

Endocrine Disruption and Reproductive Dysfunction in Freshwater Fishes Exposed to Agricultural Runoff and Synthetic Estrogens in Rural India: A Mechanistic Critical Review

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Abstract

Background: Agricultural pesticides, steroid hormones and other endocrine-active contaminants can enter freshwater networks through different and overlapping pathways. Experimental evidence establishes that estrogens and several pesticide classes can disturb fish endocrine signalling, gametogenesis and reproduction; however, chemically measured exposure and directly attributed reproductive impairment remain unevenly documented in rural Indian river systems. Objective: To synthesize evidence linking contaminant identity and route, hypothalamic–pituitary–gonadal signalling, histological outcomes, fertility, and population-level effects, while evaluating the transferability of international experimental studies to Indian freshwater fishes. Approach: A structured, source-traceable critical synthesis of Indian pesticide monitoring and fish experiments, whole-ecosystem estrogen studies, mechanistic toxicology, and standard testing methods. Results: Indian Yamuna surveys confirm historical organochlorine pesticide residues and an Indian catfish experiment provides endocrine evidence following gamma-HCH exposure; Ganga monitoring documents fish tissue injury but not estrogen-specific reproductive causation. International EE2 studies demonstrate disruption at low ng/L exposures, intersex phenotypes, lowered fertilization and even population collapse during long-term whole-lake exposure. Atrazine evidence is mixed across tests, species and conditions. Interpretation: Induced vitellogenin or gonadal lesions are valuable but non-exclusive signals; attribution requires measured mixtures, life-stage context and organism-level outcomes. Conclusion: Coordinated LC-MS/MS surveillance, validated estrogenicity assays, gonadal histopathology, controlled breeding endpoints and longitudinal ecological monitoring are needed to establish risk in rural Indian waters. Review designation: This article presents a structured mechanistic critical synthesis, not a completed systematic review; its evidence matrix organizes claims by directly measured outcomes rather than by an exhaustive PRISMA search.

Keywords: Endocrine Disruptors; Agricultural Runoff; Ethinylestradiol; Pesticides; Hpg Axis; Vitellogenin; Intersex; Gonadal Histopathology; Fertility; Ganga; Yamuna; Rural India

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Introduction: reconciling contaminant exposure and reproductive harm

Fish reproduction is governed by an integrated hypothalamic–pituitary–gonadal (HPG) axis whose outputs vary with season, photoperiod, nutrition, temperature and species-specific life history. Endocrine-active chemicals can alter receptor signalling, synthesis or clearance of sex steroids, gonadotropin secretion, hepatic vitellogenin synthesis, and gonadal differentiation. The early discovery of estrogenic alkylphenols and male-fish vitellogenesis established useful mechanistic markers, but did not alone demonstrate impaired population recruitment [1, 2, 3].

A rural-India focus adds source-attribution complexity. Crop protection chemicals may be mobilized during irrigation return flow or storms, whereas ethinylestradiol (EE2) and other synthetic estrogens are more characteristically associated with pharmaceutical or municipal wastewater, animal-use pharmaceuticals and diffuse wastewater inputs. The presence of agricultural runoff does not establish EE2 exposure. Indian monitoring from the Yamuna provides quantitative chemical occurrence evidence for organochlorine pesticides; an investigation on Ganga fish establishes contaminant-associated hepatic and branchial pathology but not steroid-specific reproductive toxicity [16, 17].

The most persuasive demonstration that low-level synthetic estrogen exposure may produce population harm comes from a seven-year whole-lake experiment with EE2 and fathead minnow in Canada [10]. This intervention cannot be treated as an Indian field measurement, but offers a mechanistic benchmark for the evidence that Indian surveillance must assemble. Subsequent recovery demonstrates that endocrine effects can be reversible at biomarker and population levels when exposure ceases, although recovery can take years [11].

Aims and scope. This mechanistic critical review (i) distinguishes agricultural pesticide inputs from wastewater-associated steroidal estrogens; (ii) synthesizes teleost HPG-axis disruption, vitellogenin induction, gonadal histopathology, and directly measured

reproductive performance; (iii) critically separates chemical monitoring and controlled Indian-species experiments from international field and whole-ecosystem evidence; and (iv) identifies design and statistical requirements for testing endocrine-linked reproductive dysfunction in Indian rural waters. The review evaluates selected literature published through 30 November 2024 and does not estimate national disease prevalence or perform a systematic-review meta-analysis.

Structured literature synthesis and evidence framework

This manuscript is a mechanistic critical review drawing on selected, traceable evidence from environmental chemistry, Indian fish ecotoxicology, controlled teleost endocrine experiments, reproductive histopathology, and whole-ecosystem studies. The central questions concern exposure provenance, HPG-axis mechanisms, evidence of actual fertility impairment, and the transferability of experimental results to Indian rivers. Studies are interpreted according to what they directly measure: chemical occurrence, endocrine biomarkers, gonadal lesions, breeding success, or population outcomes. The review does not claim comprehensive PRISMA identification, dual independent screening, formal risk-of-bias scoring, preregistration, or meta-analysis [42].

Methodological roadmap, not a completed search census. For a future formal systematic review, an auditable search could cover PubMed, Scopus, Web of Science, CAB Abstracts and appropriate Indian institutional sources. Illustrative concepts are (fish OR teleost OR catfish OR carp OR zebrafish) AND (endocrine disrupt* OR ethinylestradiol OR estrogen* OR pesticide OR atrazine OR organochlorine) AND (reproduct* OR vitellogenin OR intersex OR gonad* OR fertility), supplemented with Indian basin and runoff terms. These terms are proposed for reproducibility planning only: they were not executed as a comprehensive database search for this manuscript, and no search totals, exclusions or screening statistics are implied.

Evidence stratum	Required measurements	Valid inference	Unwarranted inference
A. Exposure	Compound confirmation in water/sediment/fish; blanks	Chemical occurrence and exposure opportunity	Reproductive disruption
B. Endocrine response	VTG, steroids, receptor transcript; controls	Altered endocrine-associated pathway	Loss of fertility
C. Gonadal harm	Blinded gonad histology; stage/season	Tissue-level reproductive injury	Population collapse
D. Organism reproduction	Egg counts, sperm quality, spawning/fertilization	Direct reproductive performance effect	National fishery decline
E. Ecological outcome	Recruitment or abundance time series; confounders	Population-level change with attribution caveats	Universal threshold

Table 1. Evidence strata and inferential boundaries for interpreting endocrine effects (author-proposed framework).

Source ecology in agricultural watersheds

3.1. Agrochemical movement, deposition and transformations

Runoff mobilizes dissolved residues and particle-bound organochlorines as functions of solubility, soil organic carbon, antecedent moisture, rainfall intensity and canal operation. Persistence differs among contemporary-use and legacy pesticides; old residues can be remobilized from sediments after use restrictions. The Yamuna survey by Kaushik et al. assessed HCH isomers, endosulfan, DDT metabolites and cyclodienes through Haryana and Delhi corridors, illustrating how chemical detection can be spatially widespread without pinpointing a contemporaneous application site [16].

The toxicological signature of a pesticide mixture need not be estrogenic. Organophosphate insecticides may produce cholinesterase inhibition alongside altered steroidogenesis; organochlorines can interact with steroid pathways and affect reproductive phases in species-dependent ways. Mixture effects can be nonadditive at receptor, enzyme and organism levels; nutrient enrichment, hypoxia and seasonal temperature also act as major confounders [14, 15, 18].

3.2. Sources and chemical distinction of synthetic estrogens

Natural estrone (E1) and 17 β -estradiol (E2), synthetic EE2, alkylphenols and pesticide-related endocrine modulators should never be pooled analytically into a single “estrogen” exposure without chemical or bioanalytical justification. EE2 is a potent synthetic steroid commonly associated with pharmaceutical wastewater. Relative potency testing found marked differences among E1, E2 and EE2 and showed that mixtures may behave according to concentration-addition principles for some assay responses [8].

Sorption to suspended material, photolysis, microbial transformation, seasonal dilution and wastewater contributions alter the spatial and temporal exposures experienced by fish. A mixture study should combine LC-MS/MS isotope-dilution methods with sampled runoff events, dry-season baselines and paired sediment measurements. Bioassay-derived estrogenic activity should be regarded as a complementary functional measure rather than a chemical identification of EE2 [1, 8, 20].

As shown in Figure 1, agricultural chemical runoff and domestic or pharmaceutical wastewater are distinct source pathways that may converge in receiving waters. The diagram illustrates exposure possibilities; it does not represent measured concentrations or confirmed endocrine effects in any particular Indian watershed.

SOURCE-PATHWAY-RECEPTOR MODEL

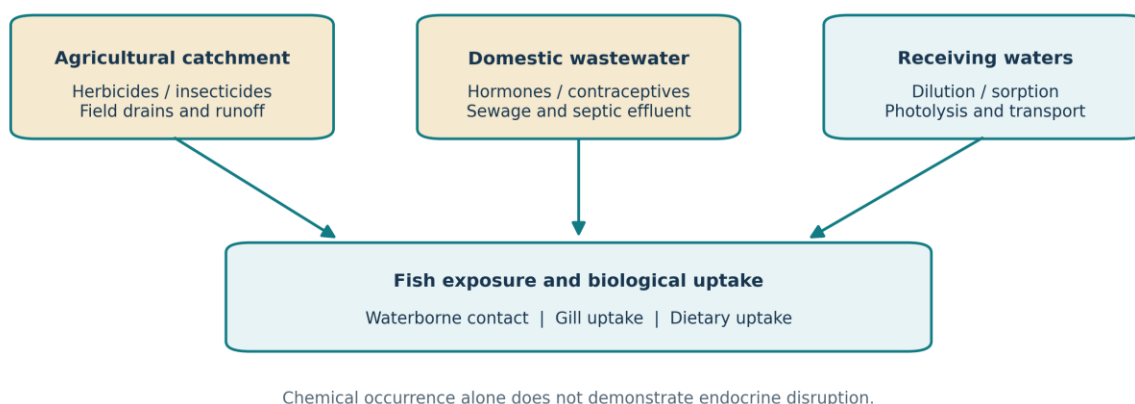


Figure 1. Conceptual distinction among agricultural and domestic sources, environmental transport, and exposure of freshwater fishes. This illustration is not a map of measured Indian concentrations.

3.3. Implications for exposure attribution. Because agricultural runoff and domestic wastewater may converge within rural drainage networks, a pesticide residue cannot serve as a proxy for EE2, nor can sample estrogenicity identify the responsible chemical without confirmatory analysis. Event-based and dry-season collections should report analyte-specific detection limits, recovery, and matrix effects; LC-MS/MS and complementary receptor bioassays answer different questions [8, 16, 18, 20]. This distinction is essential for interpreting Figure 1 and avoiding unsupported source attribution.

Agricultural and domestic effluents may mix within rural canals, drainage ditches and tributaries, complicating source apportionment. Measured estrogenic activity in a bioassay is a property of the whole sample and should not be used to assign responsibility to a named estrogen without chemical identification. Mixtures of estrone, estradiol, EE2, alkylphenols and other agonists can yield a common receptor-mediated signal despite widely different potencies [1–3, 8, 19]. Such distinction is essential for defensible rural India exposure models.

The teleost HPG axis and molecular modes of endocrine interference

4.1. Central control and feedback

The neuroendocrine axis links environmental cues to gonadotropin-releasing hormone, pituitary follicle-stimulating and luteinizing hormone output, gonadal steroidogenesis and gametogenesis. Feedback involving E2, testosterone and 11-ketotestosterone depends on sex, maturity and seasonal stage. Therefore a single plasma steroid sample may capture a transient physiological stage rather than a toxicologically induced disruption. Controlled seasonal sampling and verified sex-stage identification are obligatory [14, 18, 32]. Figure 2 summarizes the hypothalamus–pituitary–gonad signalling sequence and the distinct sites at which estrogenic ligands can interfere. Its arrows are mechanistic relationships, not a measured causal pathway in Indian river fishes; endocrine biomarkers must be interpreted alongside species, sex, reproductive stage and functional outcomes.

4.2. Estrogen receptors, hepatic vitellogenin and aromatase

Estrogens bind nuclear estrogen receptors and can modulate transcription, including hepatic vitellogenin production. VTG expression in immature or male fish is a sensitive marker of estrogenic exposure, particularly if measured in parallel with validated positive controls. However, hepatic VTG induction is an early biological response, not synonymous with testicular feminization, infertility, or reduced recruitment [1, 3, 8, 21].

Estrogenic disruption may additionally modify gonadal aromatase, steroidogenic enzymes, germ-cell proliferation, reproductive behaviour and feedback systems. A pathway claim based on transcription alone is weaker than one linking transcripts to protein, circulating steroids, blinded tissue assessment and spawning outcomes. Genomic and nongenomic mechanisms may differ by the life-history phase and duration of exposure [14, 18, 22, 33].

4.3. Pesticide-specific endocrine effects

Indigenous Indian ecotoxicology provides a particularly relevant experimental example: Singh and Canario [15] examined gamma-hexachlorocyclohexane (gamma-HCH) exposure in the freshwater catfish *Heteropneustes fossilis* during the pre-spawning period and reported endocrine-associated reproductive responses. This experiment provides Indian-species mechanistic evidence, but it does not document gamma-HCH-induced infertility or intersex prevalence in free-ranging Indian river populations. For atrazine, measured endpoints vary: goldfish studies identified changes in sex-steroid dynamics without consistent vitellogenin responses, whereas another study did not identify altered gonadal aromatase under its tested conditions [23, 24].

Some fathead-minnow atrazine experiments found reductions in egg production and histological changes, while extended reproduction assays in fathead minnow and medaka did not support impaired fecundity at environmentally realistic test conditions. A responsible review must report both groups and distinguish nominal dose, analytically measured exposure, developmental timing, replication and positive-control performance [25–29, 36, 37].

Classic field evidence of widespread intersexuality in wild roach downstream of wastewater discharges was reported by Jobling et al. [31]. This historical observation provides ecological context for the estrogenic disruption framework, but its geography and dominant source pathway differ from the agricultural settings emphasized here. The effects cannot be attributed to Indian river fishes without independent local exposure and reproductive investigations.

Histopathology, gamete integrity and phenotype

Sex differentiation and gonadal maturation are dynamic. In immature fish, undifferentiated gonads cannot automatically be labeled abnormal; in breeding fish, seasonal changes in the gonadosomatic index (GSI) are expected. Histopathological endpoints potentially associated with endocrine-active exposures include ovotestis, asynchronous oocyte development, follicular atresia, delayed spermatogenesis, disrupted reproductive ducts, germ-cell loss and altered secondary sex characteristics. Each can have infectious, nutritional, thermal, developmental or genetic explanations [4, 5, 6, 18, 34].

The roach sewage-effluent literature offers a useful endpoint hierarchy: graded effluent exposure changed gonadal duct development, field fish showed altered maturation and gamete production, and independent fertility investigations associated intersex severity with compromised male reproductive function. These studies reinforce the distinction between observing intersex and directly measuring the reproductive success of the affected fish [4, 5, 6].

For Indian fish species, tissue interpretation should record gonad position and fixation, maturity stage, full-section coverage, follicular stage distributions, spermatogenic cyst integrity, atresia grades, staining controls and observer blinding. Histology should be performed alongside endocrine and reproductive output measurements, not retrospectively interpreted from a single image. Separate reporting for males and females is mandatory [15, 17, 18].

Histological abnormality must be separated from realized reproductive dysfunction. Intersex tissue, testicular oocytes, follicular atresia or altered spermatogenic architecture provide anatomical evidence requiring stage-matched controls; they do not automatically quantify sperm fertilizing ability, eggs laid, hatch success, surviving offspring, or population recruitment. These performance endpoints must be measured directly, ideally in replicated breeding groups, before a claim of fertility impairment is made [4–6, 10, 18].

Endpoint	Indicative alteration	What it directly demonstrates	Limitations
Male VTG	Elevated circulating VTG or liver transcripts	Estrogenic response	Not proof of infertility
Ovary histology	Atresia, disordered oocytes	Altered morphology	Cycle and disease confounding
Testis histology	Germ-cell loss, ovotestis	Altered gonadal differentiation	Requires stage/species baseline
GSI	Shift in gonad/body-mass ratio	Gross reproductive investment change	Strong seasonal dependence
Sperm function	Motility, concentration, fertilization	Male gamete performance	Handling and water conditions
Egg production	Number and frequency of spawn	Direct breeding output	Tank and social-group effects

Table 2. Interpretation of commonly reported reproductive biomarkers; not all indicate endocrine causality.

5.1. Histological endpoints should be examined against reproductive stage and species-specific gonadal dynamics. Intersex denotes co-occurrence of ovarian and testicular tissues under an operational histological definition; testicular oocytes should not be equated with complete sex reversal. Oocyte atresia, spermatogenic arrest, degeneration of seminiferous tissue and delayed maturation are biologically important but not specific fingerprints of endocrine-disrupting chemicals. Sequential histology, gonadosomatic index, plasma steroid measurement and a verified exposure record should be evaluated together [4–6,18,21,22].

5.2. The inferential strength of the biomarker-to-population chain varies considerably. Male vitellogenin is a sensitive indicator of estrogenic stimulation, but it does not establish sterility. Gonadal malformation indicates anatomical disruption, but fecundity, fertilization success, recruitment and population persistence are separate outcomes requiring direct measurement. Laboratory exposures using characterized EE2 concentrations are stronger for demonstrating chemical causation; natural river surveys are stronger for establishing realistic exposure mixtures, species distributions and seasonal variation. The whole-lake intervention by Kidd et al. [10] unusually bridged these evidence levels.

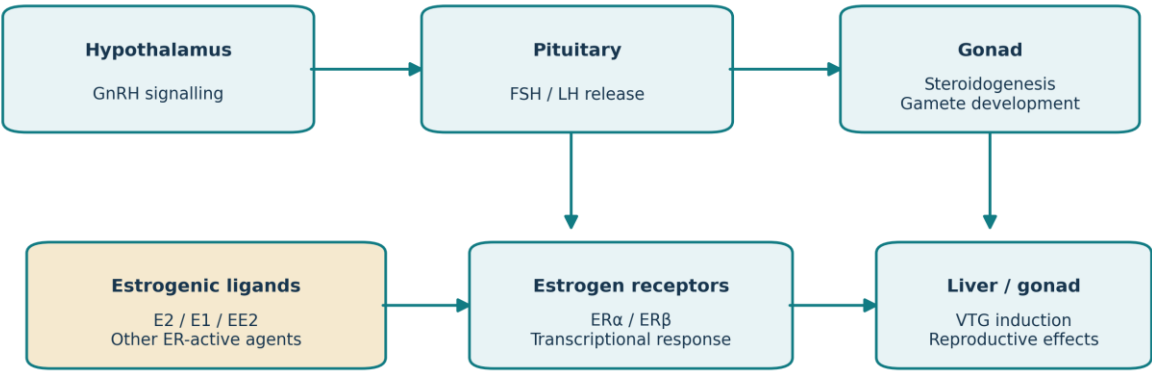
Evidence for synthetic estrogen toxicity at environmentally relevant doses

A controlled full-life-cycle fathead minnow study tested EE2 from 0.2 to 64 ng/L nominal under flow-through conditions. Gonadal differentiation and secondary sexual characteristics were adversely influenced at higher tested concentrations, and the authors interpreted the overall no-observed-adverse-effect concentration as 1.0 ng/L. It is inappropriate to quote these laboratory values as concentrations measured in Indian rural rivers [7, 43].

Shorter-term studies in zebrafish demonstrated that VTG, gonad morphology and GSI respond over different exposure windows; selected reproductive effects may recover after transfer to uncontaminated water, whereas persistent developmental exposure may produce more durable effects. Gonadal transcriptomic investigations further demonstrate that molecular changes are informative only when anchored to reproductive performance [9, 22].

The whole-lake EE2 exposure performed by Kidd et al. is particularly valuable because biochemical feminization was linked to a near-collapse of the exposed fathead-minnow population over several years at about 5–6 ng/L. Later follow-up demonstrated recovery after exposures ended; these two results together show why duration, cohort replacement and ecological context matter when interpreting a measured VTG response [10, 11, 12].

TELEOST HPG AXIS AND ESTROGENIC INTERFERENCE



A biomarker response (e.g., VTG) is not itself proof of reduced fertility.

Figure 2. Hypothalamic–pituitary–gonadal signalling and plausible points of estrogenic interference in teleosts. GnRH, gonadotropin-releasing hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; VTG, vitellogenin. Mechanistic schematic, not an experimental result.

Evidence from India: separating documented results from inference

The evidence from Indian settings is heterogeneous and should be stratified rather than presented as one national estimate. In the Yamuna system, verified organochlorine pesticide occurrence supports exposure potential. In the Ganga, comparative investigations of *Rita rita* and *Cyprinus carpio* detected gill and liver injury and altered biochemical biomarkers from contaminated locations; these endpoints do not establish EE2 exposure or direct reproductive impairment [16, 17].

The freshwater catfish gamma-HCH experiment provides Indian-species endocrine evidence under controlled exposure, which is more directly relevant to the review question but remains different from population-level data from a rural river. Together, these studies support plausibility and a surveillance agenda, not a demonstrated nationwide pattern of intersex or reduced fish recruitment specifically caused by farm-derived synthetic estrogens [15, 16, 17].

Geographic evidence boundary. For Indian waters, the cited Yamuna survey establishes organochlorine pesticide occurrence, and the cited Ganga investigation documents branchial/hepatic injury and biochemical changes in sampled fish; neither study directly demonstrates EE2-induced gonadal injury, impaired fertilization, or reduced recruitment in those rivers [16, 17]. The controlled gamma-HCH experiment on *Heteropneustes fossilis* establishes an endocrine response in an Indian freshwater species [15]. By contrast, the Canadian whole-lake EE2 experiment establishes a causally informative population response to an experimentally added synthetic estrogen [10]; its effect size, concentration and ecological context must not be represented as measurements from Indian catchments.

Study / geography	Exposure or intervention	Measured endpoint	Correct evidentiary classification
Kaushik et al.; Yamuna	Organochlorine pesticide residues	River/canal chemistry	Indian chemical occurrence; not fish fertility
Shah & Parveen; Ganga	Pesticide-contaminated sites	Gill/liver pathology; oxidative biomarkers	Indian fish injury; not EE2-specific reproduction
Singh & Canario; Indian catfish	Experimental gamma-HCH	Reproductive endocrine response	Controlled Indian-species mechanism
Kidd et al.; Canada	Whole-lake EE2	VTG, gonads, population	Strong ecological



Länge et al.; laboratory	Fish full-life- cycle EE2	Sex differentiation, reproduction	causation; international comparator Controlled dose-response; international comparator
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Table 3. Geographic provenance and strength of the principal evidence; explicitly prevents inappropriate India-specific attribution.

7.1. Evidence from rural India should be classified by endpoint rather than geography alone. Kaushik et al. [16] document pesticide residues within the Yamuna drainage, providing exposure evidence; this is not itself a demonstration of altered fish gonadal development. Singh and Canario [15] investigated reproductive endocrine effects of a pesticide in the freshwater catfish *Heteropneustes fossilis*, providing species-relevant experimental evidence but not nationwide field prevalence. Shah and Parveen [17] assessed tissue-level injury and biomarkers associated with contaminated Ganga fishes; injury markers should not be automatically ascribed to a discrete estrogen receptor mechanism.

For any basin-specific causal claim, site-level concentrations should be linked to fish sampled in the same reproductive season. Reporting should document species, sex, body size, maturity, current gonadal stage, abiotic covariates, co-pollutants and whether measured concentrations represent instantaneous water values, integrated water samples or tissue burdens. A single high downstream value cannot establish chronic exposure during the preceding gametogenic window [15–17,20].

Exposure mixtures, dose metrics and interactions

Measured concentration, chemical activity and bioavailability are not interchangeable. Farm runoff can deliver episodic pesticide pulses; wastewater-derived steroids may display different hydrological profiles. Combining analyte-specific concentration time series with effect-directed estrogenicity fractionation would distinguish receptor-active mixtures from non-estrogenic toxicants. The persistence of legacy pesticides in sediments requires event-triggered sampling, and analytical matrix effects require surrogate standards and procedural blanks [8, 16, 20, 45].

Exposure-effect thresholds also depend on life stage, receptor complement and duration. Early embryonic atrazine exposure has been linked to adult reproductive alterations in a zebrafish experimental model, while other fish studies found limited reproductive consequences under differing protocols; such contrasts emphasize that species, endpoint and assay design can drive apparent disagreement [25, 29, 30].

Advanced endpoints and recommended study design for rural India

A tiered field study should select replicated reference, upstream agricultural, irrigation-drain and mixed wastewater reaches, stratified by monsoon and dry-season flow. Site microclimate, water chemistry, nutrients, ammonia, dissolved oxygen, turbidity and land use should be recorded at each sampling. Samples for EE2, E1, E2 and selected pesticides should undergo validated extraction and LC-MS/MS with analyte-specific limits of detection, field blanks, spikes and isotope-labelled internal standards whenever available. Water and sediment measurements should be paired with tissue burdens when ethically justified [16, 20, 35, 39].

Matched fish specimens should include documented sex, total length, age proxy, nutritional state and gonad stage. Measurements should comprise plasma VTG or species-validated transcript assays, E2/testosterone/11-ketotestosterone, blinded gonad histology, sperm motility/fertilization and repeated spawning output where feasible. Tank-level replication is necessary in controlled experiments: multiple fish sharing a tank must not be misrepresented as independent exposure replicates. The OECD fish short-term reproduction assay, sexual-development test and extended-generation protocols provide useful design reference points [38–41].

Statistical caution: within a river reach, fish sampled on the same date are biological subsamples of a shared site-level exposure, not independent replicate watersheds. Repeated water bottles, analytical injections, multiple histological fields per gonad, and several offspring from the same parental tank also do not independently replicate the assigned treatment. Field comparisons should define river reach × sampling occasion (or independently exposed sites) as the appropriate exposure-level unit, model fish nested within site and occasion, and avoid inflated sample sizes and underestimated standard errors [35, 38–41].

The strongest causal test links field-observed chemical mixtures with replicated laboratory exposure using representative locally occurring species and life stages. Negative controls, solvent controls where applicable, quantified low-dose treatments, mixtures, positive estrogen controls and recovery arms are needed. Where VTG and gonad lesions do not accompany reduced fertilization or

egg production, findings must be described as endocrine or tissue signals rather than proven population harm [1, 9, 31, 41].

GONADAL ASSESSMENT AND EVIDENCE STRENGTH

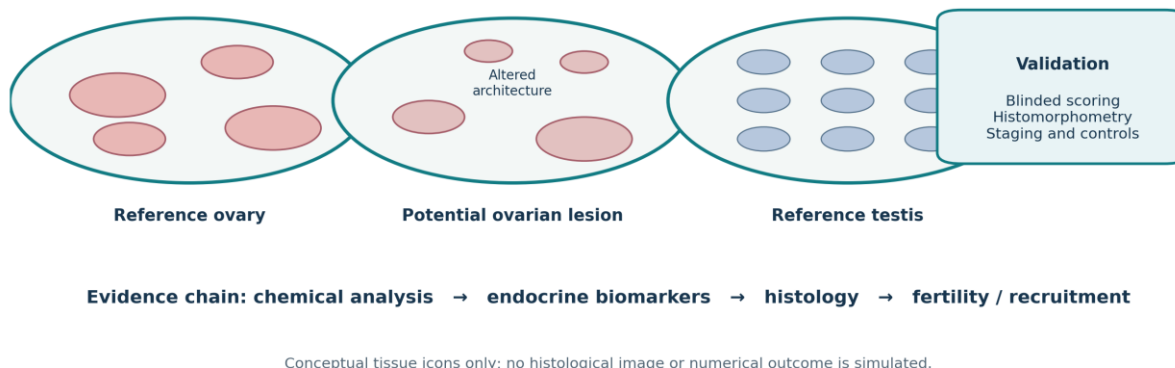


Figure 3. Conceptual representation of reproductive tissue assessment and the hierarchy from contaminant measurement to reproductive endpoints. Illustrations are schematic and do not depict actual histological sections.

Welfare, ecology and policy relevance

Reduced reproductive capacity can affect population trajectories through changes in sex ratio, sperm quality, spawning behaviour, offspring viability and recruitment. Yet a single altered laboratory biomarker is not a field recruitment estimate. Fish assemblages experience predation, flooding, temperature stress and fishing pressure that may mask or amplify contaminant effects. Ecosystem-scale work highlights both direct endocrine harm and indirect food-web consequences [10, 12, 13, 19, 44].

A rural India monitoring programme should integrate agricultural source controls with sanitation and wastewater treatment. Managing farm pesticide residues will not necessarily eliminate steroid hormones entering through domestic sewage; source categories must be matched to analytical evidence. Risk communication should avoid universal fish-estrogen threshold claims derived from one species and one experimental design [8, 16, 19].

Research priorities and evidence gaps

A first priority is geographical breadth: the literature presently does not justify a quantitative national estimate for hormone-driven intersex or reproductive failure among freshwater fishes in rural India. The second is verified chemical specificity: several compelling Indian pollutant studies concern pesticides or nonspecific tissue injury rather than EE2 quantification. Third is endpoint integration: environmental chemistry, sex-specific biomarkers, histology and breeding success need to be measured in matched fish over time. Fourth, effects of complex mixtures and environmental seasonality require controlled replication rather than single-site correlations [14, 15, 16, 17, 19].

A future systematic review can then evaluate publication bias, chemical-specific evidence, measured effect concentrations and taxon-specific sensitivity through preregistered methods. Meta-analysis should only combine studies with sufficiently comparable endpoints, genuine independent units, measured exposures and extractable effect estimates. A prospective evidence map with explicit null findings would be an appropriate intermediate step before any pooled reproductive risk estimate [27, 33, 42].

Integrated statistical analysis and transparent reporting

12.1. Study units, confounding and causal contrasts

In a rural field design, the analytical unit must reflect exposure assignment. Individual fish collected from the same reach during the same period may share a contaminant plume; treating those fish as independent replicates of catchment exposure produces pseudoreplication. A nested model should represent reach, site, season and species explicitly, and predefine whether the main contrast concerns pesticide concentration, estrogenic activity or a combined-mixture index. Fish size and reproductive stage should be handled as biologically justified covariates, while dissolved oxygen, water temperature, nutrients and wastewater proximity should be prespecified potential confounders. Post-exposure variables on a causal pathway must not be casually adjusted away as if they were baseline confounders [13, 19, 33].

A nominal count of individual fish is not the effective sample size for exposure contrasts when many fish originate from the same reach or tank. Inferential models should account for within-reach correlation and repeated measures, and report independent site, tank, or breeding-unit counts separately from fish, section and technical-replicate counts. Inference to all Indian river basins is not warranted from a few correlated locations.

Where sites are revisited, generalized mixed-effects or hierarchical Bayesian models can partition within-site temporal change from between-site difference. The primary outcome for a reproductive hazard question should be a direct endpoint such as fertilization success, eggs per unit time or spermatogenic cell fractions, not only a response biomarker. Secondary molecular pathways should be analysed with multiplicity control. Negative findings are informative when precision, exposure verification and sufficient assay sensitivity are demonstrated [25, 26, 28, 35].

12.2. Exposure-response modelling and mixture effects

Estrogen receptor agonists do not all share the same potency. A toxic-unit or bioanalytical-equivalent approach may be useful only when activity is measured in a validated system and the assumptions for potency scaling hold. Simple addition of raw pesticide masses and steroid masses is not biologically interpretable. The concentration-addition findings for steroidal estrogens provide a specific empirical example rather than proof that all agricultural mixtures behave additively [8].

Time-varying exposure requires care: event-mean concentrations after rainfall can hide intense short peaks, whereas a dry-season grab sample can miss exposure during a critical reproductive interval. Passive sampling, time-integrated composites or repeated targeted collections should be matched to fish life history and the temporal window of the biomarker. Sediment-associated legacy compounds should be analysed separately from dissolved steroid hormones, with detection limits and recovery reported for each matrix [16, 17, 20].

12.3. Reproductive adverse outcome pathways

A useful conceptual adverse-outcome chain begins with ligand-receptor interaction or altered steroid synthesis, progresses through gonadotropin or sex-steroid dysregulation, then through developmental or gametogenic lesions, and finally reaches reduced fertilization, fewer viable offspring or recruitment change. A study measuring only one early key event cannot establish downstream adversity. The whole-lake estrogen investigation comes close to connecting all levels; the many narrower assays remain valuable for mechanistic support but have a different inferential status [10, 11, 18, 19].

This distinction has practical consequences for risk communication. Sex reversal and intersex are related but not synonymous; induction of male VTG does not always accompany gonadal transformation; and a low gonadosomatic index may arise through normal seasonal regression. A coherent mechanistic diagnosis therefore requires convergent indicators and a well-described species-specific reproductive calendar [1, 4, 5, 9, 14].

Context-specific research roadmap and implementation

13.1. Basin prioritization and sentinel species

Basin surveillance should start with documented land use, pesticide practices, canal connectivity and wastewater discharge points rather than assigning all agricultural waterways an assumed estrogen burden. Among candidate sentinel taxa, selection should reflect local abundance, known spawning season, non-lethal sampling possibilities and availability of species-validated VTG or hormone assays. Native catfishes and cyprinids offer ecological relevance, but assay transfer from zebrafish or fathead minnows should be validated for each species and sample matrix [15, 16, 17].

A minimum annual sequence would encompass the pre-monsoon low-flow period, first flush after agricultural application, peak monsoon, and post-monsoon reproductive window, adapted to each basin. At every interval, field controls should include chain of custody, sterile collection handling, measured physicochemical conditions and documented analytical quality assurance. Tissue collection and reproductive testing require appropriate institutional animal-ethics approval and welfare safeguards [38–41].

13.2. Translating evidence into prevention

Interventions should target sources supported by data: responsible crop-chemical handling, buffer vegetation, runoff retention and erosion management for pesticide delivery; sanitation, sewage interception and wastewater treatment for pharmaceutical steroids; and industrial discharge controls for alkylphenols or other relevant estrogenic compounds. None of these should be claimed to have already restored Indian fish reproduction without pre/post intervention monitoring. Repeated biomarker and recruitment measurements could demonstrate benefits with stronger confidence than a single chemical survey [2, 11, 12, 16].

For journals demanding a systematic review, the present structured synthesis supplies the conceptual framework and a vetted starting bibliography, but a separate documented search and screening exercise remains necessary. Databases should be searched

independently, full texts evaluated against fixed criteria, risk of bias appraised and an auditable extraction sheet preserved. Where studies are too heterogeneous to pool, structured tables with effect direction, null findings and limitations offer more defensible conclusions than an artificially precise summary statistic [13, 19, 33, 42].

13.3. A rigorous follow-up programme should use pre-specified contrasts: upstream agricultural sites, drainage junctions receiving domestic wastewater, and downstream mixed-exposure sites. A statistically appropriate hierarchy includes river basin and sampling occasion as repeated spatial-temporal factors; fish from the same site should not be regarded as fully independent replicates for exposure effects. Seasonal water chemistry, migration, age, parasite loads and suspended solids should be considered alternative explanations [16,17,20,42].

Recommended endpoints extend from analytical chemistry (EE2, E2, E1, relevant pesticide classes) and bioanalytical estrogenic activity to male VTG, estradiol/testosterone, cyp19a1 expression, standardized gonadal histopathology, sperm traits and spawning outcomes. The same individual cannot always provide every nonlethal and terminal endpoint, so study designs should specify matched subsamples and ethical approvals before collecting fish. Interpretation should distinguish an estrogenic phenotype from reduced reproductive function, and population inference from an individual-level molecular response [18,35,38–41].

For prospective basin studies, a preregistered sampling hierarchy should identify independently selected reaches, repeated seasonal visits, and matched individual reproductive measurements. Power calculations and confidence intervals should be based on the site- or tank-level contrast rather than treating every fish, histological slice, or analytical replicate as an independent experiment.

Limitations

This synthesis is not a PRISMA-complete systematic review. It has no invented database retrieval totals, screening counts, quantitative meta-analysis or numerical risk estimates. The references represent traceable selected literature rather than a documented exhaustive search census. Many direct reproductive experiments come from other countries and are mechanistic comparators, not observations made in Indian rivers. Atrazine results are not uniformly positive across assays, and such contradictions have been retained rather than harmonized into a false consensus.

A central India-specific limitation is the scarcity of regional field investigations that jointly measure confirmed estrogen or pesticide exposures, standardized gonadal pathology, and direct reproductive output (fertilization, spawning success, viable offspring, or recruitment) within the same fish populations across independent river basins. Evidence of chemical occurrence or nonspecific tissue injury is not a substitute for such integrated reproductive data. Consequently, neither a national reproductive-risk prevalence estimate nor basin-wide EE2-specific causation can be inferred from the presently cited Indian studies [15–17].

14.1. Critical integration. Evidence for endocrine-disrupting effects on fish is robust at the level of controlled experimental exposure, including the unusually powerful whole-lake intervention [10]. It is less complete for exposure-specific reproductive causation in rural Indian river systems, where complex mixtures, hydrological pulses and variable species ecology limit extrapolation [15–17]. This contrast is a substantive scientific finding, not simply an absence of studies. Uncertainty should be represented explicitly in tables, figures and policy recommendations.

The review includes references with publication or issue dates no later than November 2024; an apparent online-first date after this cutoff should be excluded even if the journal assigns a nominal 2024 volume. This is a historical literature coverage rule, not a representation that the manuscript itself was prepared or published in 2024.

Conclusions

Fish endocrine disruption is best evaluated as a mechanistic sequence from verified exposure through receptor and steroidogenic responses to gonadal dysfunction, gamete quality, realized breeding outcomes and population change. International experiments demonstrate that EE2 can affect fish at low-ng/L concentrations and, under sustained exposure, impair entire populations. Indian river and species studies establish pesticide exposure and biologically relevant toxicological mechanisms, but currently cited evidence is insufficient to declare widespread synthetic-estrogen-induced reproductive collapse across rural India. The research priority is a coordinated, seasonally resolved and quality-assured field-to-laboratory programme capable of distinguishing pesticide toxicity, estrogenicity and other confounders while preserving measured reproduction as the central adverse outcome.

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