JAHM
ISSUES IN LAW & MEDICINE
A JOURNAL OF THE ALLIANCE FOR HIPPOCRATIC MEDICINE

Anti-Progesterone in Environmental Water

By: Elise Rose, PhD, Michael Varveris, M.D.

Abstract: Past research has shown that steroid hormones and other endocrine-disrupting compounds (EDCs) are present in environmental water, with implications for human and wildlife health. Abortion providers have shifted a large part of their practice to medication-induced abortion, using the anti-progesterone synthetic steroid hormone mifepristone. We wanted to see if mifepristone, an EDC, could be detected in water sources. Water samples were collected from sites upstream and downstream of water treatment facilities, and from municipal tap water, from each of 3 American cities. The water samples were tested for mifepristone using PR CALUX®. There were significant levels of mifepristone (up to 0.041 μgram/l) in the water of all but one of the nine sampling classes. Because of the plethora of effects of progesterone, this contaminant could affect the physiology of aquatic animals; and human health, including fertility, pregnancy and fetal development. The findings indicate the necessity for significant additional investigation into the levels of various hormones and EDCs, including mifepristone, in water sources.

Keywords: mifepristone, endocrine-disrupting compounds (EDCs), water quality

1. Introduction

Past research has shown that endocrine-disrupting compounds (EDCs), including steroid hormones, synthetic estrogens and progestins found in oral contraceptives, are present in water supplies [1, 2]. This problem remains an environmental and health concern, especially given the widespread use of contraceptives [3] and the increased prevalence of medication abortion in recent years [4]. These compounds have a number of deleterious effects on human and animal biology, particularly aquatic animals [5, 6, 7, 8, 9, 10]. Small amounts of these chemicals (parts per trillion) can affect the endocrine system of animals and humans [10]. Progestins, including specifically the anti-progesterone synthetic steroid hormone mifepristone, have been found to have disruptive effects on the fertility and reproduction of fish [6, 7]. Moreover, further highlighting the potential for environmental toxicity and contamination, the FDA recommends treating any unused mifepristone pills as hazardous waste [11].

Removal from the water supply of EDCs, including estrogens, progesterone, and other steroids, by either basic wastewater treatment or basic drinking water treatment, has proved to be problematic [12, 13, 14, 15, 16, 17], though recently hybrid water treatment methods have shown more promise, especially those including both reverse osmosis and activated carbon filters [12, 14, 17].

Abortion providers have shifted a large part of their practice from surgical to chemical (medication-induced) abortion. In 2023, 642,700 of the abortions in the US were medication abortions, forming 63% of the total [4]. The first drug given in this type of abortion is the anti-progesterone synthetic steroid hormone mifepristone, or RU-486. Mifepristone is an antagonist of progestational and glucocorticoid hormones. Mifepristone competitively binds to the progesterone receptor, thereby

inhibiting the effects of endogenous or exogenous progesterone [18, 19, 20, 21]. The drug is thought to disrupt attachment of the placenta to the uterine lining in early pregnancy [20, 22, 23]. The prostaglandin-like drug misoprostol is administered several days later to induce uterine contractions and preterm labor [24].

The increased use of the anti-progesterone hormone mifepristone indicates it might be present in water supplies. Given the potential negative impact of mifepristone on the environment, and on human and animal health, and the absence of studies addressing it, we sought to investigate the presence of unmetabolized mifepristone in water supplies at three sites in the United States. Cities with moderate populations of women of child-bearing age were selected for this initial testing. A statistically significant amount of mifepristone was found in all but one of the nine water sources.

2. Materials and Methods

We took 4 water samples from 3 sites that are upstream of water treatment facilities; 4 water samples from 3 sites downstream of water treatment facilities; and 4 samples from municipal tap water, from each of 3 American cities: Austin, Texas; Blacksburg, Virginia; and Carbondale, Illinois. The water in the upstream locations does not contain untreated sewage. In all 3 cities, municipal sewage water is routed directly to the water treatment facilities and then discharged to environmental water after treatment. Municipal drinking water comes from surface waters, not from groundwater wells [25, 26, 27, 28]. For each location, water treatment facilities that are located within the area of the highest population density and/or that treat wastewater from a university were chosen. Each sampling location was chosen based on accessibility and proximity to the discharge location of the water treatment facilities.

Sample bottles were prepared by ALS Environmental in Cincinnati, Ohio, for analysis of anti-progesterone CALUX®. Samples were collected utilizing grab sample techniques. Each city also contained a "field blank" sample filled with laboratory-supplied distilled water. All samples were shipped to SafeBridge Regulatory & Life Sciences Group overnight for next day delivery. The water samples were sent blind (without source identification) to BioDetection Systems laboratory in the Netherlands which dissolved the samples in DMSO and used PR CALUX® bioassays to test for mifepristone [29]. The PR CALUX® activity was determined after 24h exposure and benchmarked against mifepristone (Ru486). Briefly, optimized solid-phase extraction (SPE) was applied to extract water samples. The enriched extract with a mixture of compounds was exposed to genetically modified cell lines in CALUX® bioassays. Firefly luciferase gene, coupled with responsive elements (REs) such as reporter genes, is incorporated into the DNA of these cell lines. The presence of an appropriate substrate, in this case mifepristone, triggers the activation of such REs, thus initiating the creation of luciferase, which then emits light. The amount of light produced is measured by luminometer and is proportional to the amount of ligand-specific receptor binding, which is benchmarked against a reference level of mifepristone.

Statistical Analysis

For the purpose of the analysis, values that were below the detectable threshold were considered as zero (0). We calculated the t-test statistic as follows:

$$\text{t-test statistic} = \frac{\text{observed value - expected value (control)}}{\text{population standard deviation } (\sigma x)}$$

For the t-test of statistical significance, differences were considered significant at $p < 0.05$. While not considered as significant, p-values within the range of $0.05 < p < 0.1$ are indicated as tendencies towards significance. The results of that analysis are included in Tables 1 and 2 and in Appendix A.

In order to be more rigorous, we asked Dr. X to recommend a non-parametric statistical test, which would not depend on an assumption of normal distribution among the findings. We used Dr. X's method to perform an analysis based on straight probability of rankings. The results of that analysis are included in Tables 1 and 2 and in Appendix B.

3. Results

Results are given in Table 1, Mifepristone concentration vs. controls, t-test and ranked test; Table 2, Differences in mifepristone concentration between water sources, t-test and ranked test; and Figure 1, Mifepristone concentration vs. controls, below.

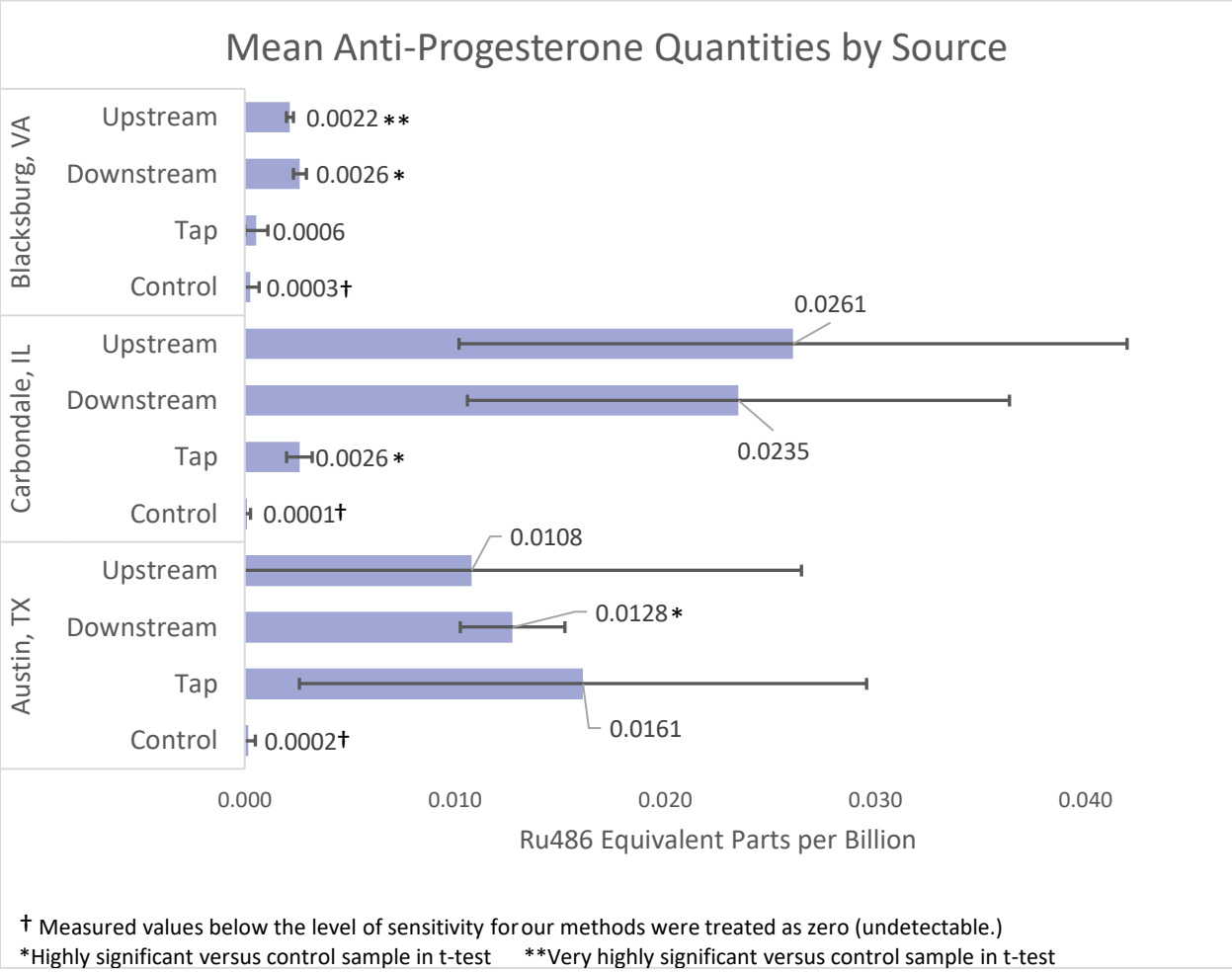
Table 1. Mifepristone concentration vs. controls, t-test and ranked test.
P = probability; S = significance; Conc = mean concentration of anti-progesterone (Ru486 equivalent), μgram/l

City	**Water Source**	**Conc**	**t-test P**	**t-test S**	**Ranked test P**	**Ranked test S**
Blacksburg VA						
	Upstream	0.0022	$p < 0.0005$	very highly significant	0.0143 $p < 0.05$	significant
	Downstream	0.0026	$p < 0.005$	highly significant	0.0143 $p < 0.05$	significant
	Tap	0.0006	$p > .20$	not significant	0.2143	not significant
	Control	0.0003				
Carbondale IL						
	Upstream	0.0261	>.10	not significant	0.0143 $p < 0.05$	significant
	Downstream	0.0235	$0.05 < p < .10$	tending towards significance	0.0143 $p < 0.05$	significant
	Tap	0.0026	$p <0.005$	highly significant	0.0143 $p < 0.05$	significant
	Control	0.0001				
Austin TX						
	Upstream	0.0108	$p > .10$	not significant	.0119 $p < 0.05$	significant
	Downstream	0.0128	$p < 0.025$	highly significant	.0079 $p < 0.01$	highly significant
	Tap	0.0161	$p > .10$	not significant	.0079 $p < 0.01$	highly significant
	Control	0.0002				

Table 2. Differences in mifepristone concentration between water sources, t-test and ranked test. U = upstream; D = downstream; T = tap; P = probability; S = significance.

City	**Water Sources**	**% change**	**t-test P**	**t-test S**	**Ranked test P**	**Ranked test S**
Blacksburg VA						
	U-D	27.9↑	<0.005	highly significant	0.0428 p < 0.05	significant
	U-T	38.1%↓	<0.0005	very highly significant	0.0143 p < 0.05	significant
	D-T	51.9%↓	<0.005	highly significant	0.0143 p < 0.05	significant
Carbondale IL						
	U-D	13.8%↓	>.10	not significant	.5	not significant
	U-T	90.0%↓	<0.0005	very highly significant	0.0716 0.05 < p < .10	tending towards significance
	D-T	88.9%↓	<0.0005	very highly significant	0.0571 0.05 < p < .10	tending towards significance
Austin TX						
	U-D	45.1%↓	>.10	not significant	0.0716 0.05 < p < .10	tending towards significance
	U-T	2.9%↓	>.10	not significant	.22	not significant
	D-T	77%↑	>.10	not significant	.5	not significant

Figure 1. Mifepristone concentration vs. controls.



† Measured values below the level of sensitivity for our methods were treated as zero (undetectable.)
*Highly significant versus control sample in t-test **Very highly significant versus control sample in t-test

3.1 Blacksburg

These statistics indicate that upstream and downstream differ significantly from each other and from both controls. The amount of mifepristone in these environmental water sources is greater than that in the controls to a highly significant degree ($p < .005$ or lower) by the t-test and significantly by the ranking test. The tap water here appears significantly cleaner than the environmental water. Two of the four tap samples were below our levels of detection.

3.2 Carbondale

These statistics indicate that upstream and downstream differ significantly from both controls but not from each other. The tap water here is significantly cleaner than both sources of environmental water but contains a significant amount more of the contaminant than do the controls by the t-test ($.05 > p > 0.025$), as well as by the ranking test.

3.3 Austin

The downstream samples have significantly more of the contaminant than do the controls by both the t-test ($.05 > p > 0.025$) and the ranking test. Neither upstream nor tap water differ significantly from controls by the t-test because of the large standard deviation in the measurements. The tap water in the Austin area may contain higher levels of mifepristone than the downstream water, but it cannot be said to be significantly different by the t-test because of the large standard deviation in the tap water measurements. However, samples from both upstream and tap water differ significantly from controls by the ranking test.

3.4 Water Treatment

The upstream locations should not be considered as water containing untreated sewage. In all 3 cities, sewage water is routed directly to the water treatment facilities and then discharged to environmental water after treatment [25, 26, 27, 28]. In all 3 cities, the level of mifepristone in the downstream water sources is not appreciably lower than the level in upstream water sources; in fact, in two of the cities it is higher. This may be expected. Untreated sewage water may not be present in the upstream samples, but it is possible that above-ground streams could become contaminated from nearby sewage lines if the latter are damaged by frost heaving or other environmental disruptions. On the other hand, treated sewage water is expected to form part of the downstream samples. At any rate, water treatment was not effective for removing this EDC. This downstream, treated environmental water is not equivalent to household tap water, which was tested in a different group.

4. Discussion

The presence of mifepristone in environmental water could have effects on wildlife and even on human health. Mifepristone could affect the reproductive and other physiology of aquatic animals such as fish and amphibians [5, 6, 7, 8, 9, 11, 12] and farm animals [10]. Steroid hormones have even been shown to have negative effects on the root growth of plants [11]. Mifepristone is considered by one manufacturer, Fass, to have “low potential for bioaccumulation” in fish, but “very high chronic toxicity”, though Fass admits that both these claims are comparative rather than quantitative. Up to 10% of mifepristone is still present in sediment after 239 days [30].

We do not know how long mifepristone stays in women’s tissues, or if it bioaccumulates. We could find no studies on levels of this hormone in human tissues such as liver or fat cells, either in the short term or with bioaccumulation. In a study of only 5 women, the half-life of mifepristone in the serum varied in different women from 24 to 44 hours [31], but serum is an aqueous milieu. The serum level is not the same as the tissue level, which was not measured in that study. Mifepristone is a steroid hormone, and therefore it is a fat-soluble molecule. Such a molecule might build up in fatty tissues rather than remain in the aqueous serum and be excreted. Further study of this possibility is warranted.

Steroid hormones have systemic effects on every cell in the body. Progesterone has a very large number of known effects [18, 19]. Its role in maintaining pregnancy by supporting placental attachment is well-known, but it is also involved in many other systems. An anti-progesterone such as mifepristone

could inhibit any actions of normal progesterone, particularly on pregnant women, but also on pre- or post-menopausal women. Presence of an anti-progesterone in the water supply could also cause previously effective clinical dosages of progesterone to be inadequate, because the progesterone is being antagonized by the anti-progesterone binding to its receptor.

Long-term use of low-dose mifepristone has been found to cause changes in the endometrium, including atypical thickening, the presence of carcinoma-like tissues [21], and to suppress ovulation and menstruation [32]. Low doses of mifepristone have also been shown to lead to amenorrhea [33]. Moreover, questions about the impact of such chemicals on maternal health and linkages to autism warrant further study [34]. Low progesterone levels in pre-menopausal, menstruating women have been associated with multiple health problems including lower fertility rates, higher rates of miscarriage, and higher rates of other medical complications during pregnancy [35, 36, 37, 8].

Mifepristone could even inhibit some actions of progesterone on men, who normally have small amounts of progesterone in their bodies. Low progesterone levels in men have been associated with multiple medical problems including alopecia, bone loss, erectile dysfunction, fatigue, and obesity [18].

Unmetabolized mifepristone could be present in the tested waters for several reasons. We have no data on the sources of mifepristone in the water, nor can we scientifically rank their likelihood. Given the high number of chemical abortions (642,700 medication abortions in the US in 2023 [4]), the most probable source is fecal and urinary excretion by women ingesting mifepristone for chemical abortion. Clearance from the body was mainly through feces (83%) [39]. The standard dose of 200 mg may represent excessive dosing for some individual women. Alternatively, mifepristone could enter the water from feces or urine of patients taking the medication for reasons other than abortion. One of the other conditions for which mifepristone is prescribed is Cushing's Syndrome or Cushing's Disease [40]. There are estimated to be fewer than 20,000 Americans with a diagnosis of Cushing's [41], so this is a far less likely contributor. Mifepristone is also sometimes prescribed for endometriosis, meningioma, and mental illness [21], but these are also far less common uses. Other sources could be improper disposal of any unused product by the patient or manufacturer. Considering the regulations governing pharmaceutical manufacturing, the latter seems very improbable.

Women who take the drug would be very likely to excrete metabolites of mifepristone as well as the intact compound. We did not test our water samples for the 5 metabolites of mifepristone, including the 3 most common: metapristone (monodemethylated mifepristone), didemethylated mifepristone, and 22-OH mifepristone [42, 43]. Future studies should investigate whether water samples contain these compounds, which are pharmacologically active, able to bind to human progesterone and glucocorticoid receptors, and therefore should be regarded as significant environmental pollutants [43, 44].

Conventional water treatment was not effective for removing this EDC from water in all cities we tested. Two cities showed significant mean concentrations even in tap water. Conventional treatment systems are primarily designed to remove infectious agents such as bacteria and viruses, with secondary treatment, according to the US Environmental Protection Agency, required to address hormones (49-99% removal) [45]. Maximum removal requires tertiary treatment, such as membrane bioreactors, oxidation, or ozonation [1, 2, 12, 13, 14, 15, 16, 17, 45].

Further investigation is necessary to provide a better understanding of the impact of any levels of mifepristone in the environment, and the efficacy of its removal by any treatment systems. Concerns about the environmental, clinical and public health impact of mifepristone raise urgent questions about the current lack of meaningful regulation of this widely-used pharmaceutical.

5. Acknowledgments

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

EDC = endocrine-disrupting compound
Ru486 = mifepristone
DMSO = dimethyl sulfoxide
SPE = solid-phase extraction
RE = responsive element
FDA = Food and Drug Administration
CALUX = chemical-activated luciferase expression

The following abbreviations are used in the tables:

Conc = concentration of anti-progesterone (Ru486 equivalent), μgram/l
σx = Population Standard Deviation
P = probability
t test statistic =observed value-expected value (control)/σx
S = significance
* = below the detection limit (level of sensitivity) for our methods; considered as 0 for statistical calculations.

Appendix A

Table 3. Mifepristone concentration vs. controls, t-test.
Conc = concentration of anti-progesterone (Ru486 equivalent), μgram/l; σx = Population Standard Deviation, P = probability; t test statistic =observed value-expected value (control)/σx; S = significance; * = below detection limit for our methods (<0.00096 Ru486 equivalent ug/l); considered as 0 for statistical calculations.

State	Water Source	Conc	Mean Conc	(σx)	t-test statistic	t-test P	t-test S
All	Lab control	*					
	Lab control	*					
			.0000				
VA	Upstream	.0023					
	Upstream	.0023					
	Upstream	.0019					
	Upstream	.0021					
			.0022	0.0002	12.952	p < 0.0005	very highly significant
	Downstream	.0024					
	Downstream	.0031					
	Downstream	.0027					
	Downstream	.0023					
			.0026	0.0003	8.467	p < 0.005	highly significant
	Tap	.0011					
	Tap	.0013					
	Tap	*					
	Tap	*					
			.0006	0.0006	1.0	p > .20	not significant
	Field blank	.0010					
	Field blank	*					
			.0005				
IL	Upstream	.0014					
	Upstream	.0390					
	Upstream	.0410					
	Upstream	.023					
			.0261	0.0159	1.642	>.10	not significant
	Downstream	.0019					
	Downstream	.0270					
	Downstream	.0360					
	Downstream	.0290					
			.0235	0.0129	1.822	0.05 < p < .10	Tending towards significance

State	**Water Source**	**Conc**	**Mean conc**	**(σx)**	**t-test statistic**	**t-test P**	**t-test S**
IL	Tap	.0024					
	Tap	.0035					
	Tap	.0018					
	Tap	.0027					
			.0026	0.0006	4.262	$p < 0.005$	highly significant
	Field blank	.0004					
	Field blank	*					
			.0002				
TX	Upstream	.0022					
	Upstream	.0380					
	Upstream	.0031					
	Upstream	*					
			.0108	0.0157	0.687	$p > .10$	not significant
	Downstream	.0170					
	Downstream	.0120					
	Downstream	.0110					
	Downstream	.0110					
			.0128	0.0025	5.12	$p < 0.025$	significant
	Tap	.0046					
	Tap	.0390					
	Tap	.0120					
TX	Tap	.0089					
			.0161	0.0122	1.193	$p > .10$	not significant
	Field blank	.0008					
	Field blank	*					
	Field blank	*					
			.0003				

Appendix B

Probability Based on Ranking Method (non-parametric test)

Modified by Dr. Élise Rose with additional data using a probability method based on ranking devised by Dr. Michael New.

Summary: Water samples were taken from three cities, Blacksburg, VA, Carbondale, IL, and Austin, TX to measure and compare the amount of anti-progesterone. For each city six comparisons were made, for a total of 18 comparisons:

1) Between upstream and controls
2) Between downstream and controls
3) Between tap water and controls
4) Between upstream and downstream
5) Between upstream and tap water
6) Between downstream and tap water

Using a non-parametric test, differences in 13 of the 18 comparisons reached conventional levels of statistical significance. Importantly, 8 of the 9 comparisons involving control samples achieved conventional levels of statistical significance. That is, probability was less than 5% that due to random chance the control samples were lower than the environmental or tap samples. In other words, we can be 95-99% confident that the environmental or tap samples had more mifepristone than the control samples. The fact that supermajority of comparisons achieved conventional levels of statistical significance means that we can be statistically confident in the findings.

Blacksburg, VA (5 of 6 comparisons are statistically significant)

1) Upstream (4 Samples) vs. Control (4 Samples)

All four upstream samples report higher levels of anti-progesterone than all 4 control samples. The likelihood that both control samples would rank last is (4/8)*(3/7)*(2/6)*(1/5) = 1/70 = .01428. This achieves statistical significance at the 98% confidence level.

2) Downstream (4 Samples) vs. Control (4 Samples)

All four downstream samples report higher levels of anti-progesterone than all 4 control samples. The likelihood that both control samples would rank last is (4/8)*(3/7)*(2/6)*(1/5) = 1/70 = .01428. This achieves statistical significance at the 98% confidence level.

3) Tap water (4 Samples) vs. Control (4 Samples)

Two tap water samples report higher levels of anti-progesterone than all 4 control samples. Two tap water samples are largely indistinguishable from the control samples. The likelihood that all 4 control samples would rank in the bottom six of 8 samples would be (6/8)*(5/7)*(4/6)*(3/5) = .2143 This result is relatively unlikely, but does not reach conventional standards of statistical significance.

4) Upstream (Four Samples) vs. Tap water (Four Samples)

All four upstream samples report higher levels of anti-progesterone than the four tap water samples. The likelihood the four upstream samples would all rank in the top four would be (4/8)*(3/7)*(2/6)*(1/5) = 24/1680 = .01428. This is statistically significant at the 98% confidence level.

5) Downstream (Four Samples) vs. Tap water (Four Samples)

All four downstream samples report higher levels of anti-progesterone than the four tap water samples. The likelihood the downstream samples would all rank in the top four would be (4/8)*(3/7)*(2/6)*(1/5)= 24/1680 = .01428. This is statistically significant at the 98% confidence level.

6) Upstream (Four Samples) vs. Downstream (Four Samples)

Analyzing and interpreting these findings is more complicated. The three highest levels of anti-progesterone all come from downstream samples. Then there are three samples that are tied (two upstream and one downstream). The two lowest come from downstream samples. The likelihood that the four upstream samples would rank (1, 2, 3, 4), (1, 2, 3, 5) or (1, 2, 3, 6) is: (4/8)*(3/7)*(2/6)*(1/5) +

(4/8)*(3/7)*(2/6)*(4/5)*(1/4) + (4/8)*(3/7)*(2/6)*(4/5)*(3/4)*(1/3) .01428 + .01428 + .1428 = .0428. This is statistically significant at the 95% level.

Carbondale, IL (5 of 6 comparisons are statistically significant)
1) Upstream (4 Samples) vs. Control (4 Samples)
All four upstream samples report higher levels of anti-progesterone than all 4 control samples. The likelihood that all 4 control samples would rank last is (4/8)*(3/7)*(2/6)*(1/5) = 1/70 = .01428. This achieves statistical significance at the 98% confidence level.
2) Downstream (4 Samples) vs. Control (4 Samples)
All four downstream samples report higher levels of anti-progesterone than all 4 control samples. The likelihood that all 4 control samples would rank last is (4/8)*(3/7)*(2/6)*(1/5) = 1/70 = .01428. This achieves statistical significance at the 98% confidence level.
3) Tap water (4 Samples) vs. Control (4 Samples)
All four tap water samples report higher levels of anti-progesterone than all 4 control samples. The likelihood that all 4 control samples would rank last is (4/8)*(3/7)*(2/6)*(1/5) = 1/70 = .01428. This achieves statistical significance at the 98% confidence level.
4) Upstream (Four Samples) vs. Downstream (Four Samples)
The means here are close, .0261 vs. .0235. There is not much discernible grouping regarding how the samples are ranked. Thus there is no statistical evidence that there is a difference between the two means.
5) Upstream (Four Samples) vs. Tap Water (Four Samples)
Three upstream samples have the highest level of anti-progesterone. The fourth upstream sample has the lowest. The likelihood of the three upstream samples being the highest is (4/8)*(3/7)*(2/6) = .0716. This achieves statistical significance at the 90% level.
6) Downstream (Four Samples) vs. Tap Water (Four Samples)
Three upstream samples have the highest level of anti-progesterone. The fourth upstream sample has the second lowest. The likelihood of the three upstream samples being the highest and the fourth not being the lowest is (4/8)*(3/7)*(2/6)*(4/5) = .0571. This achieves statistical significance at the 90% level, and approaches the 95% level.

Austin, TX (4 of 6 comparisons are statistically significant)
1) Upstream (4 Samples) vs. Control (5 Samples)
The three samples with the highest level of anti-progesterone all come from upstream. The fourth upstream sample is equal to the lowest observed concentration. The likelihood that the three samples with the highest level of anti-progesterone would come from upstream is (3/9)*(2/8)*(1/7) = .0119. This achieves statistical significance at the 98% confidence level.
2) Downstream (4 Samples) vs. Control (5 Samples)
All four downstream samples report higher levels of anti-progesterone than all 5 control samples. The likelihood that the 4 samples with the highest level of anti progesterone would come from downstream is (4/9)*(3/8)*(2/7)*(1/6) = .0079. The likelihood that all 5 control samples would rank last is the same. This achieves statistical significance at the 99% confidence level.
3) Tap water (4 Samples) vs. Control (5 Samples)
All four tap water samples report higher levels of anti-progesterone than all 5 control samples. The likelihood that all 5 control samples would rank last is (5/9)*(4/8)*(3/7)*(2/6)*(1/5) = .0079 This achieves statistical significance at the 99% confidence level.
4) Upstream (Four Samples) vs. Downstream (Four Samples)
Three upstream samples have the highest level of anti-progesterone. The fourth upstream sample has the lowest. The likelihood of the three upstream samples being the three highest is (4/8)*(3/7)*(2/6) = .0716. This achieves statistical significance at the 90% level.
5) Tap water (Four Samples) vs. Downstream (Four Samples)

The mean levels of anti-progesterone are close here (.0161 vs. .01275). There does not appear to be much clustering in the ranking of samples. This indicates a lack of statistically significant differences.

6) Tap water (Four Samples) vs. Upstream (Four Samples)

Three of the four samples with the highest level of anti-progesterone come from upstream as does the sample with the lowest level of anti-progesterone. The likelihood that three of the four highest levels of anti-progesterone would come from upstream is about 22%. While this is unlikely, it does not reach conventional standards of statistical significance.

References

1. Falconer IR, Chapman HF, Moore MR, Ranmuthugala G. Endocrine-disrupting compounds: a review of their challenge to sustainable and safe water supply and water reuse. Environ Toxicol 2006;21:181-191. doi:10.1002/tox.20172

2. Racz L, Goel RK. Fate and removal of estrogens in municipal wastewater. J Environ Monit. 2010;12:58-70. doi:10.1039/b917298j

3. Daniels K, Abma JC. Current contraceptive status among women aged 15–49: United States, 2017–2019. NCHS Data Brief 2020; Report No. 388, National Center for Health Statistics, Hyattsville, MD.

4. Jones RK, Friedrich-Karnik A. Medication Abortion Accounted for 63% of All US Abortions in 2023—An Increase from 53% in 2020. Guttmacher Institute. 2024 March. https://www.guttmacher.org/2024/03/medication-abortion-accounted-63-all-us-abortions-2023-increase-53-2020

5. Fent K. Progestins as endocrine disrupters in aquatic ecosystems: Concentrations, effects and risk assessment. Environ Int. 2015;84:115-130. doi:10.1016/j.envint.2015.06.012

6. Jenila JS, Issac PK, Lam SS, Oviya JC, Jones S, et al. Deleterious effect of gestagens from wastewater effluent on fish reproduction in aquatic environment: A review. Environ Res. 2023;236:116810. doi:10.1016/j.envres.2023.116810

7. Zucchi S, Castiglioni S, Fent K. Progestins and antiprogestins affect gene expression in early development in zebrafish (*Danio rerio*) at environmental concentrations. Environ Sci Technol. 2012; 46:5183-5192. doi:10.1021/es300231y

8. Bluthgen N, Castiglioni S, Sumpter J, Fent, K. Effects of low concentrations of the antiprogestin mifepristone (RU486) in adults and embryos of zebrafish (*Danio rerio*): 1. Reproductive and early developmental effects Aquatic Toxicology. 2013 November 15;144–145:83-95 https://doi.org/10.1016/j.aquatox.2013.09.033

9. Bluthgen N, Castiglioni S, Sumpter J, Fent, K. Effects of low concentrations of the antiprogestin mifepristone (RU486) in adults and embryos of zebrafish (*Danio rerio*): 2. Gene expression analysis and in vitro activity Aquatic Toxicology. 2013 October; 144–145:96-104. DOI:10.1016/j.aquatox.2013.09.030

10. Pocar P, Brevini T, Fischer B, Gandolfi F. The impact of endocrine disruptors on oocyte competence. Reproduction 2003 1 Mar;125(3):313–325. https://doi.org/10.1530/rep.0.1250313

11. Environmental Assessment and Finding of No Significant Impact for NDA 20-687 Mifepristone Tablets. US FDA. 1996. Available from: https:www.accessdata.fda.gov/drugs_at_fda_docs/nda/20687_Mifepristone_EA.pdf.

12. Almazrouei B, Islayem D, Alskafi F, Catacutan MK, Amna R, Nasrat S, et al. Steroid hormones in wastewater: Sources, treatments, environmental risks, and regulations. Emerging Contaminants. 2023;9. doi:10.1016/j.emcon.2023.100210

13. Silva CP, Otero M, Esteves V. Processes for the elimination of estrogenic steroid hormones from water: a review. Environ Pollut. 2012;165:38-58. doi:10.1016/j.envpol.2012.02.002

14. Katibi KK, Yunos, KF, Man HC, Aris AZ, Nor MZM, et al. Contemporary Techniques for Remediating Endocrine-Disrupting Compounds in Various Water Sources: Advances in Treatment Methods and Their Limitations. Polymers (Basel) 2021;13. doi:10.3390/polym13193229

15. Can Birth Control Hormones Be Filtered from the Water Supply? Scientific American. 2009; July 28. Available from: https:www.scientificamerican.com/article/birth_control_in_water_supply. [Accessed 2026 June 7]

16. Guerrero-Gualan D, Valdez-Castillo E, Crisanto-Perrazo T, Toulkeridis, T. Methods of Removal of Hormones in Wastewater. Water. 2023; 15. doi:10.3390/w15020353

17. Bajda T, Grela A, Pamuła J, Kuc J, Klimek A, Matusik J, Franus W, Alagarsamy SKK, Danek T, Gara P. Using Zeolite Materials to Remove Pharmaceuticals from Water. Materials (Basel). 2024 Aug 3;17(15):3848. doi: 10.3390/ma17153848. PMID: 39124512; PMCID: PMC11313275.

18. Kolatorova L, Vitku J, Suchopar J, Hill M, Parizek A. Progesterone: A steroid with wide range of effects in physiology as well as human medicine. Int J Mol Sci. 2022; 23. doi:10.3390/ijms23147989

19. Progesterone. National Center for Biotechnology Information, National Institutes for Health, National Library of Medicine. 2025. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Progesterone
20. Baulieu EE. The Antiprogestin Steroid RU 486 and Human Fertility Control. In Baulieu EE, Segal SJ, editors. The Antiprogestin Steroid RU 486 and Human Fertility Control. New York: Springer; 1985. p. 1-25.
21. Lin Q, Zhou Y. Endometrial Changes Associated with Mifepristone: A Review. Int. J Gen Med. 2025;18:0:5503–5508. https://doi.org/10.2147/IJGM.S535797
22. PubChem Compound Summary for CID 55245, Mifepristone. National Center for Biotechnology Information, National Institutes for Health, National Library of Medicine. 2025. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Mifepristone.
23. Das C, Catt KJ. Antifertility actions of the progesterone antagonist RU 486 include direct inhibition of placental hormone secretion. Lancet 1987;2:599-601. doi:10.1016/s0140-6736(87)92988-6.
24. PubChem Compound Summary for CID 5282381, Misoprostol. National Center for Biotechnology Information, National Institutes for Health, National Library of Medicine. 2025. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Misoprostol
25. Shoal Creek Conservancy. The Ins and Outs of the Austin Water System. Shoal Creek Conservancy. 2023; January. [cited 2026 May 16] Available from: https://shoalcreekconservancy.org/austin-water-system/
26. Town of Blacksburg Virginia. Drinking Water. [cited 2026 May 16] Available from: https://www.blacksburg.gov/departments/departments-a-k/engineering-and-gis/drinking-water
27. Town of Blacksburg Virginia. Wastewater. [cited 2026 May 16] Available from: https://www.blacksburg.gov/departments/departments-a-k/engineering-and-gis/wastewater
28. Carbondale Illinois. Water and Sewer. [cited 2026 May 16] Available from: https://explorecarbondale.com/174/Water-Sewer
29. BioDetection Systems BV Catalogue 15. Amsterdam: BioDetection Systems; 2024.
30. Pharmaceuticals and Environment/Mifepristone. Janusinfo Region Stockholm. [cited 2026 May 20] Available from: https://janusinfo.se/beslutsstod/lakemedelochmiljo/pharmaceuticalsandenvironment/databaseenven/mifepristone.5.30a7505616a041a09b062f91.html
31. Kekkonen R, Heikinheimo O, Mandelin E, Lahteenmaki P. Pharmacokinetics of mifepristone after low oral doses. Contraception 1996;54:229-234. doi:10.1016/s0010-7824(96)00193-x
32. Baird DT, Brown A, Critchley HOD, Williams AR, Lin S, Cheng L. Effect of long-term treatment with low-dose mifepristone on the endometrium. Hum Reprod. 2003;18:61-68. doi:10.1093/humrep/deg022
33. Kumari K, Malik R, Hada A, Goel H. Effectiveness of low dose mifepristone in medical management of fibroids. J S Asian Fed Ob and Gyn. 2024;16:98-102. doi:10.5005/jp-journals-10006-2398.
34. Lu J, Wang Z, Liang Y, Yao P. Rethinking autism: the impact of maternal risk factors on autism development. Am J Transl Res. 2022;14:1136-1145.
35. Ku CW, Allen JC Jr, Lek SM, Chia ML, Tan NS, Tan TC. Serum progesterone distribution in normal pregnancies compared to pregnancies complicated by threatened miscarriage from 5 to 13 weeks gestation: a prospective cohort study. BMC Pregnancy Childbirth. 2018 Sep 5;18:360. doi: 10.1186/s12884-018-2002-z
36. Ucyigit A, Fuller JL, Poon LC, Johns J, Ross JA. The significance of low first trimester serum progesterone in ongoing early pregnancies presenting as pregnancies of unknown location. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2021 March; 258:294-298.
37. Stavridis K, Kastora SL, Triantafyllidou O, Mavrelos D, Vlahos N. Effectiveness of progesterone rescue in women presenting low circulating progesterone levels around the day of embryo transfer: a systematic review and meta-analysis. Fertility and Sterility. 2023;119:954-963.
38. Low Progesterone. Cleveland Clinic. 2025. Available from: https://my.clevelandclinic.org/health/diseases/24613-low-progesterone
39. Sarkar NN. Mifepristone: bioavailability, pharmacokinetics and use-effectiveness. Eur J Obstet Gynecol Reprod Biol. 2002 Mar 10;101(2):113-20. doi: 10.1016/s0301-2115 (01)00522-X. PMID: 11858883.
40. Cushing Syndrome Diagnosis and treatment. Mayo Clinic. cited 2026 May 16] Available from: https://www.mayoclinic.org/diseases-conditions/cushing-syndrome/diagnosis-treatment/drc-
41. Cushing's Syndrome. NIH. National Institute of Diabetes and Digestive and Kidney Diseases. [cited 2026 May 16] https://www.niddk.nih.gov/health-information/endocrine-diseases/cushings-syndrome#howCommon
42. Heikinheimo O, Laähteenmaäki P L A, Koivunen E, Shoupe D, Croxatto H, Luukkainen T, Laähteenmaäki P. Metabolism and serum binding of Ru 486 in women after various single doses.
Human Reproduction, 1987 1 July;2(5):379–385. https://doi.org/10.1093/oxfordjournals.humrep.a136554

43. Lähteenmäki P, Heikinheimo O, Croxatto H, Spitz I, Shoupe D, Birgerson L, Luukkainen T**.** Pharmacokinetics and metabolism of RU 486. Journal of Steroid Biochemistry 1987 Jan 1;27(4-6): 859-863
DOI: 10.1016/0022-4731(87)90160-9
44. Szpot P, Wachelko O, Jurek T, Zawadzki M. Determination of Mifepristone (RU-486) and Its Metabolites in Maternal Blood Sample after Pharmacological Abortion. Molecules. 2022 Nov 6;27(21):7605.
doi: 10.3390/molecules27217605
45. Characteristics and treatment of pharmaceuticals and personal care products in wastewater. 2024 Aug. Available from: https://www.epa.gov/system/files/documents/2024-08/ppcp_technology_brief.pdf. [Accessed 2026 June 8]