Sluggo® group (4/20 died compared to 1/20 in control group), statistical analysis of mortality values were not provided; Earthworms exposed to Sluggo® gained significantly less weight compared to the control group, however the starting weight of the Sluggo® group was below the control group and this may have affected survival and weight gain; Sluggo® was applied at a rate 8× the recommended rate because of the small size of the funnels used in the study
Luhrs (2009)	W. Neudorff GmbH KG	Total earthworm abundance, biomass, and species dominance were measured in a field treated with an experimental $FePO_4$ bait and compared with a positive and negative control; Samplings occurred pre-treatment, and 1 month, 7 months, and 1 year after application	Treatment with the iron phosphate bait did not cause biologically relevant acute or long-term effects on a natural earthworm population	The full formulation of the iron phosphate pellets was not provided; Treatment occurred under realistic conditions using recommended application rates; Any immediate effects of the iron phosphate bait on earthworm mortality may not have been detected because the first sampling did not occur until 1 month after treatment which may have given the population time to recover

3. Does the EDTA as used with ferric phosphate pose the same concerns as the EDTA that was reviewed as part of Sodium Ferric Hydroxyl EDTA?

The EDTA used with ferric phosphate poses the same concerns that were raised for EDTA as part of the review of sodium ferric hydroxyl EDTA.

The NOSB Crops Committee voted to reject sodium ferric hydroxyl EDTA (SFH EDTA) for use as a slug and snail bait in 2007 (NOSB Crops Committee, 2007). The reasons cited for rejection were that ferric phosphate is already listed for that use, concerns about potential harm to humans and the environment, and inconsistency with organic farming and handling. The Crops Committee concluded that EDTA clearly has the potential to be harmful to the environment and can result in the detrimental movement of metals in soils and river sediments. Furthermore, the Crops Committee was concerned about EDTA's slow rate of biodegradation and its persistence in the environment. The EU Commission risk assessment on EDTA (EC, 2004) was cited as the reference for this conclusion. The potential harmful effects of EDTA on human health were also a concern to the Crops Committee. In particular, the Committee concluded that "EDTA is a very strong metal chelating agent, especially for calcium. It is poorly absorbed in mammalian GI tract and concerns have been raised that excessive usage in food could deplete the body of Ca and other minerals" (NOSB Crops Committee, 2007).

In their review of SFH EDTA, the Crops Committee drew conclusions on EDTA compounds in general. Therefore, their conclusions apply to the EDTA compound as it is used with ferric phosphate just the same as their conclusions apply to SFH EDTA.

4. Are there any unbiased studies that back up the findings of Edwards et al. (2009) as cited in the technical report or with contrasting results? Does the Edwards study seem biased?

There are three available studies that evaluate the potential toxicity of ferric phosphate molluscicides containing EDTA to earthworms (Edwards et al., 2009; Langan and Shaw, 2006; Luhrs, 2009). Each study is summarized and reviewed below.

Edwards et al. (2009)

Edwards et al. (2009) is a peer-reviewed, published study that examined the effects of ferric phosphate, EDTA, EDDS, and combinations of these ingredients on earthworms in both an OECD (Organization for Economic Cooperation and Development) artificial soil test and a microcosm test. The OECD artificial soil earthworm toxicity test is a standard laboratory test using *Eisenia fetida*. Seven different treatments were tested in artificial soil and included a control, metaldehyde, iron phosphate, EDDS, EDTA, iron phosphate + 3% EDDS, and iron phosphate + 3% EDTA. The authors tested a range of concentrations for each test chemical (0.1 to 10,000 mg of chemical/kg soil). For comparison purposes, the approximate soil concentrations of ferric phosphate and EDTA or EDDS that would result from a typical single application of Sluggo® or Ferramol® are: ferric phosphate - 0.083 mg/kg soil, EDTA - 0.09 mg/kg soil, and EDDS - 0.18 mg/kg soil (Neudorff, 2010). Ten earthworms were released into each jar of soil and the median lethal dose (LD_{50}) was estimated for each treatment. In the microcosm test, earthworms from the *Lumbricus terrestris* species were confined in containers with soil and exposed to molluscicide pellets for 14 days in order to determine feeding, body weight, and mortality rates. Pellet applications included an untreated control, commercial metaldehyde pellets, commercial Sluggo® pellets, iron phosphate pellets (no chelating agent), EDTA pellets, and EDDS pellets. Each treatment was tested at 1× and 5× the recommended application rate.

Results from the OECD test in Edwards et al. (2009) showed that iron phosphate combined with either EDTA or EDDS had the greatest adverse effect on earthworm survival compared with the other treatments. Estimated LD_{50} values were 78.16 mg/kg for iron phosphate combined with EDTA, 82.98 mg/kg for iron phosphate combined with EDDS, 156.46 mg/kg for EDTA, and 145.57 for EDDS. These median lethal doses were around 1000 times greater than the expected soil concentrations that would result from a single application of Sluggo® or Ferramol®. Indeed, earthworm numbers were not significantly affected at relevant soil concentrations. Iron phosphate by itself was not toxic to earthworms in the OECD artificial soil test with a calculated LD_{50} value greater than 10,000 mg/kg.

Results from the microcosm test showed that earthworm mortality and body weights did not significantly differ from control for any of the treatments tested. The study report stated, "there were considerable differences in earthworm weights, although none of them differed significantly ($P \leq 0.05$) from the control earthworm mean weights" (Edwards et al., 2009). Earthworms exposed to Sluggo® at both 1× and 5× the recommended application rate displayed slightly reduced body weight gains over the course of the study. However, because these differences were not statistically different from the control treatment, it should be concluded that Sluggo® did not produce evidence of toxicity to earthworms in the microcosm test.

Edwards et al. (2009) was sponsored by Lonza Ldt., which also provided the experimental pellets used in this study (except for the commercial Sluggo® pellets). Lonza Ldt. is a direct competitor of Neudorff, which manufactures a slug and snail bait containing metaldehyde.

Langan and Shaw (2006)

Langan and Shaw (2006) is a peer-reviewed, published study that examined the effects of Sluggo® pellets on the survival and behavior of the earthworm *Lumbricus terrestris* in artificial burrows. The authors compared the effects of Sluggo® pellets (8× the recommended application rate) to metaldehyde (35× the recommended application rate) and a bran based pellet control (all funnels had the same number of pellets). The artificial burrows were constructed with funnels and soil. Apple leaves were cut and placed on the surface of the soil in each funnel. The funnels were monitored daily for 33 days to record the number of leaves and pellets remaining on the surface. Leaves and pellets were replaced every 10 days. The body masses of surviving earthworms were recorded at the end of the study.

The study authors reported that iron phosphate pellets (Sluggo®) caused negative effects on earthworm survival and growth compared to metaldehyde and control pellets (Langan and Shaw, 2006). The data showed that a significantly higher amount of pellets and leaves remained on the soil surface for iron phosphate pellets compared with control pellets. The difference in leaf removal was described as reduced surface activity in the earthworms exposed to iron phosphate pellets. Mortality was higher in the iron phosphate group (4/20 earthworms died) compared with the other two groups that both had a mortality rate of only 1/20, however statistical data on mortality values were not provided. Survivors of the iron phosphate group gained significantly less mass than the control group.

Langan and Shaw (2006) was not sponsored by Lonza Ltd. (the manufacturer of metaldehyde bait), however, the authors acknowledged the help of two Lonza employees for providing experimental treatments and advice about the funnels used to make artificial burrows. This study does provide possible (not definitive) evidence of toxicity to earthworms resulting from the use of ferric phosphate pellets containing EDTA.

Luhrs (2009)

Luhrs (2009) is a field study sponsored by Neudorff to evaluate the effects of Neu1166M on a natural earthworm population. It was submitted by Neudorff to the NOSB for review (Neudorff, 2010). The study report describes Neu 1166M as containing 0.9944% ferric phosphate, but does not identify any other ingredients in the formulation. The experiment was performed in a pasture over the period of about a year and Neu 1166M pellets (200 kg/ha) were compared to an untreated control and carbendazim pellets (the positive control). A single application was performed in each plot of the field using a movable plot sprayer, and earthworm samplings of the soil occurred pre-treatment, 1 month, 7 months, and 1 year after application. The parameters examined with each sampling were total earthworm abundance, total earthworm biomass, and species dominance. Treatment with Neu 1166M did not cause any biologically relevant effects on the parameters examined in a natural earthworm population. Therefore, it can be concluded that a single application of Neu 1166M to soil did not appear to cause a significant increase in earthworm mortality or affect the growth or species distribution of earthworm populations over a long period of time. By contrast, treatment with the positive control significantly and negatively affected each of the parameters measured at every sampling point except for one.

The Luhrs (2009) study may be considered more relevant than the other two available earthworm toxicity studies because it is a field study conducted under realistic conditions. It measured several parameters to assess the health of an entire earthworm population over time. The study report does not indicate if EDTA or EDDS is part of the ferric phosphate bait formulation Neu 1166M. However, since all of Neudorff's marketed slug and snail baits contain a chelating agent, the experimental bait probably does as well.

Based on the available studies (summarized in Table 2), there is not enough evidence to definitively conclude whether ferric phosphate molluscicides containing EDTA are toxic to earthworms following typical rates of application. All of the studies have strengths and limitations. Edwards et al. (2009) demonstrated in an OECD artificial soil test that a combination of ferric phosphate with EDTA or EDDS is more toxic to earthworms than ferric phosphate alone, which did not produce toxic effects in earthworms. However, the purpose of this study was solely to estimate the lethal dose and no effects were observed at relevant soil concentrations. When combinations of ferric phosphate and EDTA or EDDS were tested at relevant soil concentrations, no significant toxic effects were observed on earthworms (Edwards et al., 2009; Luhrs, 2009).

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