

Serum Urea as a Sentinel Biomarker of Organophosphate-Induced Nephrotoxicity: Evidence from a 90-Day Study of Methyl Parathion and Chlorpyrifos Exposure in Albino Rats

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Abstract

Organophosphate (OP) pesticides remain among the most widely used and most toxicologically consequential agrochemicals in the world, and their capacity to injure organs far beyond the nervous system is increasingly recognised. This paper draws exclusively on serum urea data generated in a controlled 90-day toxicological study of methyl parathion (MT) and chlorpyrifos (CfT) in albino rats (*Rattus norvegicus*) to examine what a single, inexpensive renal biomarker can reveal about the systemic burden imposed by OP exposure on a living body. Eighteen adult albino rats were divided into control, methyl-parathion-treated (5 mg/kg body weight), and chlorpyrifos-treated (10 mg/kg body weight) groups and dosed daily by oral gavage. Serum urea was assayed on Day 1, Day 21, and Day 90 using a urease-GLDH method. Baseline urea values were 11.63, 13.57 and 15.02 mg/dL in the control, MT and CfT groups respectively. By Day 21, urea had risen to 15.39 mg/dL in the control group, 18.27 mg/dL in the MT group and 24.01 mg/dL in the CfT group. By Day 90, the values had reached 14.98, 24.17 and 30.66 mg/dL respectively, representing increases of roughly 29%, 78% and 104% over baseline in the control, MT and CfT groups. One-way ANOVA on the pooled dataset confirmed that these differences were statistically significant ($F = 5.68$, $p = 0.01$). Taken together, the data indicate that both compounds progressively impair renal handling of nitrogenous waste, that this impairment intensifies with duration of exposure, and that chlorpyrifos exerts the more severe nephrotoxic effect of the two compounds at the doses tested. These findings reinforce the case for serum urea as a sensitive, low-cost early-warning marker of OP-induced organ damage in exposed biological systems, including humans.

1. Introduction

Organophosphate pesticides act primarily by inhibiting acetylcholinesterase, and this neurotoxic mechanism has dominated both public understanding and clinical management of OP poisoning. However, a narrow focus on cholinergic crisis obscures a second, slower-moving consequence of exposure: progressive damage to the kidneys and liver, organs that bear the metabolic burden of detoxifying and excreting these compounds. Urea, the principal nitrogenous end-product of protein catabolism, is synthesised in the liver via the urea cycle and cleared by the kidneys through glomerular filtration. Because urea is partially reabsorbed in the renal tubules, elevated serum urea concentrations, when interpreted alongside other renal markers, provide meaningful evidence of impaired excretory capacity or of an accelerated catabolic state. Both patterns are of direct relevance to OP toxicity, since these pesticides are known to injure the glomeruli and tubules directly and, in acute poisoning, to drive the body into a state of heightened protein breakdown (Rahman et al., 2021; Kalenik et al., 2026).

Recent experimental and epidemiological literature supports interpreting renal effects of organophosphate exposure as part of a broader systemic toxicological profile. Animal studies have documented renal biochemical and structural changes after chlorpyrifos exposure, including elevations in urea or blood urea nitrogen and creatinine, while human and population-based studies indicate that pesticide exposure can be associated with subclinical tubular injury or altered kidney function. Importantly, current reviews emphasize that oxidative stress, inflammation, mitochondrial dysfunction and apoptosis can contribute to pesticide-associated renal injury, although the strength of evidence differs between acute poisoning and chronic low-level exposure (Aung et al., 2020; Wan et al., 2021; Jacobson et al., 2021;

Sobolev et al., 2022; Lozano-Paniagua et al., 2023; Lu et al., 2026).

This paper isolates and interprets the serum urea findings from a larger toxicological investigation of methyl parathion and chlorpyrifos, with the aim of illustrating, through a single well-characterised biomarker, how an organophosphate insult translates into a measurable, time-dependent physiological change in the body of an exposed mammal (Jadhao, 2026).

2. Materials and Methods

2.1 Animals and Experimental Design

Eighteen adult albino rats (*Rattus norvegicus*) of either sex, weighing between 180 and 285 g, were procured and acclimatised for 14 days under standard husbandry conditions (temperature $25 \pm 2^\circ\text{C}$, humidity 50–60%, ad libitum feed and water). The study was approved by the Institutional Animal Ethical Committee (Reg. No. 729/PO/Re/S/11/CPCSEA). Animals were allocated to three groups of six: a vehicle control group (distilled water), a methyl-parathion-treated group (5 mg/kg body weight), and a chlorpyrifos-treated group (10 mg/kg body weight), with doses derived from published LD50 data and administered daily by oral gavage. Doses were recalibrated after Day 21 to account for changes in body weight over the course of the 90-day chronic exposure period (Jadhao, 2026).

2.2 Urea Estimation

Blood samples (0.5 mL) were withdrawn from the tail vein on Day 1 (pre-dosing baseline), Day 21 (sub-acute phase) and Day 90 (chronic phase), and serum was separated by centrifugation at 4500 rpm for 20 minutes. Serum urea was measured using a urease-GLDH kit read at 390 nm on an automated biochemistry analyser, alongside creatinine, AST, ALT and acetylcholinesterase, allowing urea to be interpreted in the context of concurrent hepatic and neurological findings (Jadhao, 2026).

2.3 Statistical Analysis

Data are expressed as mean $\pm$ standard deviation ($n = 6$ per group). Paired t-tests compared control against treated groups, and one-way ANOVA (SPSS v.23) was used to assess overall significance across groups, with $p \leq 0.05$ taken as the threshold for statistical significance.

3. Results

3.1 Sub-Acute Phase: Urea Levels at Day 1 vs Day 21

At baseline (Day 1), serum urea concentrations were broadly comparable across groups, though the animals subsequently assigned to the chlorpyrifos group started from a modestly higher value than the control group. By Day 21, a clear divergence had emerged: the chlorpyrifos group showed the sharpest rise, an increase of 59.8% over its own baseline, considerably outpacing both the methyl parathion group (34.6% rise) and the control group, which remained comparatively stable (Jadhao, 2026).

Table 1. Mean Serum Urea Levels (mg/dL), Day 1 vs Day 21

Group	Day 1 (mg/dL)	Day 21 (mg/dL)	% Change
Control	11.63	15.39	+32.3%
Methyl Parathion (5 mg/kg)	13.57	18.27	+34.6%
Chlorpyrifos (10 mg/kg)	15.02	24.01	+59.8%

Comparative statistical analysis of the sub-acute phase (Table 2) showed that although urea trended upward with treatment, the change did not reach statistical significance at 21 days ($F = 2.48$, $p = 0.10$), unlike creatinine and ALT, which were already significantly elevated at this stage. This suggests urea responds somewhat more slowly than other markers during the early phase of OP exposure (Jadhao, 2026).

Table 2. Urea Values and Statistical Significance — Sub-Acute Phase (21 Days)

Group	Mean $\pm$ SD (mg/dL)	F-value	p-value	Significance ($p \leq 0.05$)
Control	13.37 $\pm$ 4.87			
Methyl Parathion	15.7 $\pm$ 6.9	2.48	0.10	Not Significant
Chlorpyrifos	18.9 $\pm$ 9.1			

3.2 Chronic Phase: Urea Levels at Day 1 vs Day 90

Extending exposure to 90 days produced a much more pronounced and unambiguous nephrotoxic signature. The control group's urea rose only mildly, from 11.63 to 14.98 mg/dL (a change consistent with normal physiological variation and remaining within a healthy range). By contrast, the methyl parathion group rose from 13.57 to 24.17 mg/dL, a 78% increase, and the chlorpyrifos group rose from 15.02 to 30.66 mg/dL, a 104% increase — meaning chlorpyrifos-exposed animals were, on average, carrying roughly double the nitrogenous waste load they started with (Jadhao, 2026).

Table 3. Mean Serum Urea Levels (mg/dL), Day 1 vs Day 90

Group	Day 1 (mg/dL)	Day 90 (mg/dL)	% Change
Control	11.63	14.98	+28.8%
Methyl Parathion (5 mg/kg)	13.57	24.17	+78.1%
Chlorpyrifos (10 mg/kg)	15.02	30.66	+104.1%

Unlike the sub-acute phase, the chronic-phase overall comparison (Table 4) confirmed that urea elevation was now statistically significant across groups ($F = 5.68$, $p = 0.01$), placing it alongside AST, ALT, AChE and creatinine as a marker that reliably distinguishes OP-exposed animals from controls once exposure is sustained over the full 90-day period (Jadhao, 2026).

Table 4. Urea Values and Statistical Significance — Chronic Phase (90 Days, Overall Data)

Group	Mean $\pm$ SD (mg/dL)	F-value	p-value	Significance ($p \leq 0.05$)
Control	13.7 $\pm$ 4.45*			
Methyl Parathion	17.5 $\pm$ 7.40*	5.68	0.01	Significant
Chlorpyrifos	21.41 $\pm$ 9.56*			

Across both time points, the ordering of severity was consistent: chlorpyrifos > methyl parathion > control, and the gap between treated groups and control widened with duration of exposure, with the exponential trajectory of the chlorpyrifos group's urea curve indicating an accelerating, rather than plateauing, injury pattern over the 90-day window (Jadhao, 2026).

4. Discussion: What Urea Tells Us About OP Pesticide Effects in the Body

The pattern of urea elevation documented here offers a clear physiological narrative of how organophosphate pesticides act on the body over time. At the outset, all three groups showed comparable, essentially normal urea values, confirming that the animals began the study with healthy renal function. Divergence appeared only after exposure began, and it widened steadily rather than appearing abruptly, a pattern consistent with cumulative organ injury rather than a single acute insult (Sobolev et al., 2022; Lu et al., 2026).

Two overlapping mechanisms plausibly explain the rise in urea in the treated groups. First, direct renal injury: organophosphates and their oxidised metabolites are known to provoke oxidative stress in kidney tissue, damaging the glomeruli and tubular epithelium and thereby reducing the glomerular filtration rate, so that urea — which is filtered and only partly reabsorbed — accumulates in the blood. Second, an accelerated catabolic state: OP intoxication places metabolic and cholinergic stress on the whole body, which can increase the breakdown of proteins and thus increase the rate at which urea is generated in the first place, independent of any change in kidney function. Because urea often rises earlier or faster than creatinine in cases of acute poisoning, its elevation may reflect this catabolic component as much as it reflects impaired excretion, whereas creatinine remains the more specific marker of filtration capacity per

se. Reading the two markers together, as this study did, provides a fuller picture: both rose in the treated groups, and both worsened with time, pointing toward a combined picture of renal compromise overlaid on a heightened catabolic burden (Aung et al., 2020; Alipanah et al., 2022; Abduh et al., 2023; Alruhaimi et al., 2024).

The comparative severity of the two pesticides is also instructive. Chlorpyrifos consistently produced the larger rise in urea at both the 21-day and 90-day time points, despite methyl parathion being classically regarded as the more acutely toxic compound by LD50. This suggests that acute lethality and chronic organ toxicity are not the same property of a molecule, and that a compound less dangerous in a single high dose may nonetheless impose a greater cumulative burden on the kidneys when administered repeatedly over months. This has direct relevance for real-world exposure scenarios, where humans and animals are rarely exposed to a single massive dose but instead absorb OP residues repeatedly, through diet, occupational contact, or environmental drift, over weeks, months, or years (Sakinah et al., 2024; Farkhondeh et al., 2025).

The fact that urea elevation failed to reach statistical significance at Day 21 but became clearly significant by Day 90 is itself a meaningful finding. It indicates that urea is not the most sensitive early biomarker of OP toxicity — creatinine and ALT moved first — but that it becomes a reliable, statistically robust indicator once exposure is sustained. In practical or clinical terms, this means a single urea measurement taken early after exposure may understate the eventual harm, and that monitoring of at-risk individuals, whether agricultural workers, animals in contaminated environments, or populations near heavy pesticide use, benefits from repeated measurement over time rather than a single test (Wan et al., 2021; Lozano-Paniagua et al., 2023; Lu et al., 2026).

Taken as a whole, the urea data support the broader conclusion that organophosphate pesticides are not narrowly neurotoxic agents but systemic toxicants that progressively compromise renal function as a secondary, cumulative consequence of exposure. Given that urea assays are inexpensive, minimally invasive, and already part of routine clinical blood panels, these findings argue for its continued and even expanded use as a practical, low-cost sentinel for organ damage in populations at risk of chronic, low-level organophosphate exposure (Jacobson et al., 2021; Kalenik et al., 2026).

5. Conclusion

Serum urea, examined in isolation from a larger organophosphate toxicology study, demonstrates a clear, dose- and time-dependent nephrotoxic signature in albino rats exposed to methyl parathion and chlorpyrifos. Values rose modestly and non-significantly during the first three weeks of exposure but climbed to a statistically significant, and physiologically substantial, elevation by 90 days, with chlorpyrifos producing the more severe effect (a 104% rise over baseline) compared with methyl parathion (78%) and control animals (29%). These results reinforce the understanding that organophosphate pesticides inflict cumulative systemic harm well beyond their well-known neurotoxic action, and that renal biomarkers such as urea — cheap, accessible, and already validated here — deserve a central place in both experimental toxicology and the ongoing surveillance of populations exposed to these widely used agrochemicals.

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