CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

022577Orig1s000

ENVIRONMENTAL ASSESSMENT

Food and Drug Administration
Center for Drug Evaluation and Research
Office of Pharmaceutical Science/Immediate Office

Memorandum

Date: November 8, 2011

From: Raanan A. Bloom, Ph.D.
OPS/IO

To: Jeannie David, RPM
OPS/ONDQA

Through: Nakissa Sadrieh, Ph.D.
OPS/IO/SRS

Subject: **NDA 22-577** Viread (Tenofovir Disoproxil Fumarate; Tenofovir DF) Oral Powder

Sponsor: Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404

Review of Environmental Assessment (dated April 14, 2011; cover page: May 24, 2011)

A. Background

Gilead Sciences, Inc. requests approval for the expanded use of Tenofovir DF oral powder to include HIV infected patients 2 to < 12 years old for the treatment of HIV. An Environmental Assessment (EA) has been submitted pursuant to 21 CFR part 25.

B. Discussion

Note: Stephen Page, Ph.D., assisted in the scientific evaluation of the submitted study reports and results submitted in the EA.

Executive Summary

NDA 22-577 requests approval of Tenofovir DF oral powder for the treatment of HIV. An EA for related application NDA 21-356 for Tenofovir Disoproxil Fumarate (Tenofovir DF) Tablets was previously reviewed and approved 26 October 2001. The EA submitted with this application (NDA 22-577) is intended to expand the use of Tenofovir DF oral powder to HIV

infected patients 2 to < 12 years old for the treatment of HIV. This EA was prepared in accordance with 21 CFR Part 25 by Gilead Sciences, Inc.

Tenofovir DF, a prodrug of Tenofovir (TFV), is a fumaric acid salt of a *bis*-isopropoxycarbonyl-oxymethyl ester derivative of TFV that is rapidly absorbed and converted to TFV. The sponsors estimate that approximately 70 to 80% of doses are excreted unchanged in urine following intravenous administration. This estimate was extrapolated to include oral administration. Tenofovir DF is expected to enter predominately into the aquatic environment as a result of patient use and is likely to partition primarily into water based on its water solubility, octanol/water partition coefficient and adsorption coefficient.

The sponsor estimates an EIC of 1.28 µg/L for Tenofovir DF assuming no metabolism and the worst case scenario of all drug API entering the aquatic environment. This estimate is based on a production of (b) (4) kg Tenofovir DF used for patient use from this and the applicant's related applications for Tenofovir DF Tablets. Since the EIC is greater than 1 ppb, an EA was submitted and reviewed. The submitted information was as recommended in the CDER/CBER Guidance for Industry: Environmental Assessment of Human Drug and Biologics Applications (July 1998).

The sponsor uses acute ecotoxicology data to estimate an assessment factor (using the most sensitive EC_{50} from a *Pseudokirchneriella subcapitata* 72 hour acute toxicity test and 5th year EIC estimates) of approximately 36,719. Since this factor is greater than 1000, we conclude that approval of this application is not expected to have a significant impact on the environment.

A Finding of No Significant Impact (FONSI) is recommended for this application.

C. Environmental Assessment Review

1. **Date:** October 16, 2011
2. **Applicant:** Gilead Sciences, Inc.
3. **Address:** 333 Lakeside Drive, Forest City, CA 94404
4. **Proposed Action:** Gilead Sciences, Inc. is filing an NDA pursuant to section 505(b) of the Federal Food, Drug and Cosmetic Act for Viread (Tenofovir Disoproxil Fumarate) oral powder for expanded use by HIV infected patients 2 to < 12 years of age for the treatment of HIV-1.

5. **Identification of Chemicals**

(i) Established Name: Tenofovir disoproxil fumarate
(ii) Brand/Proprietary Name/Tradename: Tenofovir DF is used in several brand name products including Viread®, and combination products, Truvada®, and Atripla®.
(iii) Chemical Name: 9-[(R)-2-[[bis[[(isopropoxycarbonyl)oxy]methoxy]-phosphinyl]methoxy]propyl]adenine fumarate (1:1) (IUPAC), and

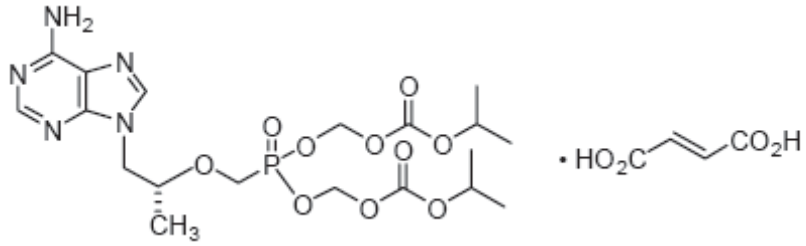
(R)-5-[[2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]-2,4,6,8,-tetraoxa-5-phosphanonanedioicacid, bis(1-methylethyl)ester, 5-oxide, (E)-2-butenedioate (1:1) (CAS)

(iv) Chemical Abstract Registration Number: 202138-50-9

(v) Molecular Formula: $C_{23}H_{34}O_{14}N_5P$

(vi) Molecular Weight: 635.52

(vii) Chemical Structure:



6. Environmental Characterization

Physical/Chemical Values

Water solubility
13.4 mg/L

Dissociation constants (pKa)
3.75

Octanol/Water Partition Coefficient
Log K_{ow} = 0.992 and 1.18 at pH 4 and pH 7, respectively

Vapor Pressure
Assumed to be nonvolatile.

Sorption/Desorption
Log K_{oc} = 1.3

Environmental Depletion Mechanisms

Photodegradation: The authors indicate that Tenofovir DF is not photolabile

Hydrolysis: The sponsor reports that Tenofovir DF is a diester prodrug of TFV that dissociates and is readily hydrolyzed to yield TFV.

Aerobic Biodegradation in Water: A ready biodegradability closed bottle test (OECD 301D) indicates that TDF does not meet the OECD criteria for "ready biodegradability" and therefore is not considered to be readily biodegradable.

Aerobic and Anaerobic Transformation in Aquatic Sediment Systems : Testing for aerobic and anaerobic transformation of the parent compound (OECD 308) indicates that Tenofovir DF rapidly undergoes primary degradation under aerobic and anaerobic water sediment conditions. By day 31 of the 102 day study, radioactivity was reduced to nondetectable levels in both aerobic and anaerobic sediments suggesting that Tenofovir DF and its degradants are not persistent in the environment. A small amount of degradation to $^{14}CO_2$ in aerobic sediments was observed ranging from 1.0 to 1.3% in the two river sediments tested, while the amounts of ^{14}C volatiles in anaerobic river sediments was negligible (< 1%). The half-life of degradation products ranged from 0.50 to 2.24 days under aerobic conditions and 6.08 to 8.33 days under anaerobic conditions.

Environmental Concentrations

The sponsor assumes all drug products produced in a year are used and enter the publicly owned treatment works (POTWs) with even distribution throughout the US per day. Based on these assumptions, and the Guidance Document EIC formula, the EIC entering the aquatic environment is estimated to be 1.28µg/L from patient use without consideration of metabolism or depletion mechanisms.

Environmental Fate and Effects

Environmental Fate

Aquatic Environment: As a result of human metabolism TDF approximately 70-80% of the delivered dose of Tenofovir DF will be introduced into the environment primarily through municipal sewage treatment plants. Tenofovir DF is expected to partition mostly into water and less likely into sludge and sediment based on water solubility (13.4mg/mL), octanol/water partition coefficient (log P_{ow} = 0.992 and 1.18 at pH 4 and pH 7, respectively), and adsorption coefficient (log K_{oc} = 1.3).

Terrestrial Environment: Based on the octanol/water partition coefficient (log P_{ow} = 0.992 and 1.18 at pH 4 and pH 7, respectively) and the adsorption coefficient (log K_{oc} = 1.3), Tenofovir DF is not expected to partition into sludge or sediment.

Atmospheric Environment: Since Tenovofir DF has a molecular weight of 635.52 and a water solubility of 13.4mg/L, volatilization of this substance is unlikely.

Environmental Effects

Since Tenofovir DF is expected to partition primarily into the aquatic environment, the sponsor conducted acute toxicity studies with freshwater green alga *Pseudokirchneriella subcapitata*, water fleas *Daphia manga,* and rainbow trout *Oncorhynchus mykiss.* Chronic toxicity studies were also conducted with fathead minnows *Pimephales promelas* and water fleas *Daphia manga.*

Aquatic organisms: A 72-hour study was conducted to determine the effect of Tenofovir DF on the growth of freshwater green alga according to OECD guideline 207. The range of concentrations tested was 0 (control) through 92 mg a.i./L Tenofovir DF. Freshwater green alga growth and biomass was monitored. The 72-hour EC_{50} value for freshwater green alga growth was estimated to be 47 mg a.i./L. With a reduction in biomass yield in treatment levels ≥ 20 mg a.i./L, the NOEC was estimated to be 14 mg a.i./L Tenofovir DF.

A 48-hour immobilization test was conducted to determine acute toxicity of Tenofovir DF to water fleas *Daphia manga* according to OECD guideline 202. The range of concentrations tested was 0 (control) though 98 mg a.i./L. Water flea immobilization was monitored. Since no concentration tested resulted in ≥ 50% immobilization, the 48-hour EC_{50} for water flea immobilization was estimated to be > 98 mg a.i./L, the highest concentration tested. The NOEC was determined to be 98 mg a.i./L since the highest concentration tested produced 0% immobilization.

A 96-hour study was conducted to determine acute toxicity (LC_{50}) of Tenofovir DF to rainbow trout *Oncorhynchus mykiss* under static-renewal test conditions according to OECD guideline 203. The range of concentrations tested was 0 (control) to 92 mg a.i./L. No adverse effects or mortality were observed at any treatment levels among rainbow trout throughout the duration of the study. The 96-hour LC_{50} value for Tenofovir DF on rainbow trout was estimated to be > 92 mg a.i./L, the highest concentration tested. The NOEC was determined to be 92 mg a.i./L.

The values obtained from these studies were compared to assessment factors as per the FDA guidance document and indicate further tiered studies were not required. Although not required, a Tier 3 assessment was conducted.

A 32-day, life-stage toxicity test was conducted to determine the NOEC and LOEC of Tenofovir DF to fathead minnow *Pimephales promelas* embryos and larvae under flow-through conditions according to OECD guideline 210. The range of concentrations tested was 0 (control) through 1.9 mg a.i./L. A conservative estimate of 1.9 mg a.i./L was determined for the NOEC since no lethality or sublethal effects were observed for the highest concentration tested. The LOEC was determined to be > 1.9 mg a.i./L.

A 21-day full life cycle test was conducted to determine the chronic effects of Tenofovir DF on the survival, growth, and reproduction of water fleas *Daphia manga* under flow-through conditions according to OECD guideline 211. The range of concentrations tested was 0 (control) through 96 mg a.i./L. The 21-day EC_{50} for *Daphia manga* survival was estimated to be > 96 mg a.i./L, the highest concentration tested, since no concentrations tested resulted in ≥ 50% immobilization. Mean total body length among daphnids was monitored at each treatment level and not considered to be significant. The mean cumulative offspring released per female was monitored following 21 days of exposure. A significant reduction in offspring per female was observed among organisms exposed to treatment levels of 25, 50, and 96 mg a.i./L. The 21-day EC_{50} for *Daphia manga* reproduction was estimated to be 21 mg a.i./L. The sponsor estimated the NOEC for

Tenofovir DF and *Daphia manga* to be 13 mg a.i./L based on the most sensitive indicator of toxicity (mean cumulative offspring released per female).

The EC_{50} concentration / MEEC ratios on all three Tiers of assessment were > 1000. These data indicate that Tenofovir DF poses low risk to the environment based on current use patterns.

Summary Table of Physical/Chemical Characterization and Depletion Studies:

Tenofovir DF Data Summary Table	
Physical/Chemical Characterization	
Water Solubility	13.4 mg/mL
Dissociation Constants	$pK_a = 3.75$
Octanol/Water partition Coefficient (Log K_{ow}) (OECD 107)	Log K_{ow} = 0.992 (pH 4) and 1.18 (pH 7)
Vapor Pressure	Presumably non-volatile
Sorption/Desorption (K_{oc}) (OECD 121)	Log K_{oc} = 1.3 (or K_{oc} = 18)
Depletion Mechanisms	
Hydrolysis	Hydrolyzes to TFV
Aerobic Biodegradation in Water (OECD 308)	Does not biodegrade
Photolysis	Does not appear to undergo photolysis as UV max is 260 nm
Metabolism Aerobic and Anaerobic Transformation in Aquatic Sediment Systems (OECD 308)	At least 70-80% of the delivered dose reaches the environment primarily via urine Suggests that Tenofovir DF and its degradants will not last long in the aquatic environment

Summary of environmental effects:

The following ecotoxicity effects studies were conducted in accordance with OECD guidelines:

Environmental Effects		
Study	**Exposure**	**Result**
Microbial Inhibition Activated Sludge	3 hours OECD 209	NOEC = 600 mg/L EC_{50} = 940 mg/L
Acute Toxicity		
Study	**Exposure**	**Result**
Freshwater Green Alga *Pseudokirchneriella subcapitata*	72 hours OECD 201	NOEC = 14 mg/L EC_{50} = 47 mg/L
Water Fleas *Daphia manga*	48 hours OECD 202	NOEC = 98 mg/L EC_{50} > 98 mg/L
Rainbow Trout	96 hours	NOEC = 92 mg/L

Oncorhynchus mykiss	OECD 203	EC_{50} > 92 mg/L
Chronic Toxicity		
Study	**Exposure**	**Result**
Fathead Minnow *Pimephales promelas*	32 days Fish Early Life Cycle OECD 210	NOEC = 1.9 mg/L LOEC > 1.9 mg/L
Water Fleas *Daphia manga*	21 days Reproduction OECD 211	NOEC = 13 mg/L EC_{50} > 21 mg/L

A list of the study reports is provided under item 9. Full study reports are in the Confidential Appendix P.

Cumulative Environmental Fate and Effects

The combined production and EIC of Tenofovir DF from all sources is listed below:

NDA NO.	Name	Production (kg/year)	EIC (ppb or µg/L)
22-577, 21-356, 21-752, 21-937	Viread, Truvada, Atripla	(b) (4)	(b) (4)
202-123	Complera		

Total production is (b) (4) kg/year with a cumulative EIC of (b) (4) ppb. Despite an increase in the cumulative production and EIC, there still remains to be an acceptable margin between environmental exposure and biological effect. These data indicate that Tenofovir DF is not expected to pose a significant risk to the environment based on current use patterns.

7. Mitigation Measures and Alternatives

Since no adverse environmental impact is expected, no mitigation methods are addressed.

8. Literature Reviewed

1. Kohler, J. et. al. Tenofovir renal proximal tubular toxicity is regulated by OAT1 and MRP4 transporters. *Lab Inves*. 91(2011): 852-858.
2. Hall, A.M. et. al. Tenofovir associated kidney toxicity in HIV-infected patients: A review of the evidence. *Am J Kindey Dis.*. 5(2011): 733-780.
3. Long, Y. et. al. Molecular characterization and functions of zebrafish ABCC2 in cellular efflux of heavy metals. *Comparative Biochemistry and Physiology*. 153(2011): 381-391.
4. Kohler, J. et al. Tenofovir renal toxicity targets mitochondria of renal proximal tubules. *Lab Invest*. 2009, 89(5): 513-519.
5. Cotter, A. et. al. Endocrine complications of human immunodeficiency virus infection: Hypogonadism, bone disease and tenofovir-related toxicity. *Best Practice and Research Clinical Endocrinology & Metabolism*. 25(2011): 501-515.
6. Reichel, V. et. al. Transport of a fluorescent cAMP analog in teleost proximal tubules. *Am J Physiol Regul Integr Comp Physiol*. 293(2007): R2382-R2389.

Findings: The sponsor has reported that the primary mode of elimination of Tenofovir DF is excretion in urine with approximately 70-80% of Tenofovir DF dose. It logically follows that kidney cells may be exposed to high concentrations of TDF; a concept that various reports have supported (1,2,4,5). Additional investigations have reported Tenofovir DF associated renal toxicity in both clinical studies and animal models and suggested that the greatest toxicity is localized to the proximal tubule (1,2,4,5). Although corroborating data does not exist for aquatic species, the sponsor has indicated that Tenofovir DF will partition primarily into the aquatic environment. With this in mind, it is important to note that both renal function as well as the ATP-binding cassette (ABC) transporter MRP4, a renal cell membrane transporter protein that has been shown to transport Tenofovir DF into the urine, are conserved in aquatic species such as *Danio rerio* (zebrafish) (6). Although the sponsors findings that Tenofovir DF poses low risk to the aquatic environment are supported, these assumptions are based upon traditional endpoints. It may prove valuable for future ecotox investigations to consider non-traditional endpoints such as renal toxicity.

9. EA Study Reports

   The following table of study reports is provided as Table 10.2-1 on page 23 of the submitted EA.

10. Comments and Conclusions

Based on an evaluation of the information provided in this EA and previous EAs, on FDA Guidance, and on the scientific validity of the "no effects" conclusions of the EA, no significant adverse environmental impacts are expected from the approval of Tenofovir DF for the treatment of HIV infection.

A Finding of No Significant Impact (FONSI) is recommended for this application.

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/s/

RAANAN A BLOOM
11/08/2011

NAKISSA SADRIEH
11/08/2011

Environmental Assessment
Finding of No Significant Impact

NDA 22-577
Viread (Tenofovir Disoproxil Fumarate) Oral Powder

Food and Drug Administration
Center for Drug Evaluation and Research

November 8, 2011

FINDING OF NO SIGNIFICANT IMPACT

NDA 22-577

Viread (Tenofovir Disoproxil Fumarate) Oral Powder

The National Environmental Policy Act of 1969 (NEPA) requires all Federal agencies to assess the environmental impact of their actions. The Food and Drug Administration (FDA) is required under NEPA to consider the environmental impact of approving certain drug product applications as an integral part of the regulatory process.

NDA 22-577 requests approval for Tenofovir DF Oral Powder. This NDA is intended to expand the use of Tenofovir DF use to HIV infected patients 2 to < 12 years old for the treatment of HIV. In support of its application, Gilead Sciences, Inc., prepared an environmental assessment (attached) in accordance with 21 CFR Part 25, which evaluates the potential environmental impacts of Tenofovir DF.

The Food and Drug Administration, Center for Drug Evaluation and Research, has carefully considered the potential environmental impact due to approval of this application and has concluded that this action is not expected to have a significant impact on the environment. Therefore, an environmental impact statement will not be prepared.

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Office of Pharmaceutical Science

Attachment: April 14, 2011, Environmental Assessment

SECTION 1.12.14

ENVIRONMENTAL ANALYSIS

TENOFOVIR DISOPROXIL FUMARATE

GILEAD SCIENCES, INC.

24MAY2011

CONFIDENTIAL AND PROPRIETARY INFORMATION

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LIST OF IN-TEXT TABLES

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

°C	degrees Celsius
µg	microgram
^{14}C	carbon 14
$^{14}CO_2$	carbon dioxide
a.i.	active ingredient
CAS	chemical abstracts service
CFR	code of federal regulations
Ch.	chapter
CHMP	Committee for Medicinal Products for Human Use
CMC	Chemistry and Manufacturing Control
EA	environmental assessments
EC_{50}	median effect concentration
EEC	expected environmental concentration
EIC	environmental introduction concentration
FDA	Food and Drug Administration
GLP	good laboratory practice
HBV	hepatitis B virus
HHS	Health and Human Services
HIV	human immunodeficiency virus
HPLC	high performance liquid chromatography
hr	hour
IND	FDA investigational new drug application
IUPAC	International Union of Pure and Applied Chemistry
kg	kilogram
K_{oc}	adsorption coefficient
K_{ow}	octanol: water partition coefficient
L	liter
LC/MS/MS	Liquid Chromatography – Tandem Mass Spectrometry
LC_{50}	median lethal concentration
LOEC	lowest observed effect concentration
K_{oc}	adsorption coefficient
K_{ow}	octanol/water partition coefficient
log P	partition coefficient
log P_{ow}	octanol/water partition coefficient
m.w.	molecular weight
MEEC	maximum expected environmental concentration
mg	milligram
mL	milliliter
NDA	FDA new drug application

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS (CONTINUED)

NEPA	National Environmental Policy Act of 1969
nm	nanometer
NOEC	no observed effect concentration
O_2	oxygen
OECD	Organization for Economic Co-operation and Development
pH	measure of acidity of a solution
pKa	acid dissociation constant
POTW	publicly owned treatment works
ppb	parts per billion
ppm	parts per million
TFV	tenofovir
TDF	tenofovir disoproxil fumarate (tenofovir DF)
TR	Taunton River
WR	Weweantic River
US	United States
UV	ultraviolet
WWTP	waste water treatment plant

1. ENVIRONMENTAL RISK ASSESSMENT

1.1. Date

April 14, 2011

1.2. Name of Applicant/Petitioner

Gilead Sciences, Inc.

1.3. Address

333 Lakeside Drive
Foster City, CA 94404

2. DESCRIPTION OF PROPOSED ACTION

2.1. Requested Approval

Gilead Sciences, Inc. (Gilead) has previously filed and received approval of a New Drug Application (NDA) (NDA 21-356, approved 26 October 2001) pursuant to section 505(b) of the Federal Food, Drug, and Cosmetic Act for Tenofovir Disoproxil Fumarate (Tenofovir DF, TDF). The current NDA (NDA 22-577) and supplemental NDA to NDA 21-356 are to expand its use to HIV infected patients 2 to <12 years old. The National Environmental Policy Act of 1969 (NEPA) requires all Federal agencies to assess the environmental impacts of their actions and to ensure that the interested and affected public is informed of environmental analyses. The Food and Drug Administration (FDA) is required under NEPA to consider the environmental impacts of approving drug and biologics applications as an integral part of its regulatory process. FDA's regulations in 21 CFR Part 25 specify that environmental assessments (EAs) must be submitted as part of certain NDAs, abbreviated applications, applications for marketing approval of a biologic product, supplements to such applications, investigational new drug applications (INDs) and for various other actions (see 21 CFR 25.20), unless the action qualifies for categorical exclusion.

Tenofovir DF is a nucleotide analog HIV-1 reverse transcriptase inhibitor and an HBV reverse transcriptase inhibitor that is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in adults and adolescent patients 12 years of age and older. Tenofovir DF is also indicated for the treatment of chronic hepatitis B in adults.

The following is submitted according to the requirements under the Food and Drug Administration Guidance for Industry, Environmental Assessment of Human Drug and Biologics Applications, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research, July, 1998, CMC 6, Revision 1 (“FDA Guidance Document”). In addition to meeting the FDA Guidance Document requirements Gilead has conducted additional environmental studies as part of their product stewardship of TDF and submits these data pursuant to 21 CFR Part 25. Gilead requests continued approval of the use of TDF as requirements under the Guidance and per regulatory requirements for environmental assessment have been met as follows.

2.2. Need for Action

Tenofovir DF, a prodrug of tenofovir (TFV), is a fumaric acid salt of a *bis*-isopropoxycarbonyl-oxymethyl ester derivative of TFV. In vivo, TDF is converted to TFV, an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5’-monophosphate. Tenofovir is a potent and selective inhibitor of HIV and HBV in vitro. The recommended dose for the treatment of HIV-1 or chronic hepatitis B in adults: 300 mg once daily taken orally without regard to food, and for the treatment of

HIV-1 in adolescent patients (≥12 years of age and ≥35 kg) is also 300 mg once daily taken orally without regard to food. As TFV is the primary and only known pharmacologically active metabolite and the excipients are inert substances which have no pharmacological or toxicological effects, this assessment will focus on the environmental impact of the active ingredient only.

2.3. Locations of Use

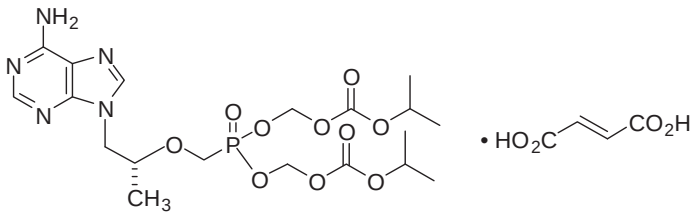
Tenofovir DF is administered orally and may be used by individuals throughout the United States (US) in hospitals, clinics, and/or homes. It is not expected to be concentrated in any particular geographic region.

2.4. Disposal Sites

In US hospitals, pharmacies or clinics, empty or partially empty bottles will be disposed of according to hospital, pharmacy or clinic procedures. In the home, empty or partially empty containers will typically be disposed of by a community's solid waste management system, which may include landfills, incineration and recycling, although minimal quantities of unused drug may be disposed of in the sewer system.

3. IDENTIFICATION OF SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION

3.1. Physical and Chemical Properties

Established Name:	Tenofovir disoproxil fumarate
Brand/Proprietary Name/Tradename:	Tenofovir DF is used in several brand name products including Viread®, Truvada®, and Atripla®.
Chemical Names:	9-[(R)-2-[[bis[[(isopropoxycarbonyl)oxy]methoxy]-phosphinyl]methoxy]propyl]adenine fumarate (1:1) (IUPAC) (R)-5-[[2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]-2,4,6,8,-tetraoxa-5-phosphanonanedioic acid, bis(1-methylethyl)ester, 5-oxide, (E)-2-butenedioate (1:1) (CAS)
Chemical Abstracts Service (CAS) Registration Number:	202138-50-9
Molecular Formula:	$C_{23}H_{34}O_{14}N_5P$
Molecular Weight:	635.52
Chemical Structure:	

4. ENVIRONMENTAL ISSUES

4.1. Environmental Fate of Released Substances

4.1.1. Identification of Substances of Interest

Tenofovir DF, a prodrug of TFV, is a fumaric acid salt of a *bis*-isopropoxycarbonyl-oxymethyl ester derivative of TFV. In vivo, TDF is converted to TFV, an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5'-monophosphate. Tenofovir is a potent and selective inhibitor of HIV and HBV in vitro.

Following oral administration of TDF to HIV infected patients, TDF is rapidly absorbed and converted to TFV. Tenofovir is primarily excreted by the kidney by both filtration and an active tubular transport system with approximately 70 to 80% of the dose excreted unchanged in urine following intravenous administration, which is also assumed for the purposes of this assessment to be the primary excretion product of oral administration.

The excipients are inert substances which have no pharmacological or toxicological effects and therefore, this assessment will focus on the environmental impact of the active ingredient only.

4.1.2. Physical and Chemical Characterization

The physical and chemical properties of TDF that are environmentally relevant are summarized as follows, and in *Appendix 11* (a) *Tenofovir DF Data Summary Table.*

Water Solubility:	13.4 mg/mL
Dissociation Constants:	$pK_a = 3.75$
Partition Coefficient:	log P (as determined for pharmaceutical purposes) = 1.25
	log K_{ow} = 0.992 and 1.18 at pH 4 and pH 7, respectively as determined by OECD Guideline 107
	log K_{ow} not determined at pH 10 due to the instability of TDF in the buffer phase
	Both determinations are in agreement that the primary partition is into water rather than octanol

The partition coefficient (*n*-octanol/water) was determined using the flask-shaking method following OECD Guideline 107 and according to Good Laboratory Practices (GLP) (AD-104-2005). These data suggest partitioning of TDF into the aquatic environment rather than sediment.

Adsorption Coefficient (K_{oc}):	log K_{oc} determined for TDF was = 1.3 (or K_{oc} = 18).

The adsorption coefficient (K_{oc}) for TDF was determined following the OECD Guideline 121 and according to GLP (AD-104-2008). As TDF is unstable in some aqueous conditions, determination of the adsorption coefficient according to the OECD 106 protocol could not be conducted.

Using HPLC analyses and comparison to compounds with known K_{oc} values, the mean log K_{oc} determined for TDF was = 1.3 (or K_{oc} = 18). This also suggests that TDF will partition to the water compartment.

Vapor Pressure:	Assumed to be nonvolatile
Ultraviolet -Visible Absorption Spectrum:	TDF does absorb in the UV range (UV max at 260 nm) but it is not photolabile.

4.1.3. Environmental Depletion Mechanisms

Ultraviolet-Visible Absorption Spectrum:	TDF does absorb in the UV range (UV max at 260 nm) but it is not photolabile.
Hydrolysis:	TDF (m.w. 635.52) is a diester prodrug of TFV (m.w. 287.21). TDF dissociates and is readily hydrolyzed to yield TFV.
Aerobic Biodegradation in Water:	Not considered readily biodegradable.

Tenofovir DF was tested to assess its degree of ready biodegradability using the procedure outlined in the OECD guideline 301D and according to GLP for the "Ready Biodegradability Closed Bottle Test" (TOX0340). The results indicate that TDF is not readily biodegradable under the conditions tested.

In addition to the above standard tests to determine environmental depletion mechanisms, Gilead has conducted an additional study as part of its product stewardship of TDF.

Aerobic and Anaerobic Transformation in Aquatic Sediment Systems: Suggests that TDF and its degradants will not last long in the aquatic environment.

The rates of aerobic and anaerobic transformation of parent [^{14}C]tenofovir DF were studied at a concentration of 1.0 mg/L and a temperature of 20 ± 2°C for 102 days in two aerobic and two anaerobic sediments varying in pH, textural characteristics, organic matter content and microbial content (Taunton River (TR) and Weweantic River (WR) sediments). The study was conducted according to OECD Guideline 308 and according to GLP (AD-104-2007).

Tenofovir DF rapidly underwent primary degradation converting to several degradation products under the aerobic and anaerobic water sediment conditions of this study.

Average material balance ranged from 91.2 to 107.3% of the applied radioactivity throughout the 102-day study. Tenofovir DF decreased from an average of 91.6 and 90.4% of the applied radioactivity on Day 0 to nondetectable levels by Day 31 for the aerobic TR and WR sediments, respectively. A small amount of radioactivity (1.0% and 1.3%, respectively) was converted to $^{14}CO_2$ in the TR and WR aerobic sediments, respectively.

Tenofovir DF decreased from an average of 92.5 and 90.5% of the applied radioactivity on Day 0 to nondetectable levels by Day 31 for the anaerobic TR and WR sediments, respectively. Negligible amounts of ^{14}C volatiles were observed (< 1%) from the anaerobic systems.

Several major degradation products (> 10% of the applied radioactivity) were observed in the HPLC analyses at retention times of approximately 4 minutes, 11 minutes and 12 minutes. The half-life of these products ranged from 0.50 to 2.24 days under aerobic conditions and 6.08 to 8.33 days under anaerobic conditions. At 4-minutes, TFV, a known metabolite, was identified at concentrations ranging from 7.9 to 13.7%. At 11-minutes and 12-minutes, peaks ranging from 7.2 to 39.0 and 19.5 to 36.4% of radioactivity, respectively, were identified and underwent extensive LC/MS/MS analysis, however structural assignments could not be made for these components.

Although the specific chemical structures could not be determined for two of the degradants, predicted environmental concentrations of the parent drug and degradants will be orders of magnitude below the known effect concentrations of the parent drug in chronic studies (see Tier 3 testing, Section 7.2.3).

The findings would suggest that TDF and its degradants will not last long in the environment. These data and other data (such as the low K_{ow}) would suggest that TDF is

not a risk to the sediment as it would not partition to the sediment and would also not last long in sediment, even if it reached this part of the environment.

4.1.4. Environmental Concentrations

The metabolism of TDF will result in excreted drug substance being introduced into the environment primarily through municipal sewage treatment plants or septic tanks.

Environmental Introduction Concentration (EIC): The EIC entering the aquatic environment from patient use is calculated without including consideration of metabolism or environmental depletion mechanisms that occur in the waste treatment process. The EIC from patient use is based on the highest annual quantity of the active moiety expected to be produced for use during the next five years; the quantity used in all dosage forms and strengths included in this application; and the quantity used in related applications for TDF.

The calculation of the EIC for the aquatic environment assumes all drug products produced in a year are used and enter the publicly owned treatment works (POTWs), even distribution throughout the US per day, and no metabolism or depletion mechanisms:

EIC - Aquatic (ppb) = A x B x C x D

where: A = kg/year production (as active moiety)
B = $1/1.214 \times 10^{11}$ (1/liters per day entering POTWs)
C = year/365 days
D = 10^9 µg/kg (conversion factor)

Using this calculation, the EIC from patient use of TFV is approximately 1.28 µg/L (refer to section 10.1, *Confidential Appendix: Calculation of Environmental Introduction Concentration*).

Because TDF is not readily biodegradable (to CO2) and although it does not persist as TDF in the environment, a conservative/protective assumption of this environmental risk assessment is that the EIC, Expected Environmental Concentration (EEC) and Maximum Expected Environmental Concentration (MEEC) for TDF are similar.

In summary, based on production data, the MEEC is 1.28 µg/L (1.28 ppb), which would be considered requiring an Environmental Assessment under the FDA Guidance Document.

4.1.5. Summary of Environmental Fate of the Substance

Aquatic Environment: Tenofovir DF is expected to enter predominantly into the aquatic environment as a result of patient use and is likely to partition mostly into water and less likely into sludge or sediment based on its water solubility, octanol/water

partition coefficient and adsorption coefficient. An additional study indicated that it would not persist in the aquatic environment.

Terrestrial Environment: The octanol/water partition coefficient of TFV log P_{OW} was determined per OECD 107 and was 0.992 and 1.18 at pH 4 and pH 7, respectively and the adsorption coefficient (K_{OC}) is 18.

These parameters predict TDF would not partition into sludge soils or sediment.

Atmospheric Environment: The solubility in water of TDF and presumed low vapor pressure preclude the air compartment from being affected by volatilization of this substance at the public sewage treatment plant. Manufacturing controls prevent significant releases to the air during the manufacturing process.

As the MEEC exceeded 1 ppb, as per the FDA Guidance Document, an evaluation of the environmental effects of TFV was performed including an assessment of risk based on the MEEC and comparison to Assessment Factors as per the FDA Guidance Document.

4.2. Environmental Effects of Released Substances

A tiered approach to environmental effects testing of TDF as per the FDA Guidance Document, including a risk assessment evaluation compared to Assessment Factors. As TDF partitions to water based on the above data, effects of released substances on aquatic organisms was conducted for potential acute and chronic effects.

4.2.1. Respiratory Inhibition Study

A respiratory inhibition study was conducted according to OECD 209 and GLP (AD-104-2006) with TDF to determine the concentration of the test substance which would inhibit the respiratory activity of WWTP activated sludge microorganisms. Tenofovir DF was tested at concentrations of 75, 150, 300, 600 and 1200 mg a.i./L in the test system, and an appropriate control (3,5-dichlorophenol) was tested to ensure test validity.

Compared to the controls, respiratory inhibition for TDF was 0.0, 0.0, 0.0, 0.0 and 86.7%, at the increasing concentrations tested, respectively. Oxygen consumption in the test substance test vessels (75, 150, 300, 600 and 1200 mg a.i./L) was 13.5, 13.3, 16.9, 15.4 and 1.8 mg O_2/L/hr, respectively. The NOEC for TDF was 600 mg a.i./L and the EC_{50} was determined to be 940 mg a.i./L.

4.2.2. Tier 1 and Tier 2 Acute Aquatic Ecotoxicity Testing and Assessment

Tenofovir DF was evaluated in acute studies in algae, daphnia and fish (OECD 201, 202 and 203, respectively). All studies were conducted in accordance with GLPs. Data are summarized in the table below.

Table 4.2.2-1. Acute toxicity studies in aquatic organisms

Study	Result	Reference
Freshwater Green Alga (OECD 201) *Pseudokirchneriella subcapitata*	NOEC = 14 mg/L EC_{50} = 47 mg/L	TX-104-2002
Water Fleas (OECD 202) *Daphnia magna*	NOEC = 98 mg/L EC_{50} > 98 mg/L	TX-104-2003
Rainbow Trout (OECD 203) *Oncorhynchus mykiss*	NOEC = 92 mg/L LC_{50} > 92 mg/L	TX-104-2004

In assessing the above studies for environmental impact, the values obtained were compared to Assessment Factors as per the FDA Guidance document indicated that there would be no environmental impact of the use of TDF in marketed products.

Test Tier 1 Assessment

The Test Tier 1 Assessment compares the lowest EC_{50} of acute toxicity studies to the MEEC. As per the FDA Guidance Document, if the ratio is $\geq$1000, no environmental impact is anticipated. For TDF, the following values were employed.

EC_{50} = 47 mg/L from the OECD 201 study in algae (OECD 201)

MEEC = 0.00128 mg/L (1.28 ppm or 1.28 µg/L)

$$\frac{47\ \text{mg/L}}{0.00128\ \text{mg/L}} = \text{approx. } 36719$$

As this ratio $\geq$ 1000, no environmental impact is anticipated.

Test Tier 2 Assessment

The Test Tier 2 Assessment compares the lowest EC_{50} of a battery of acute studies to the MEEC. As per the FDA Guidance Document, if the ratio is $\geq$100, no environmental impact is anticipated. For TDF, the following values were employed.

EC_{50} = 47 mg/L from the OECD 201 study in algae (OECD 201)

MEEC = 0.00128 mg/L (1.28 ppm or 1.28 µg/L)

$$\frac{47\ \text{mg/L}}{0.00128\ \text{mg/L}} = \text{approx. } 36719$$

As this ratio $\geq$ 100, no environmental impact is anticipated.

4.2.3. Tier 3 Aquatic Ecotoxicity Testing and Assessment

As part of Gilead's product stewardship efforts, chronic studies in fish (fathead minnow) and reproductive (water flea) studies were conducted. All studies were conducted in accordance with GLPs. Data are summarized in the Table 4.2.3-1.

Table 4.2.3-1. Chronic and reproductive toxicity studies in aquatic organisms

Study	Result	Reference
Fathead Minnow (OECD 210) *Pimephales promelas* Fish Early Life Cycle	NOEC = 1.9 mg/L LOEC > 1.9 mg/L (highest dose tested)	TX-104-2005
Water Fleas (OECD 211) *Daphnia magna* Reproduction	NOEC = 13 mg/L EC_{50} = 21 mg/L	TX-104-2007

Based on these data, (though not obligated to be performed under the FDA Guidance Document), a Tier 3 Assessment was performed.

Test Tier 3 Assessment

The Test Tier 3 Assessment compares the lowest EC_{50} or LOEC of chronic or reproductive studies to the MEEC. As per the FDA Guidance Document, if the ratio is $\geq$10 and there are no effects at the MEEC, no environmental impact is anticipated. For TDF, the following values were employed.

LOEC > 1.9 mg/L (highest dose tested) from the OECD 210 study in fathead minnow – (lowest value of the chronic or reproductive studies)

MEEC = 0.00128 mg/L (1.28 ppm or 1.28 µg/L)

$$\frac{1.9 \text{ mg/L}}{0.00128 \text{ mg/L}} = \text{approx. } 1484$$

As this ratio $\geq$ 10, no environmental impact is anticipated.

4.3. Summary

A standard battery of environmental fate and effects studies has been conducted to evaluate the environmental risk associated with the use of TDF. Tenofovir DF is likely to partition mostly into water, based on its octanol/water partition coefficient and adsorption coefficient.

A battery of acute, chronic and reproductive effects studies were conducted in aquatic organisms. The results of these studies showed low to moderate toxicity. The results of these studies were compared to the MEEC. The EC_{50} concentration / MEEC ratios at all

three tiers of assessment based on one (1) acute study (Tier 1), a battery of studies (Tier 2), and chronic studies (Tier 3) were greater than 1000. Based on the above data and risk assessment conclusions, the current data indicates that TDF is of low risk to the environment based on current use patterns. Gilead therefore requests approval for the anticipated use based on no environmental impact of this clinical use.

5. MITIGATION MEASURES

Gilead has provided guidance to its contract manufacturing organization to establish programs and procedures to anticipate and prevent potential adverse environmental impacts associated with this proposed action. All plant operations at their contract manufacturing organizations, including distribution and waste management operations, are carried out by trained personnel under the supervision of qualified personnel with training in both normal and emergency operations. Any incident that would require additional specialized expertise would be provided by local fire, rescue, medical and emergency authorities or emergency response contract specialists.

6. ALTERNATIVES TO THE PROPOSED ACTION

No potential adverse environmental effects have been identified for the proposed action and therefore no alternatives are proposed.

7. LIST OF PREPARERS

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8. REFERENCES

The following references were used in the preparation of this Environmental Assessment and are generally available. Specific citations from these references may be obtained upon request.

21 CFR Ch. 1
Part 25 Environmental Impact Considerations
Food and Drug Administration, HHS

Environmental Assessment Technical Assistance Handbook
Food and Drug Administration
March 1987

Guidance for Industry, Environmental Assessment of Human Drug and Biologics Applications
U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Center for Biologics Evaluation and Research
July 1998
CMC 6
Revision 1

9. TENOFOVIR DF DATA SUMMARY TABLE APPENDIX

Tenofovir DF Data Summary Table	
Physical/Chemical Characterization	
Water Solubility	13.4 mg/mL
Dissociation Constants	3.75
Octanol/Water partition Coefficient (Log Kow) (OECD 107)	log K_{ow} = 0.992 and 1.18 at pH 4 and pH 7, respectively
Vapor Pressure	Presumably non-volatile
Sorption/Desorption (K_{oc}) (OECD 121)	log K_{oc} determined for TDF was = 1.3 (or K_{oc} = 18).
Depletion Mechanisms	
Hydrolysis	Hydrolyzes to TFV
Aerobic Biodegradation in Water (OECD 308)	Does not biodegrade
Photolysis	Does not appear to undergo photolysis as UV max is 260 nm
Metabolism	At least 70-80% of the delivered dose reaches the environment primarily via urine
Aerobic and Anaerobic Transformation in Aquatic Sediment Systems (OECD 308)	Suggests that TDF and its degradants will not last long in the aquatic environment
Environmental Effects	
Microbial Inhibition Activated Sludge (OECD 209)	NOEC = 600 mg/L EC_{50} = 940 mg/L
Acute Toxicity Freshwater Green Alga (OECD 201) *Pseudokirchneriella subcapitata*	NOEC = 14 mg/L EC_{50} = 47 mg/L
Water Fleas (OECD 202) *Daphnia magna* Acute Toxicity	NOEC = 98 mg/L EC_{50} > 98 mg/L
Rainbow Trout (OECD 23) *Oncorhynchus mykiss*	NOEC = 92 mg/L EC_{50} > 98 mg/L
Chronic Toxicity Fathead Minnow (OECD 210) *Pimephales promelas* Fish Early Life Cycle	NOEC = 1.9 mg/L LOEC > 92 mg/L (highest dose tested)
Water Fleas (OECD 211) *Daphnia magna* Reproduction	NOEC = 13 mg/L EC_{50} = 21 mg/L

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/s/

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