Finding of No Significant Impact (FONSI)

in support of a New Animal Drug Application
for
Revalor-XR
(trenbolone acetate and estradiol extended-release implant)
for
increased rate of weight gain and improved feed efficiency during 70 to 200 days after implantation in steers and heifers fed in confinement for slaughter

Intervet Inc.
(d/b/a/ Merck Animal Health)
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The Center for Veterinary Medicine (CVM) has considered the potential environmental impact of this action and has concluded that this action will not have a significant impact on the quality of the human environment and, therefore, an environmental impact statement will not be prepared.

Intervet Inc. (doing business as Merck Animal Health) is requesting the approval of a new animal drug application (NADA) for the use of Revalor-XR (trenbolone acetate and estradiol extended-release implant) for increased rate of weight gain and improved feed efficiency during 70 to 200 days after implantation in steers and heifers fed in confinement for slaughter. The Revalor-XR implant consists of 10 coated pellets, each containing 20 mg trenbolone acetate (TBA) and 2 mg 17β-estradiol (17β-E2) for a total of 200 mg TBA and 20 mg 17β-E2. The product is to be administered via subcutaneous implantation on the posterior aspect of the middle of the ear by means of an implant gun. The product will be dispensed over the counter.

In support of the application, Intervet has provided an environmental assessment (EA) dated December 2, 2016. A copy of the EA is attached. We have reviewed the EA and find that it supports a FONSI. This EA was a collaborative effort between Intervet, their contractors, and CVM; it was developed through an iterative process over several years and includes data from several new fate and effects studies contracted by Intervet. CVM worked in concert with Intervet to determine the relevant exposure pathways for assessment and effects endpoints of concern, and to develop appropriate approaches, methods, and assumptions for use in the EA, including development of the exposure analysis methods for watershed scale evaluations.

The purpose of the EA was to assess the fate, effects, and potential impacts of the environmentally-relevant metabolites and transformation products of the active ingredients (TBA and 17β-E2) contained in Revalor-XR. Based on available data from fate studies and monitoring of manure storage structures on feedlots, it was determined that the primary metabolite of 17β-E2 in beef cattle manure and entering the environment is 17α-estradiol (17α-E2), with minor amounts of 17β-E2 and estrone (E1) also found. These three compounds are collectively referred to as estradiol-related compounds. Likewise, the primary metabolite or transformation product of TBA is 17α-trenbolone (17α-TB), with minor amounts of 17β-trenbolone (17β-TB) and trendione (TBO) also found. These three are collectively referred to as trenbolone-related compounds. Therefore, the EA evaluated the potential risk from these six primary metabolites of concern.

The EA includes three primary sections: 1) an exposure assessment in which predicted environmental concentrations (PECs) were developed for various scenarios using environmental fate modeling, 2) an effects assessment in which the predicted no effect concentrations (PNECs) were derived based on chronic fish reproductive effects data, and 3) a risk characterization that utilized the risk quotient (RQ) method, which is based on the ratio of a PEC to a PNEC. An overview of the risk assessment process is presented in Figure 1-1 of the EA.

A review of the published literature supports that the primary metabolites of estradiol (E2) and TBA are endocrine disrupting compounds (EDCs) that are known to cause effects on the reproduction of aquatic organisms (i.e., fish) when chronically exposed to low concentrations (parts-per-trillion, ppt). Therefore, the exposure assessment focused on the potential exposure in the aquatic environment and the effects assessment focused on effects on fish reproduction endpoints, such as fecundity, fertility, etc. Effects on terrestrial organisms (i.e., plants, invertebrates, etc.) were not evaluated because exposure in the terrestrial environment is expected to be limited due to very rapid degradation in soil (<7 days). In addition, fish were found to be more sensitive to exposure to estradiol- and trenbolone-related compounds than other aquatic species; therefore, effects on aquatic invertebrates and amphibians were not evaluated.

Exposure Assessment

Active metabolites of E2 and TBA are excreted in cattle manure and have the potential to enter the aquatic environment through several different sources and pathways. In the exposure assessment, the exposure in the aquatic environment (i.e., PEC values) was quantitatively estimated first for individual farm-scale (single pathway) scenarios and subsequently for aggregate watershed-scale scenarios using advanced computer modeling developed by the United States Environmental Protection Agency (US EPA). In the EA, an aggregate exposure is defined as exposure to a single chemical by multiple pathways and routes of exposure.

The impacts of the six primary E2 and TBA metabolites were evaluated in this assessment. However, preparing a collective environmental exposure and risk assessment on all six of these compounds was found to be difficult and scientifically challenging because certain key data were not available. Specifically, there was a lack of data on 1) the proportion of each metabolite in feces and urine, 2) fish reproductive toxicity to estrone and trendione, and 3) fate and mobility of some metabolites. Moreover, modeling each of the six TBA and E2 metabolites in three affected environmental compartments (manure, soil and water) for multiple farm-scale and aggregate watershed-scale scenarios would have been complex and difficult to perform and interpret given the limitations of the fate and transport models currently available. Therefore, instead a simpler, conservative surrogate compound approach was used in which a single surrogate estradiol compound[1] and a single surrogate trenbolone compound[2] were defined and modeled in the exposure assessment and used for

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[1] In the EA, a surrogate estradiol compound is defined as one estradiol-like compound with the physical-chemical and environmental fate properties that conservatively represents a composite of the parent compound, 17β-E2, and its metabolites.
[2] In the EA, a surrogate trenbolone compound is defined as one trenbolone-like compound with the physical-chemical and environmental fate properties that conservatively represents a composite of the primary metabolites of TBA.

characterizing risk from use of Revalor-XR. This was done because the molecular structures and many of the physical-chemical properties of 17α-E2, 17β-E2, and E1 are quite similar, therefore, it could be assumed that they would be transported, transformed, and degraded similarly in the environment. For the same reasons, the same assumption and approach was also used for 17α-TB, 17β-TB, and TBO. Using this surrogate compound approach, the environmental concentration (i.e., PEC values) estimated for the surrogate compounds are considered to represent the total residue of all three individual metabolites of estradiol or trenbolone in the relevant compartments modeled (manure, soil, and water).

To model the fate, transport, and exposure of the surrogate compounds in aquatic and terrestrial environments, data on the physical-chemical properties, mobility in soil, and rates of degradation in soil, water, manure, and sediment were required. The physical-chemical and environmental fate properties used in the environmental modeling of the surrogate compounds were selected using conservative approaches and assumptions. This ensured that PEC values determined for water would not be underestimated. Parameters used in the modeling were selected using data obtained from acceptable literature studies and/or Intervet-owned studies that were conducted in accordance with Organization for Economic Co-operation and Development (OECD) Guidelines and Good Laboratory Practices (GLP). Several criteria were used by CVM to evaluate whether the data from a published literature study were acceptable for use in deriving environmental fate parameters, such as whether: 1) the experimental methods and procedures were adequate or similar to OECD Guidelines and/or other acceptable guidelines, 2) the analytical methods were adequate and the analytical results were within acceptable standards, and 3) the data analysis methods used were adequate to determine the final endpoint of interest. Once all acceptable data were identified, conservative methods recommended in the US EPA Guidance for Selecting Input Parameters in Modeling the Environmental Fate and Transport of Pesticides[3] were used to derive the environmental fate parameters for use in the modeling of the surrogate compounds. Specific methods used to derive each fate parameter are discussed in Section 4.5 of the EA.

The physical-chemical properties and environmental fate data obtained for the estradiol- and trenbolone-related compounds suggest that they will be slightly to moderately mobile in soil [soil organic carbon adsorption coefficient (Koc) values are 1930 and 1618 L/kg for the surrogate estradiol and trenbolone compounds, respectively] and degrade rapidly via microbially-mediated processes in aerobic soils [soil degradation half-life (DT_{50}) values are 6.8 and 1.8 days for the surrogate estradiol and trenbolone compounds, respectively]. In addition, further binding and degradation of the metabolites is expected in the aerobic and anaerobic water-sediment environment. The surrogate estradiol and trenbolone compounds were estimated to degrade in water-sediment environments with an aerobic DT_{50} of 15.1 and 33.4 days, respectively, and an anaerobic DT_{50} of 66.2 and 60.5 days, respectively. Metabolites of both estradiol and trenbolone have been found to photodegrade rapidly in clear waters; however, little photodegradation data is available for waters with high turbidity. As a result, hydrolysis and photodegradation of the estradiol- and trenbolone-related compounds were not accounted for in the environmental fate modeling, which likely resulted in an overestimate of exposure. A summary of the environmental fate data endpoint values that were used in the exposure modeling is provided in Table 4-33 and 4-34 of the EA.

Based on studies sponsored by Intervet, degradation of the estradiol and trenbolone-related compounds is expected to occur in manure after it is excreted, either when held on the

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[3] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-selecting-input-parameters-modeling

feedlot (e.g., stockpiled in a feedlot pen or other surface on the feedlot) or in a storage pond, and prior to application to cropland. However, the situation is complicated because degradation is greatly reduced under anaerobic conditions. Anaerobic conditions appear to occur rapidly in the manure environment following excretion, including on the feedlot surface, in stockpiles and in storage ponds. In addition, non-extractable residues (NER) may be formed in manure. Although NER appear to be tightly bound to manure, it is unknown whether these residues could be released and become bioavailable as organic matter in manure degrades or when manure is applied to cropland and mixed with soil. As a result, a conservative approach was used in the EA in which the anaerobic DT_{50} values for manure were used (instead of aerobic DT_{50} values) and NERs were assumed to be as bioavailable as the extractable residues. In addition, during later sampling time periods of the manure degradation studies, it was found that degradation in manure slowed or stopped (i.e., plateaued). This plateau effect was taken into consideration in the exposure assessment when applying the manure degradation data. See Section 4.3.5 in the EA for a summary of manure degradation data and the discussions in Section 4.5.2.5.6 and Appendix 15.4 of the EA for further details on the use of the NER data and plateau approach.

Exposure Modeling

Once approved, Revalor-XR can be legally used anywhere in the US; therefore, estradiol- and trenbolone-related compounds could potentially be introduced into any freshwater aquatic ecosystem. However, in order to determine the potential impacts to the aquatic environment for this assessment, geographical areas at high risk for potential impacts were identified to aid in calculating conservative PECs in surface water (PEC_{sw}) for use in the risk characterization. These areas are meant to represent locations where reasonable worst-case exposures may occur in the US (i.e., geographic areas where cattle production is concentrated and aquatic ecosystems are most likely to be impacted). Using information on beef cattle statistics, beef cattle management practices used in the US, regulation of beef cattle feedlots, and region-specific data (e.g., precipitation, soil characteristics, etc.), two geographic areas in the US were identified for in-depth evaluation: Midwest (Iowa) and Great Plains (Texas). These areas were chosen due to the intense beef cattle production on animal feeding operations (AFOs) at these locations, as well as their unique weather and soil characteristics.

Once the two geographic areas were identified, three generic farm-scale exposure scenarios were developed to represent hypothetical feedlot operations in the two areas of interest. These generic exposure scenarios encompassed various feedlot cattle production and manure management practices (Midwest 1, Midwest 2, and Great Plains). The potential sources and routes of exposure were determined for each of these three generic exposure scenarios. Based on beef cattle management practices in the US and the conditions of use for Revalor-XR, nine exposure pathways were identified. These pathways included:

1. Runoff and leaching from manure-amended agricultural soils. This pathway has four subparts:
    a. Direct exposure:
        i. Runoff following solid manure amendment
        ii. Runoff following irrigation with liquid manure from a manure storage pond
    b. Indirect exposure:
        i. Leaching to subsurface tile drains and subsequent migration/entry to surface water
        ii. Leaching (infiltration) to groundwater and subsequent migration/entry to surface water

2. Leaching from an unpaved feedlot to groundwater, and subsequent migration/entry to surface water
3. Leaching from stockpiled solid manure to groundwater, and subsequent migration/entry to surface water
4. Leaching from unlined storage ponds to groundwater and subsequent migration/entry to surface water
5. Direct runoff from feedlot
6. Overspill from storage pond

The selection of the geographic areas of interest and development of these generic exposure scenarios is explained in Chapter 5 of the EA and in Chapters 2 through 5 of the Exposure Assessment (Exponent, 2016) contained in Attachment 16.2 of the EA.

These pathways were evaluated individually at a farm-scale, and if they were found to contribute a significant amount of the surrogate compounds to the aquatic environment, they were included in the aggregate watershed-scale analysis of the generic Midwest (Iowa) and Great Plains (Texas) exposure scenarios.

The farm-scale PEC values for the individual exposure pathways were determined using one of the following software programs or other acceptable direct-calculation methods: 1) US EPA's Exposure Analysis Modeling System (EXAMS)-Pesticide Root Zone Model (PRZM) Simulation Shell (EXPRESS) was used to model surface runoff from solid and liquid manure-amended cropland, 2) US EPA's PRZM-3 was used to model leaching to tile-drains, 3) US EPA's Screening Concentration in GROund Water (SCI-GROW) was used to model leaching from feedlot, storage pond and cropland surfaces, and 4) mathematical calculations were used to estimate direct runoff from a feedlot. Information on these exposure models and the exposure assessment analyses that were conducted for the individual farm-scale exposure pathways are summarized in Chapter 5.3 of the EA and presented in greater depth in Chapter 6 of the Exposure (Attachment 16.2 to the EA).

A total of 96 PEC values were estimated for each surrogate compound for the generic farm-scale exposure scenarios, including five PEC values for groundwater and leachate for leaching scenarios, one PEC value for direct runoff from a feedlot, 42 PEC_{sw} values for runoff from cropland irrigated with storage pond water, and 48 PEC_{sw} values for runoff from cropland amended with solid manure based on till (42 PECs) and no-till (6 PECs) application methods. Table 5-4 of the EA lists the maximum PEC values obtained for each pathway. Based on these PEC values it was determined that the leaching pathways would not contribute significantly to the PEC_{sw}; therefore, all five leaching pathways were eliminated from evaluation in the watershed-scale modeling. In addition, overspill of a storage pond following a 25-year, 24-hour rain event was excluded because this event would be rare and would result in only acute exposures. Thus, the watershed-scale modeling evaluated only the three surface runoff exposure pathways, including direct runoff from a feedlot and runoff from cropland amended with solid manure and irrigated with liquid manure from a storage pond.

Aggregate Watershed-scale Modeling

The two large watersheds chosen to be modeled on the watershed-scale exposure were: 1) the Maquoketa River watershed in eastern Iowa in the Midwest and, and 2) the Tierra Blanca Creek watershed in the Texas panhandle in the Great Plains. In the Texas watershed, a combination of the following exposure pathways was simulated to determine the aggregate (combining all pathways) PEC_{sw} at the watershed pour point: runoff after land application of solid manure and runoff after irrigation of land with liquid manure from a storage pond. For the Iowa scenario, a slightly different combination of exposure pathways was simulated to determine the aggregate PEC_{sw} at the pour point: runoff after land application of solid manure and direct runoff from a feedlot.

To simulate the fate of the surrogate compounds on a watershed scale, the US EPA Better Assessment Science Integrating point & Non-point Sources (BASINS) model was used in combination with Hydrologic Simulation Program-Fortran (HSPF/WinHSPF) model. Key high-level assumptions used in the modeling for all evaluated exposure pathways include:

- The number of feedlots and cattle in the watershed was estimated based on the US Department of Agriculture (USDA) Census of Agriculture data from 2007 and were randomly distributed in the watershed based on the default random generator algorithm of ArcGIS 10 (ACM collected algorithm 599)
- All of the beef cattle (100%) in the watershed were assumed to be implanted with Revalor-XR
- Beef cattle implanted with Revalor-XR were assumed to be held on the feedlot all 365 days a year; it was assumed that cattle (and the manure they produce) would be contained for a holding period of 182 days, then the cattle and manure would be removed and replaced with a new herd of cattle that also were implanted with Revalor-XR and held for the remaining duration of the year (183 days)
- Application of the surrogate compounds (accumulated over 182 days) to cropland occurs twice per year via manure application and through irrigation of storage pond water, once during the spring (May 1-7) and once during the fall (October 1-7), each over a consecutive seven-day period
- The runoff scenarios were simulated every day over a ten year period with the highest rainfall (1999 and 2009), which is considered representative of a realistic conservative runoff scenario
- Transport is instantaneous from the application site to the watershed pour-point
- The ground surface throughout the watershed was considered to be impermeable during runoff events (i.e., no infiltration or leaching was assumed in the model)
- Degradation was accounted for in soil during dry periods only (i.e., when no runoff occurred)
- All chemicals in runoff and in surface water were assumed to be freely dissolved, bioavailable, and not subject to adsorption or degradation

A detailed description of the approaches used, as well as a list of all assumptions and calculations, is presented in Chapter 5.4 of the EA and in Chapter 7 of the Exposure Assessment (Attachment 16.2 of the EA). When the BASINS/HSPF model is used with the above assumptions, it acts primarily as an estimator of dilution in the watershed. That is, except during dry periods, the chemical is only subject to transport (via rainfall or stream flow) where it is diluted by the volume of water. Environmental fate processes such as adsorption and degradation are not accounted for in soil and aquatic-sediment systems (except for degradation in soil during dry periods), resulting in conservative exposure estimates (i.e., higher concentrations than would otherwise be expected).

In order to conduct the modeling in BASINS/HSPF, it was necessary to calculate the application rates of the surrogate compounds for each exposure pathway (i.e., runoff from solid manure application to cropland, runoff from irrigation with water from a storage pond, and subsequent runoff and direct runoff from a feedlot). Key high-level assumptions used to determine the application rates of the surrogate compounds for all exposure pathways included:

- During the year, liquid and solid manure would be subject to storage, runoff, and application to cropland
- The volume of manure excreted (both liquid and solid) and mass of drug excreted was calculated based on the number of cattle in the watershed, which was also used to determine the number of acres amended with manure (and therefore, surrogate compound) and the size of the storage pond
- The surrogate compound was partitioned between liquid and solid manure based on the organic-carbon normalized partition coefficient derived from sorption experiments and the mass fraction of the manure that was organic carbon (see Chapters 7.2.5.1, 7.2.6.1, 7.3.5.1, and 7.3.6.1 of the Exposure Assessment in Attachment 16.2 of the EA for a listing of all assumptions and calculations used to determine the distribution of excreted compounds)
- Annual runoff percentages of 60 and 25% obtained from USDA-National Resource Conservation Service (NRCS) for paved feedlots in Iowa and unpaved feedlots in Texas, respectively, were used to determine the percentage of liquid manure on a feedlot surface available to runoff directly to surface water (Iowa scenario) or the storage pond (Texas scenario); the remaining liquid manure was stored with the solid manure
- Degradation was assumed to occur in the manure held on the feedlot (either in a stockpile or storage pond) over a 182 day storage period based on anaerobic degradation half-life values
- Non-extractable residues (NER) in the manure were not subject to degradation and were bioavailable after application to land
- The value where the plateau occurred, described above, is the minimum amount of chemical remaining in the manure at the end of storage (regardless of the extent of degradation)

The application rates of the surrogate compound contained in solid and liquid manure were determined as follows.

In both watersheds, the application rate for the surrogate compounds in solid manure was determined by assuming that all manure remaining on a feedlot after the 182-day[4] holding period would be applied to cropland. During this period, the extractable portion of the surrogate compound was subject to anaerobic degradation. NERs were not subject to degradation, but were assumed to be bioavailable and added to the remaining surrogate compound after degradation was accounted for. At the end of the 182-day manure storage period, all solid manure was applied to cropland and incorporated to a depth based on the tillage practices for that region, i.e., conventional till practices (manure and surrogate compound incorporated uniformly in the top 15 cm) or conservation till practices (e.g., no-till; manure and surrogate compound incorporated uniformly in the top 5 cm). For the Iowa watershed, it was assumed that conventional tillage practices (15 cm incorporation depth) were used on 29% of the cropland, and that conservation till practices (5 cm incorporation depth) were used on the remaining 71% (see Chapter 7.1.3 of the Exposure Assessment, Attachment 16.2 of the EA). For the Texas watershed, it was assumed that only conventional tilling practices (15 cm incorporation depth) were used.[5] After incorporation, a portion of the top 2 cm was allowed to runoff during rain events in accordance with the PRZM-3 non-uniform extraction model (see Chapter 7 of the Exposure Assessment, Attachment 16.2 of the EA). For a complete list of assumptions and calculations, see

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[4] Solid manure (and remaining liquid manure that did not runoff) was assumed to be collected daily and held for a storage period of up to 182-d (Chapter 4.1 of the Exposure Assessment [Attachment 16.2 to the EA]).
[5] The percent of cropland using till and no-till application practices for Iowa and Texas were based on USDA-Economic Research Service (ERS) data.

Chapters 7.2.5.3, 7.2.6.3, 7.3.5.3, and 7.3.6.3 of the Exposure Assessment (Attachment 16.2 to the EA).

The application rate for surrogate compound in liquid manure was different in the two watersheds due to two assumptions: 1) the feedlots in Texas were assumed to be unpaved, whereas the Iowa feedlots were paved, and 2) the Texas feedlots were assumed to be subject to US EPA concentrated animal feeding operation (CAFO) regulations (40 CFR 412.31), which would require them to contain their wastewater runoff on the feedlot (e.g., storage pond), whereas it was assumed that Iowa feedlots were not in compliance (i.e., direct runoff to surface waters would occur). Therefore, in the Iowa watershed, the application rate for the surrogate compound in liquid manure is equivalent to the fraction of liquid manure assumed to directly runoff to surface waters (i.e., 60% based on the USDA-NRCS annual runoff percentages). The liquid manure in runoff was not subject to degradation or adsorption. In the Texas watershed, the fraction of liquid manure available to runoff (i.e., 25% based on the USDA annual runoff percentage) was assumed to be captured in a storage pond. In the pond, the liquid manure was assumed to be stored for the same period as solid manure (up to 182 d) and was subject to anaerobic degradation during this period of time. At the end of the holding period, liquid manure from the pond was applied to cropland at an irrigation rate of 2 in/A with an incorporation depth of 5 cm. This application occurred concurrently with that for the solid manure. For a complete list of assumptions and calculations, see Chapters 7.2.5.2, 7.2.6.2, 7.3.5.2, and 7.3.6.2 of the Exposure Assessment.

According to the BASINS/HSPF, the results of the simulations for each watershed are the predicted watershed pour point concentrations experienced by a non-mobile receptor organism on a daily time step for the ten year period. These values are equivalent to the PEC_{sw}. The PEC_{sw} results from the aggregate watershed-scale modeling for the Iowa and Texas scenarios are presented in Table 1. The values are presented for the individual pathways, followed by the values for the combined aggregate exposure resulting from all pathways. Due to limitations of the BASINS/HSPF models, these PEC values do not account for adsorption to soil, sediment or suspended particles, or degradation/transformation in aquatic systems. Therefore, these PEC values represent conservative estimates (likely overestimates) of the surface water concentrations in the Iowa and Texas watersheds due to the use of Revalor-XR.

The PEC_{sw} values presented in the bottom row of Table 1 represent the 90^{th} percentile monthly maximum 21-d moving average PEC_{sw} at the watershed pour-point during the 10-year simulation period. Typically the US EPA models use the yearly maximum concentrations to derive the 90^{th} percentile PEC_{sw} values. However, in this EA, the method to derive the PEC_{sw} was modified to use monthly maximum averages rather than yearly maximum averages because fewer years were simulated (10 years instead of the 30 years typically simulated by US EPA). If the 90^{th} percentile PEC_{sw} was calculated based on yearly maximum concentrations it would be biased by extreme values due to the small sample size (i.e., only 10 values total), which would result in an overestimation of the PEC_{sw}. The use of the daily concentrations was also considered to calculate the 90^{th} percentile PEC_{sw}, but many days during the 10-year period had very low concentrations (at or around zero), which would result in the 90^{th} percentile PEC_{sw} being biased towards low values and potentially underestimate the PEC_{sw}. Basing the calculation of the 90^{th} percentile PEC_{sw} values on the monthly maximum 21-d moving average concentrations reduces bias from both extremes and uses more of the modeled data than the yearly maximum method; thus, increasing the sample size (i.e., 120 values total). In addition, the monthly period better approximates the time period that was used to calculate the moving average PEC_{sw}

values (21 days). Therefore, the monthly maximum 21-d moving average concentrations were used to calculate the 90th percentile PEC_{sw}.

Table 1: Summary of the PEC_{sw} for the aggregate watershed-scale modeling in the Texas and Iowa scenarios

Exposure pathway	Comments	Surrogate estradiol compound [ng/L]		Surrogate trenbolone compound [ng/L]	
		Texas	Iowa	Texas	Iowa
Direct runoff from feedlot [a]	Maximum daily concentrations, not considering buffers or in-stream degradation and partitioning	NA	0.202	NA	1.580
Direct runoff from manure-amended soil [a]	Maximum daily concentrations, solid manure applied to the field over a 7-day period in spring (May 1-7) and fall (October 1-7) each year for 10 years	1.454	0.375	3.635	2.466
Direct runoff after irrigation with manure storage pond water [a]	Maximum daily concentrations, liquid manure applied to the field over a 7-day period in spring (May 1-7) and fall (October 1-7) each year for 10 years	0.0014	NA	0.0060	NA
Aggregate exposure of all pathways [b]	90th percentile of the monthly maximum 21-d moving average concentrations for the 10-year period	0.0125	0.0108	0.0149	0.0815

[a] The PEC_{sw} values presented are the maximum daily concentrations for the 10-year period 1999 – 2008 without accounting for dissipation of the surrogate compounds in runoff or surface water or adsorption to soil, sediment or suspended particles.
[b] The PEC_{sw} values presented are the 90th percentile of the monthly maximum 21-d moving average concentrations at the watershed pour-point for the 10-year period 1999–2008 without accounting for dissipation of the surrogate compounds in runoff or surface water or adsorption to soil, sediment or suspended particles.
NA – not applicable.

The Iowa scenario had higher aggregate PEC_{sw} values compared to the Texas scenario due to the high concentration of surrogate compounds introduced into the surface water from direct runoff from feedlots. Direct runoff from feedlots was evaluated in the Iowa scenario only; whereas, in the Texas scenario it was assumed that all feedlot runoff would be captured in a storage pond and applied to cropland via irrigation (i.e., no direct runoff to surface waters). Direct runoff from the feedlot to surface waters resulted in considerably higher PEC_{sw} values that were similar to those resulting from runoff from manure-amended soils (1.580 and 2.466 ng/L, respectively, for the surrogate trenbolone compound). The PEC_{sw} values resulting from irrigation with storage pond water were much lower (0.0060 ng/L for the surrogate trenbolone compound) (Table 1). It was assumed that 100% of AFOs in the Iowa scenario were directly discharging feedlot wastewater (mobile liquid manure runoff and rainfall) to surface waters. This percentage is considered to be an overestimation (see Chapter 5.2.2 of the EA) and, as a result, the PEC values for the Iowa watershed scenario are also expected to be overestimated.

The aggregate exposure (combining all pathways) was found to be intermittent, primarily driven by seasonal manure application and rainfall events (see Chapters 7.2.7 and 7.3.7 of the exposure assessment; Attachment 16.2 of the EA). This was especially evident for the Texas scenario, where manure application (either solid or liquid) was the only pathway modeled. During the 10-year simulation period (1999–2008) in the Texas watershed, there were 3,508 of 3,653 days (96.03%) when the concentration of surrogate estradiol compound in the stream was close to zero (<0.001 ng/L), and 3,554 of 3,653 days (97.29%) when the concentration of the surrogate trenbolone compound was close to zero. For the Iowa scenario, direct runoff from feedlots resulted in more frequent exposure because runoff was accounted for on a daily basis and driven by rainfall events (see Chapters 7.2.5 and 7.2.6 of the exposure assessment; Exponent, 2016; Attachment 16.2 of the EA). As this pathway contributed significantly to the aggregate watershed-scale exposure, the percentage of days with an in-stream concentration close to zero (less than 0.001 ng/L) was less in the Iowa scenario; 2,761 of 3,653 days (75.58 %) for the surrogate estradiol compound and 2,259 of 3,653 days (61.84%) for the surrogate trenbolone compound. However, the number of days that the instream concentration was higher than 0.1 ng/L over the 10-year simulation period was still very low; 19 of 3,653 days (0.52%) for the surrogate estradiol compound and 163 of 3,653 days (4.46 %) for the surrogate trenbolone compound. The fact that direct runoff from feedlots in the Iowa watershed resulted in more days with exposure, which were distributed relatively evenly over the modeling period, explains the difference in the 90th percentile monthly maximum 21-d moving average PEC_{sw} obtained for the Iowa and Texas scenarios.

Effects Assessment

In the effects assessment, PNEC values were derived for the most potent metabolites of concern (17α-E2, 17β-E2, 17α-TB, and 17β-TB) based on effects on fish reproductive endpoints. From a thorough review of the published literature, including acute and chronic effects data for various endpoints for aquatic plants, aquatic invertebrates, amphibians, and fish, it was determined that fish are the most sensitive and sentinel taxonomic group for understanding and evaluating the impact of estradiol- and trenbolone-related compounds in the aquatic environment. Furthermore, these published studies also demonstrated that fish reproductive endpoints, such as fecundity, fertility, hatching success, etc., are the most sensitive effects endpoints to assess population-relevant impacts. Therefore, effects on fish reproductive-endpoints based on chronic exposures to estradiol- and trenbolone-related compounds were used to derive the PNEC values used in the EA for risk characterization.

The PNEC values were derived for the 17α and 17β isomers of E2 and TB using 1) conservative no observed effects concentration (NOEC) or EC_{10} (the effects concentration that results in a 10% reduction in fecundity) value(s) for the most sensitive fish reproduction endpoints estimated from chronic studies (≥21 days), and 2) a conservative assessment factor (AF) of 10 to account for uncertainty in laboratory data and other issues; except for 17β-E2, in which a smaller AF of 2 was used because a considerable amount of chronic and multigenerational effects data were available. The PNEC is equal to the effects concentration divided by the AF; e.g., NOEC/10. The NOEC and EC_{10} values were selected from acceptable chronic studies obtained from the published literature[6] or Intervet-sponsored studies. Several effects values were supported by multiple studies in several different fish species. These effects values are thought to conservatively represent potential

[6] It is important to note that the daily tabulated data for apical effects endpoints (e.g., daily egg production, fertility, etc.) were obtained for the chronic effects studies for 17α-TB and 17β-TB conducted by the US EPA (Ankley et al., 2003 and Jensen et al. 2006), which allowed additional statistical analysis to determine conservative effects values. Therefore, additional studies were not conducted by Intervet for these compounds.

effects in a variety of fish species over chronic durations from 21 to 280 days. In addition, the use of the AF to derive the PNEC is expected to conservatively protect against potential effects that could occur in later generations. The PNEC values were determined to be 19.9, 1.4, 3.2, and 0.2 ng/L for 17α-E2, 17β-E2, 17α-TB, and 17β-TB, respectively. Detailed information on how the PNEC values were derived and the data on which they are based is contained in Chapter 6 of the EA.

Risk Characterization

In the risk characterization, the risk quotient (RQ) method (PEC/PNEC) was used to estimate the potential risk to fish reproduction endpoints when exposed to the surrogate estradiol compound or the surrogate trenbolone compound. The RQ values for the surrogate estradiol compound and surrogate trenbolone compound were estimated using the same methods.

For the surrogate estradiol compound, the RQs for fish reproduction-related endpoints were calculated by assuming that the toxicity of the surrogate compound was equal to the toxicity of the 17α isomer, which is expected to be the primary metabolite in manure applied to land. Thus, to derive the RQ values for the surrogate estradiol compound, the PEC_{sw} values for the surrogate estradiol compound were compared to the PNEC of 17α-E2 (19.9 ng/L). However, it is also expected that a small portion of the surrogate estradiol compound represents 17β-E2 and estrone. Based on available data, the 17β-E2 is a more potent endocrine disruptor in fish than 17α-E2 and estrone. Therefore, a separate set of RQ values were calculated where it was conservatively assumed that the toxicity of the surrogate estradiol compound was equal to the toxicity of 17β-E2; i.e., the PEC_{sw} values for the surrogate estradiol compound were compared to the PNEC of 17β-E2 (1.4 ng/L). A similar approach was used for the surrogate trenbolone compound using its PEC_{sw} values and the PNEC values for 17α-TB and 17β-TB, which are 3.2 and 0.2 ng/L, respectively. Although estrone and trendione are also expected to be present in cattle manure and the environment, RQ values were not derived for these compounds because the α- and β-isomers of E2 and TB are expected to be much more potent, and therefore, sufficiently address the potential risks posed by estrone and trendione.

In CVM's Guidance for Industry #166[7], an RQ value of ≤1 is used as a preliminary screening level (i.e., assessment value) to determine if additional analysis and refinement of the risk assessment may be needed. Because of the many conservative assumptions used throughout the exposure and effects assessment in the EA for Revalor-XR (as discussed further below), CVM thinks that an RQ value of approximately 1 or less indicates that significant environmental effects are highly unlikely at the predicted level of exposure.

In total, the highest RQ values representing thousands of PEC values were determined for each of the surrogate estradiol and trenbolone compounds for the farm-scale crop scenarios simulating solid manure application and liquid manure application via irrigation with storage pond water, and also for the two aggregate watershed-scale scenarios that were modeled. Table 2 below presents the highest PEC_{sw} and RQ values for the surrogate estradiol and trenbolone compounds for these different scenarios.[8] For the generic farm-scale exposure scenarios, no RQ exceeded the assessment value of 1, except for 17β-TB for the runoff

[7] CVM. 2006. Environmental Impact Assessment (EIAs) for Veterinary Medicinal Products (VMPs) – Phase II (VICH GL 38).

[8] RQ values for the individual farm-scale modeling of the leaching pathways were not calculated in the EA because the modeling estimated concentrations in groundwater, not surface water, and therefore, could not be compared to the PNEC for fish because fish would not exist in groundwater.

resulting from solid manure application to agricultural land; none of the RQs for the surrogate estradiol compound exceed 1. Based on conventional tillage practices, eleven of 42 crop scenarios (approximately 26%) modeled had RQs greater than 1 (maximum RQ = 2.1; see Table 7-2 of EA), while for no till practices, RQs for all six crop scenarios modeled exceed a value of 1 (RQ range = 2.13 – 6.30), all for the surrogate trenbolone compound. However, all of these RQ values are based on the conservative assumption that 100% of the surrogate trenbolone compound is as toxic as 17β-TB. When considering available data on the proportion of 17β-TB excreted by cattle and potentially entering the environment (Chapter 4.1.1 of the EA), the percentage of the surrogate compound attributed to 17β-TB is likely very low. Thus, the RQ values for 17β-TB presented in Table 2 are considered to be significantly overestimated.

Table 2: Surface water RQs for the surrogate estradiol and trenbolone compounds for the generic farm-scale exposure scenarios and aggregate watersheds

Modeling	Scenario/ Watershed	Pathway	RQs for the surrogate compounds based on the PNEC for:			
			17α-TB	17β-TB	17α-E2	17β-E2
Generic (farm-scale) exposure scenarios[a]	Direct runoff soil	Runoff from solid manure (conventional tillage)	0.130	2.10	0.004	0.050
		Runoff from solid manure (no-till)	0.390	6.30	0.010	0.145
		Runoff from irrigation with storage pond water	0.020	0.350	0.004	0.050
Aggregate watersheds[b]	Midwest (Iowa)	Direct runoff from a feedlot Runoff from solid manure (conventional tillage and no-till)	0.025	0.410	≤0.001	0.008
	Great Plains (Texas)	Runoff from solid manure (conventional tillage) Runoff from irrigation with storage pond water	0.005	0.070	≤0.001	0.009

[a] Based on either the highest PEC_{sw} of the 42 US EPA crop scenarios modeled (conventional tillage or irrigation) or the highest PEC_{sw} of six US EPA crop scenarios modeled (no till), where the PEC_{sw} is the 90th percentile of the maximum yearly 21-d moving average concentration over a 30-year simulation.
[b] Based on the 90th percentile of the monthly maximum 21-d moving average PEC_{sw} at the watershed pour-point.

Because some of the RQs for the generic farm-scale exposure scenarios were greater than 1, additional exposure analyses were conducted by Intervet using the watershed-scale modeling approach previously described. The aggregate watershed-scale modeling approach is considered an exposure refinement in the EA. CVM agrees and considers the watershed-scale results to best represent the risks likely to occur for aquatic receptors, specifically fish in this case, as they are the group of animals most likely to be affected. For both of the aggregate watershed-scale scenarios, Iowa and Texas, the RQs did not exceed the assessment value of 1, for either the trenbolone- or the estradiol-related compounds. Even if the RQ was calculated using the single highest 21-d moving average PEC_{sw} for the entire 10-year simulation period (0.181 ng/L for the surrogate trenbolone compound[9]), which is based on a total of 3633 PEC_{sw} values, this would only result in an RQ of 0.905 based on the PNEC for 17β-TB. For comparison, the RQs based on the 90th percentile of the maximum monthly 21-day moving average PEC_{sw} values for the aggregate watersheds, which are those that CVM considers most appropriate for use in risk characterization, range from ≤0.001 to a maximum of 0.410 (Table 2). The highest RQ is for the Iowa watershed

[9] This PEC value is the single highest 21-d moving average PEC_{sw} out of all 3633 values calculated for the Iowa aggregate watershed.

scenario for the surrogate trenbolone compound when the PNEC is based on 17β-TB. None of the RQs for the aggregate watershed scenarios exceed the assessment value of 1, supporting a conclusion that no further assessment is needed.

These risk characterization results collectively indicate that no environmental impacts are expected from the approval of REVALOR-XR. It is also important to note that these RQ values are considered overestimates due to the number of conservative assumptions that were used in the exposure and effects assessments (see the following section).

Conservative Assumptions Used in the EA

Many conservative assumptions were used throughout the Revalor-XR EA and risk assessment. As a result, the PEC and RQ values reported in the EA are considered to overestimate the actual exposures and risks for fish in the aquatic environment. However, because the RQs were ultimately found to be acceptable in magnitude (i.e., <1), no further refinement of, or changes to, these conservative assumptions is needed at this time. The most important conservative assumptions are listed below. These and many others are described in greater depth in Chapter 9.2 of the EA.

- The compounds' properties and input values used for modeling were conservatively selected in order to maximize transport to surface water and not underestimate PEC_{sw}.
- The modeled watersheds have some of the highest densities of beef cattle and feedlots in the country.
- It was assumed that 100% of the feedlot cattle in the watersheds would be treated with Revalor-XR.
- For the Iowa scenario, it was assumed that all feedlots are paved.
- A worst-case was evaluated for the Iowa watershed by assuming that 100% of AFOs are directly discharging to surface water when this percentage is expected to be substantially lower.
- It was assumed there were no vegetative buffers between the treated fields/feedlots and the receiving stream.
- During runoff, it was assumed that overland transport in the entire watershed occurs on a ground surface that is impervious (i.e., no infiltration of water or surrogate compound into soil layers is assumed in the computer model).
- Adsorption to soil, sediment, or manure was not taken into account in the watershed-scale modeling due to limitation of the model and available data. The use of these values would have reduced the PEC_{sw} values.
- Degradation/transformation in the aquatic-sediment systems was not included in the watershed-scale evaluations. Accounting for these processes would have reduced the PEC_{sw} values.
- A highly conservative method for calculating RQs was used that assumed the more toxic β-isomers are expected to be present in the environment in much higher amounts than actually anticipated. It is expected that the β-isomers will exist in the environment at much lower concentrations than the α-isomers and to contribute less to the PEC values and RQs for the surrogate compounds. Thus the reported RQs based on the PNEC values for the two β-isomers are overestimated, perhaps substantially.

Cumulative Assessment

In addition to the quantitative assessment described above, Intervet also considered the potential for the introduction of steroid hormones from multiple natural and anthropogenic sources, such as excretion by humans, livestock (e.g., cattle, pigs, poultry, etc.), aquaculture (fish), and wildlife (both terrestrial and aquatic). Using data available in the published literature, estimates were made for the yearly mass (kg/year) of estrogens and androgens excreted by humans, livestock, and wildlife that could potentially enter the environment in the US. These estimates were compared to the estimated maximum mass of the estradiol and trenbolone metabolites associated with the use of all Revalor products to determine their potential contribution to the overall load of estrogens and androgens entering the environment. Based on these conservative, estimates, it was determined that the annual contribution of estradiol and trenbolone-related compounds associated with all Revalor products will be less than 1% of the overall load of estrogens and androgens entering the environment in the US from human, livestock, aquaculture, wildlife, and other sources.

Regulatory Conclusion

All of the RQ values determined for the two watershed-scale aggregate exposure assessments, Great Plains (Texas) and Midwest (Iowa), were found to be below the threshold value of 1 used to determine the need for further assessment. This was true even when basing the RQs on the maximum 21-d moving average PEC_{sw} for the entire 10-year simulation period (a total of 3633 PEC values for each compound), not just the 90^{th} percentile maximum monthly values. These watersheds represent areas of the intensive beef cattle production in large and small feedlots, and therefore, are representative for a conservative nationwide exposure assessment and for regulatory decision making. In addition, the assessment incorporated many conservative assumptions (see Chapter 9.2 of the EA) and likely resulted in an overestimation of the potential environmental risk to sensitive fish populations.

Based on the information and analysis presented in the EA, CVM has determined that no significant impacts on the human environment are expected from the proposed use of Revalor-XR in steers and heifers fed in confinement for slaughter for increased rate of weight gain and improved feed efficiency during 70 to 200 days after implantation.

{see appended electronic signature page}

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Electronic Signature
Addendum for Submission ID

Signing Authority (Role)	Letter Date
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