Abstract This research study delved into the synthesis, physicochemical properties, and toxicological effects of synthetic diazo dyes, specifically focusing on two compounds, ZA-31 and ZA-32, based on 4-OH coumarin. These dyes are widely used in various industries, including textiles, food production, and medicine, for their coloring properties, but concerns regarding their potential health impacts have arisen. The synthesis involved the preparation of these diazo dyes from mono-azo dyes based on J-acid, yielding compounds with distinct characteristics. The solubility and absorbance of these dyes were examined in different solvents namely (H2O, DMSO, DMF, EtOH, CH3Cl, EtOAc), revealing minimal influence on their absorbance profiles based on solvent choice. Dyes ZA-15 and ZA-16 were subjected to various tests, including melting point determination, percentage yield calculation and spectroscopic techniques (UV-VIS, and FTIR), which collectively verified the formation of these compounds. The high melting points observed for these compounds indicated their ionic nature, and it was noteworthy that the melting points increased with the structural complexity of the molecules. Furthermore, the higher λmax values observed in UV spectroscopy indicated the brighter coloration of these dyes. The FT-IR spectra exhibited distinct peaks corresponding to specific functional groups, including O-H, -N=N-, C=C, S=O, and N-H. These findings provided additional confirmation of the presence of these functional groups within the synthesized compounds. Toxicity assessments were carried out, including acute and chronic studies in albino mice, along with histopathological examinations of vital organs (kidney, liver, heart &brain). Acute toxicity tests indicated LD50 values value of 3.1 g/Kg/po for the diazo dye ZA-31 and 4 g/Kg/po for ZA-32 and observed behavioral abnormalities in mice exposed to these dyes. Chronic toxicity studies revealed significantly decrease in the levels of hematological parameters (platelet count, hematocrit percentage, and hemoglobin concentration) in albino mice, leading to substantial alterations in their blood composition due to dye ZA-31 compared to ZA-32. These findings indicate that the p-Cl substituted group produces more toxicity than the p-Br substituted dye in albino mice. Additionally, synthetic diazo dyes ZA-31 and ZA-32 both exhibited notable increase on the serological parameters (glucose, cholesterol, creatinine, ALP, SGOT, SGPT, and

TB) of the mice, indicating potential repercussions on their immune systems because of the presence of functional groups like p-Cl, p-Br, O-H, -N=N-, S=O, and N-H. Histopathological analyses identified effects on the kidney, liver, and heart tissues, particularly attributed to ZA-31, with notable differences between the two dyes in their toxicological profiles. The findings of this research highlight the higher toxicological risk posed by ZA-31 compared to ZA-32, emphasizing the need for caution when using these dyes at higher doses in various industries. Further investigations are warranted to explore genotoxicity and fetotoxicity aspects related to these synthetic diazo dyes.