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Insights into toxicity of polychlorinated naphthalenes to multiple human endocrine receptors: Mechanism and health risk analysis

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ABSTRACT

This study explored the combined disruption mechanism of polychlorinated naphthalenes (PCNs) on the three key receptors (estrogen receptor, thyroid receptor, and adrenoceptor) of the human endocrine system. The intensity of PCN endocrine disruption on these receptors was first determined using a molecular docking method. A comprehensive index of PCN endocrine disruption to human was quantified by analytic hierarchy process and fuzzy analysis. The mode of action between PCNs and the receptors was further identified to screen the molecular characteristics influencing PCN endocrine disruption through molecular docking and fractional factorial design. Quantitative structure–activity relationship (QSAR) models were established to investigate the toxic mechanism due to PCN endocrine disruption. The results showed that the lowest occupied orbital energy (E_{LUMO}) was the most important factor contributing to the toxicity of PCNs on the endocrine receptors, followed by the orbital energy difference (ΔE) and positive Millikan charge (q^+). Furthermore, the strategies were formulated through adjusting the nutritious diet to reduce health risk for the workers in PCN contaminated sites and the effectiveness and feasibility were assessed by molecular dynamic simulation. The simulation results indicated that the human health risk caused by PCN endocrine disruption could be effectively decreased by nutritional supplementation. The binding ability between PCNs and endocrine receptors significantly declined (up to −16.45%) with the supplementation of vitamins (A, B_2, B_{12}, C, and E) and carotene. This study provided the new insights to reveal the toxic mechanism of PCNs on human endocrine systems and the recommendations on nutritional supplements for health risk reduction. The methodology and findings could serve as valuable references for screening of potential endocrine disruptors and developing appropriate strategies for PCN or other persistent organic pollution control and health risk management.

1. Introduction

Polychlorinated naphthalenes (PCNs) were widely used in various industry applications as high-temperature boiling solvents, impregnation materials for waterproofness, flame retardants, insecticides, etc. (Falandysz, 1998; Ayala-Cabrera et al., 2021). PCNs have been identified as a persistent organic pollutant due to their toxicity, bioaccumulative ability, long-distance mobility and environmentally persistency (Fernandes et al., 2010; Stockholm Convention on Persistent Organic Pollutants, 2013). It is reported that PCNs can accumulate in human bodies and cause health issues, such as embryotoxicity, hepatotoxicity, immunotoxicity, skin damage, teratogenesis and carcinogenesis (Blankenship et al., 2000; Villeneuve et al., 2000; Li et al., 2020). Although PCNs have been banned in commercial production by 1977 (Wang et al., 2012), they are still ubiquitous in the global environment and have been detected in the diverse media and organisms as a result of thermal and other industrial processes in the presence of chlorine (Falandysz and Fernandes, 2020) and by-products in commercial polychlorinated biphenyl (PCB) formulation (Falandysz, 2007; Huang et al., 2015). Thus, PCNs still pose a great risk to human health and ecosystems

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worldwide calling for better understanding and more research efforts on their toxicological effects and risk reduction strategies (Niu et al., 2021).

However, the toxic mechanism of PCNs to humans has not been thoroughly investigated. The previous efforts have mainly concluded that the toxic mechanism of PCNs were caused by the aryl hydrocarbon hydroxylase and ethoxyresorufin-O-deethylase effects (Brinkman and Reymer, 1976; Kim et al., 2018). Studies have disclosed that PCNs may have other toxic mechanisms in addition to their dependence on aromatic receptors. Studies on porcine ovaries have shown that PCN destroyed the production of steroid by affecting its antiestrogen and androgen activities (Barć and Gregoraszczuk, 2014; Rak et al., 2017; Stragierowicz et al., 2021). PCNs have also been confirmed with embryotoxicity, fetal toxicity and even teratogenicity on pregnant female rats (Kilanowicz et al., 2011; Kilanowicz et al., 2015; Rak et al., 2017). Besides, PCNs can be detected in human liver, adipose tissue (Kunisue et al., 2009), serum (Wang et al., 2022), breast milk (Li et al., 2020) and umbilical cord blood (McCoy et al., 2020), and its toxicity reached 25% of the total toxic equivalent value in some cases (such as in serum and umbilical cord blood) (Stragierowicz et al., 2018). Nevertheless, the toxic mechanism of PCNs to human is not well studied, but it should not be ruled out that it may be caused by endocrine disruption (Stragierowicz et al., 2018). Limited studies are available on exploring the endocrine disrupting mechanism of PCNs on human due to the difficulty of in vivo experiments. In addition, existing mechanism studies on endocrine disruptors interfering with endocrine signaling pathway commonly focus on single endocrine receptor, such as hypothalamic pituitary regulation of thyroid, gonad, adrenal gland and other endocrine glands (Boas et al., 2006; Miller et al., 2009; Bushnell et al., 2010; Li et al., 2018; Zhu et al., 2020; Gifford et al., 2021). Few studies have considered multiple endocrine receptors. Identification of the mode of action (MoA) between PCNs and multiple endocrine receptors can comprehensively reveal the toxic pattern of PCNs to humans, providing better understanding and important guidance for reducing the associated human health risk caused by PCNs. Thus, such comprehensive research efforts on the mechanism of PCNs toxicity to humans are necessary with growing demand.

Quantitative structure–activity relationship (QSAR) prediction models have been employed to investigate endocrine disruption of compounds according to the MoA between compounds and endocrine receptors (Peric et al., 2015). The QSAR models established based on the MoA have been approved as an important and effective approach to correlate the molecular structures of chemicals with their reactivity. Li et al. (2010) have explored the MoA between 20 anthraquinone compounds and estrogen receptor using molecular docking, and constructed a QSAR model of estrogen disruption based on the MoA, providing a guidance for virtual screening of anthraquinones as potential endocrine disruptors. However, the integration of MoA and QSAR models in most studies has only been applied to screen potential endocrine disruptors, and there lacks research on the quantification of factors interfering endocrine disruption and toxicity. Moreover, to reduce the toxic impacts of PCNs on endocrine receptors and the associated human health risk, molecular dynamics (MD) simulation assisted by molecular docking have been used to realize the toxicity regulation by changing external stimulation conditions (e.g., nutrients) based on its combined disrupting mechanism (Gu et al., 2021). MD simulation is a theoretical method to study the interaction between the receptor and ligand (Li et al., 2021). Few studies have been conducted to determine the change of binding patterns through changing the simulated environment of ligand-receptor. There is still a general lack of mechanistic insights on how the prioritized electronic parameters, together with the MD simulation data, could cooperate to result in the PCN endocrine disrupting toxicity endpoints. Moreover, there is no report on the use of MD method to simulate the changes of ligand-receptor interaction mode based on an in-depth molecular orbital theory.

A nutritious diet is an effective method to control human health risks. Studies have proved the appropriate diet composition may reduce the human health risk of exposure to chemicals. Joseph et al. (2015) indicated that the uptake of arsenic in the gastrointestinal tract can be influenced by diet composition. Li et al. (2022b) recommended supplementary diet plans to reduce the abortion risk caused by synthetic musks. Diet regulation schemes of neonic derivatives were simulated through molecular dynamic simulation method by Zhao et al. (2021). They found that the carcinogenic and mutagenic risk of neonic derivatives can be inhibited by the consumption of wolfberry, spinach, tea, walnut, apple, and corn. Thus, it is necessary to find a proper nutrition scheme to reduce the human health risks caused by PCNs endocrine disruption. Besides, the health risk control effects of various delivery routes and doses of nutrients have barely been tackled before.

Therefore, this study aims to explore the combined disrupting mechanism of PCNs on key receptors of the human endocrine system through QSAR modeling and examine the risk reduction strategies by adjusting the nutritious diet for the people exposed to PCN contamination. In this study, we selected endocrine receptors based on the two main types of hormones (i.e., amino acid-based and steroid-based hormones) (Hutchinson et al., 2017). The representative endocrine receptors of amino acid-based (i.e., thyroid receptor and adrenoceptor) and steroid-based hormones (i.e., estrogen receptor) were thus selected as the signal receptors of PCN endocrine disruption. The binding ability between PCNs and these endocrine receptors was investigated through MD simulation. The individual and combined endocrine disruption intensities between PCNs and these receptors were determined. The parameters affecting PCN endocrine disruption were screened based on the information of its chlorine substituents and MoA with the receptors. Four QSAR models were constructed to quantify the main factors and their toxicity effects and reveal the mechanism of PCNs as endocrine disruptors. Seventy-five PCN homologues were prioritized from the perspective of endocrine disruption. The recommended nutrition scheme for reducing health risk for the workers in PCN-contaminated sites was established based on the mechanism analysis. This study will be able to reveal the toxic mechanism of PCNs on the human endocrine system and develop appropriate strategies for PCN related health risk management.

2. Materials and method

2.1. Molecular structures of PCNs and source of proteins

Seventy-five PCN homologues were selected as target molecules, and their structures were collected from the PubChem molecular library of National Institutes of Health (NIH). Estrogen receptor (PDB ID: 2JJ3), thyroid receptor (PDB ID: 1N46) and adrenoceptor (PDB ID: 6IN0) were considered as target proteins in humans. The protein structure of 2JJ3, 1N46 and 6IN0 was derived from Protein Data Bank.

2.2. Characterization of binding ability between PCNs and endocrine hormone receptors

LibDock scores obtained using the molecular docking method can be used to represent the human health risk of PCNs. Docking score is a simple and fast chemical flag-based statistical scoring and has been widely used in previous studies to determine binding affinity (Zsoldos et al., 2007; Rao et al., 2007; Singh et al., 2016; Padhi et al., 2021). A higher LibDock score means a stronger binding ability (Ren et al., 2019; Gu et al., 2020a; Gu et al., 2020b; Wang et al., 2021). The reliability of the LibDock score has been verified using experimental data in various studies. Fang et al. (2021) found that a tetrapeptide ligand (i.e., YEHF) had a high LibDock Score and the highest absolute values of van der Waals interaction energy and interaction energy, indicating that YEHF was the optimal screened resin for antibody purification. They also conducted the static adsorption experiments and the results showed that YEHF resin had higher Human immunoglobulin G (hIgG) binding ability and lower bovine albumin binding ability, which could fit the results

obtained from LibDock and MD simulation. Xu et al. (2021) found the chlorogenic acid derived from seaweeds was an inhibitor of histone acetyltransferase by using the molecular docking method. This finding was further proved by animal experiments. Besides, an in vitro cell experiment showed that tangeretin might regulate the cell cycle and apoptosis by regulating core targets, thus inhibiting the growth of cancer cells. This finding further validates the previous molecular docking results that citrus peel can treat oral squamous cell carcinoma through vital active ingredients and critical target genes (Yu et al., 2022). Lv et al. (2021) screened the novel inhibitors of penicillin-binding protein 2a (PBP2a) from a commercial database (containing 197,258 compounds) by using LibDock scores of the compounds-PBP2a. The biological activity tests of screened inhibitors were further conducted. The experimental results reflected good antibacterial properties of the screened inhibitors, which verified the results of LibDock scores. Thus, in this study, the LibDock score of 75 PCNs and three target proteins (2JJ3, 1N46, and 6IN0) were used to reflect the endocrine disruption of PCNs to the selected hormone receptors.

The reliability of LibDock scores obtained by molecular docking was also validated through their comparison with associated experimental data on rats. The DR-CALUX-REP $\log EC_{50}$ (concentration effective in producing 50% of the maximal response) value of PCNs relative to the 2, 3, 7, 8 - TCDD was selected as its toxicity parameter (Behnisch et al., 2003). The toxicity of PCNs to each endocrine hormone receptor was determined through the linear fitting relationship between the $\log EC_{50}$ and LibDock score. Thus, the LibDock scores were used to represent the endocrine disruption of PCNs and were further used as the inputs for constructing the QSAR model.

The molecular docking between 75 PCNs and three target proteins (2JJ3, 1N46 and 6IN0) was conducted by the Discovery Studio® 2021 software. In this process, the selected protein as the receptor molecule was defined by the "LibDock module". The possible binding sites, and modify and define the binding sites can be found through the "Find Sites from Receptor Cavities" under the "Define module". And then the ligand molecules were incorporated into the resulting protein binding cavity for rapid docking with receptor proteins. Moreover, "Docking Preferences" was selected "User Specified", the "Max Hit to Save" was set to 10, the other were the default settings. In the form of LibDock score, the change of the binding ability is analyzed. The higher the LibDock score is, the stronger the bonding strength is obtained. (Gu et al., 2020a; Gu et al., 2020b).

2.3. Characterization of endocrine disruption of PCNs

Analytic hierarchy process (AHP) is a multi-objective decision-making analysis combining quantitative analysis with qualitative analysis. To accurately judge the comprehensive interference effect of PCNs on human endocrine receptors, the AHP method was used to set the weights of the effect between PCNs and different hormone receptors based on the linear fitting results of $\log EC_{50}$ and LibDock score (Saaty, 1987; Jing et al., 2013; Gu et al., 2021). Firstly, a hierarchical model was constructed (Kułakowski, 2020). The comprehensive interference effect of PCNs on human endocrine hormones were set to the target layer, and the binding ability between PCNs and target proteins (2JJ3, 1N46 and 6IN0) was set to the index layer. The paired comparison method and the 1–9 comparison scale were then used to construct the paired comparison array of hormone receptors (Table S1) (Gu et al., 2021). After that, the weight can be calculated through the n-order pairwise comparison matrix (Eq. (1)), and a consistency test was conducted. The satisfactory consistency was acceptable, when the random consistency index (Eq. (4)) is smaller than 0.1.

n-order pairwise comparison matrix:

$$A = \begin{bmatrix} a_{11} & a_{12} & \cdots & a_{1n} \\ a_{21} & a_{22} & \cdots & a_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ a_{n1} & a_{n2} & \cdots & a_{nm} \end{bmatrix} \tag{1}$$

consistency test:

$$\lambda = \frac{1}{n}\sum_{i=1}^{n}\frac{AW}{W} \tag{2}$$

$$CI = \frac{\lambda - n}{n-1} \tag{3}$$

$$CR = \frac{CI}{RI} \tag{4}$$

where A, W, n and λ represent the judgment matrix, weight, nth order matrix and the largest eigenvalue, respectively; CI, RI and CR are the indexes of deviation consistency, average consistency and random consistency, respectively.

Based on the degree of action of PCNs to estrogen (2JJ3), thyroid (1N46) and adrenoceptor (6IN0) determined by the analytic hierarchy process method, the comprehensive interference response index of PCNs to above proteins was calculated by fuzzy comprehensive evaluation method (Ren et al., 2019). The fuzzy comprehensive evaluation method can transform qualitative evaluation into quantitative evaluation based on the membership degree theory of fuzzy mathematics, that is to use fuzzy mathematics to make a general evaluation of things or objects restricted by many factors. In this study, the integration of AHP and fuzzy comprehensive evaluation methods were first applied to obtain the comprehensive interference response index of PCN with three endocrine hormone receptors (i.e., 2JJ3, 1N46 and 6IN0). The calculated index can be used to reflect the binding capacity of all three endocrine hormone receptors at the same time. To obtain the comprehensive interference response index, the single factor r_i (the intensity of the interaction between PCNs and endocrine receptors) is firstly evaluated by single factor, and the evaluation set of single factor r_i (Eq. (5)) is obtained. Thus, the evaluation set of single factor constitutes a total fuzzy evaluation matrix (Eq. (6)). Then, the comprehensive evaluation method is established based on the weight vector (Eq. (7)) obtained by the analytic hierarchy process method: B = W * R. The calculation steps of fuzzy comprehensive evaluation are as followed (Zhang and Feng, 2018):

$$R_i = (r_{i1}, r_{i2}, \cdots, r_{im}) \tag{5}$$

$$R = (r_{ij}) = \begin{bmatrix} r_{11} & r_{12} & \cdots & r_{1j} \\ r_{21} & r_{22} & \cdots & r_{2j} \\ \vdots & \vdots & \ddots & \vdots \\ r_{i1} & r_{i2} & \cdots & r_{ij} \end{bmatrix} \tag{6}$$

$$W = \{a_1, a_2, \cdots, a_n\} \tag{7}$$

$$r_{ij} = \frac{c_{ji} - min\{c_{ji}\}}{\max\limits_{j}\{c_{ji}\} - \min\limits_{j}\{c_{ji}\}} \tag{8}$$

where r_{ij} indicates the priority of the j-th molecule with respect to the i-th side effect; c_{ij} represents any value of the evaluation parameters in the comprehensive evaluation system.

2.4. Analysis methods of endocrine disrupting mechanism of PCNs

2.4.1. Determination of factors influencing PCN endocrine disruption

The position of PCNs' chlorine substituent (Fig. S1) was selected as the factor of factorial experiment design, and the amount of chlorine

substituents in the substituent sites was the experimental level. According to the characteristics of PCNs' structure, the design of experiment module was used to establish the factorial experiment design (V) with 8 factors (position-1: A; position-2: B; position-3: C; position-4: D; position-5: E; position-6: F; position-7: G; position-8: H) and two levels (0, 1) through Minitab®. The molecular structure of PCNs is shown in Fig. S1. All factors in the design scheme were controllable. In other words, they were all fixed factors, so the fixed effect model in Minitab® was used to analyze the variance and effect of the main and the second-order interaction effects (sig. = 0.05). In addition, the LibDock scores were substituted and matched in different positions, and finally 64 substitution types were obtained, each substitution type corresponding to only one PCN homolog. This method can accurately analyze the main and the second-order interaction effects of the chlorine substituents in each position, and further determine the law of between PCNs substituent characteristics and single and combined endocrine disruption in QSAR model.

2.4.2. Screening of key factors influencing PCN endocrine disruption

According to the MoA between PCNs and endocrine hormone receptors (2JJ3, 1N46, 6IN0) obtained by molecular docking (Section 2.2) and chlorine substituent analysis (Section 2.4.1), the appropriate descriptors were selected to construct the QSAR model of PCNs' endocrine disrupting-mechanism. Before the construction of QSAR model, the principal component analysis (PCA) method (by SPSS 22.0®) was applied to analyze the relationship between the molecular docking scores and the PCNs descriptors to get the principal components, which helps to explain the importance of principal factors' physical–chemical properties (Gu et al., 2021). The Gaussian 09 software was used to optimize the structure of PCN molecules at the level of B3LYP/6-31G* base group through density functional theory (DFT) (Qu et al., 2017; Qu et al., 2018; He et al., 2020). Then, the descriptors (quantization parameters) of PCNs were obtained, specifically: dipole moment (μ, Debye), quadrupole moment (Q_{xx}, Q_{yy}, Q_{zz}, Q_{xy}, Q_{yz}, Q_{xz}), highest occupied orbital energy (E_{HOMO}, eV), lowest occupied orbital energy (E_{LUMO}, eV), $E_{LUMO\text{-}EHOMO}$ (ΔE, eV), energy gap (eV) molecular energy (total energy, eV), the most negatively charged charge number (q^-, e), the most positively Millikan charge number (q^+, e), the most positive dense root hydrogen atom charge number (qH^+, e).

2.4.3. Modeling of PCNs endocrine disrupting mechanism

The QSAR models of single and combined endocrine disruption of PCNs using SPSS 22.0® based on the multiple linear regression and partial least squares method. To construct the models, LibDock scores and the CI values between endocrine hormone receptors (i.e., 2JJ3, 1N46 and 6IN0) and PCNs were used as the dependent variables. The independent variables are the main influencing factors of PCN-based endocrine disruption selected by the principal component analysis.

In addition, the application of the QSAR model should be clear. The prediction of compounds by using the model within the application domain is considered reliable. This study used the standardized residuals δ and leverage values h_i to draw the Williams diagram (Yang et al., 2020). The Williams diagram showed an intuitive application scope of reaction model, provide the judgement of the QSAR model's reliability and robustness, and determining the outliers and influential compounds. Equations of the standardized residuals δ and leverage values h_i are as follow (Gramatica et al., 2007):

$$\widehat{\mathrm{I}}' = \frac{Y_{exp} - Y_{pre}}{\sqrt{\widehat{a}\left(Y_{exp} - Y_{pre}\right)^2 \Big/ (n - p - 1)}} \tag{9}$$

$$h_i = X_i\left(X^T X\right)^{-1} X_i^T \tag{10}$$

where Y_{exp}, Y_{pre}, and n are the experimental value, predicted value, the number of the compounds; p is the number of model descriptors; X_i is the descriptor matrix of the i^{th} compound; X is the matrix of all compound descriptors; X^T is the transpose matrix of X; and X_i^T is the transpose matrix of X_i.

The warning lever h* is the warning leverage value. When h_i is less than h^*, are considered as structural normal values. The vertical boundaries are $0 < hi < h^*$ (considered as the normal value), in which h is the warning leverage threshold calculated as below:

$$h^* = 3(p+1)/n \tag{11}$$

where n is the number of molecules and p means the number of descriptors.

2.5. Determination of regulation scheme to reduce PCN endocrine disruption in PCN-contaminated areas

2.5.1. Protein-protein docking

The protein–protein docking of endocrine receptor (1N46, 2JJ3 and 6IN0) was conducted using the ZDock method in Discovery Studio® 2021. Firstly, the 1N46 and 2JJ3 proteins were defined as a receptor molecule and ligand molecule, respectively in the software. Then, parameters of "RMSD Cutoff", "Interface Cutoff" and "ZRank" were set to a cluster radius of 6.0 Å, 9.0 Å, and false, respectively. Finally, the combined complex was named as "ZDock.pdb". The above method was repeated to finish the docking between ZDock.pdb and 6IN0 protein. The ZDock.pdb was docked to CN-71 (with the strongest endocrine disruption) through the molecular docking method (Section 2.2).

2.5.2. Reduction of endocrine disruption by nutritious schemes assisted with Taguchi experiment design and MD simulation

Studies showed that workers in the environment of metal smelting and benzene compounds should eat more foods with high protein, low fat and rich vitamins (Pu, 2010). Referenced experiment results have also proved that vitamins can prohibit the adverse impacts of PCNs on rats (Shaw and Chen, 2009). On the other hand, Choi et al. (2018) reported that Vitamins C and E could inhibit the endocrine disruption of rats by di(2-ethylhexyl) phthalate. Thus, a proper nutrition scheme could be beneficial for lowing the endocrine disruption of PCNs to exposure workers. To prove such a hypothesis, multiple nutrition schemes were selected in this study to investigate whether these supplements can reduce the endocrine disruption of PCNs through MD simulation.

MD simulation method can accurately simulate real-world scenarios by using the proper choice of periodic boundary conditions (Bader, 2012). The binding energy obtained through MD simulation reflects the strength of the interaction between proteins and molecules, and the complex with a higher absolute value of binding energy will have a stronger binding ability (Takamatsu et al., 2006; Yao et al., 2013; Guo et al., 2020; Qian et al. 2020). Thus, the appropriate nutrients for reducing PCN endocrine disruption from three different types of typical foods (i.e., high protein: low-fat milk and eggs; vitamins: apple and kiwifruit; anticancer: tea, Lycium barbarum, ginseng) were screened by using the integration of Taguchi experimental design and MD simulation method.

Firstly, the complex between CN-71 molecule and the protein–protein complex (1N46, 2JJ3 and 6IN0) were simulated by MD simulation (Section 2.5.3) under the condition of adding the main components of above food (low-fat milk, apple, Lycium barbarum and ginseng). And then the foods that significantly reduced the binding ability between PCNs and endocrine receptors were determined. Moreover, Taguchi experimental design, a statistical method to optimize the experimental runs (Li et al., 2021) was used in this study to design the experimental runs efficiently. The experiment with four factors and two levels was designed to determine the main components' combination scheme in the selected food through the Minitab®. After the design, an orthogonal table will be generated with the characteristics of the equal

combination. The foods that significantly reduced the binding ability were used as variables, adding/ not adding variables was used as the experimental level, and eight matching schemes were finally obtained.

2.5.3. Mitigation strategies to reduce the binding ability between PCNs and endocrine receptors

MD simulation was carried on using the Gromacs® in the Dell PowerEdge R7425 server. One control group without control stimulus conditions and experimental group with control stimulus conditions (the main components of low-fat milk, egg, apple, kiwifruit, tea, Lycium barbarum and ginseng) were established to simulate binding between CN-71 and protein–protein complex. The binding ability between the complexes under designed scenarios was then simulated. After that, the MD simulation process was repeated according to the food matching scheme determined by Taguchi experiment design to determine the optimal combination scheme that significantly reduced the binding ability between PCNs and endocrine receptors. Finally, the best recommended scheme was determined which was conducive to prevent the health risk of workers in PCN-contaminated areas, so as to provide guidance for reducing its health hazards to human. The binding energy of the control and experimental groups was calculated through the molecular mechanics/Poisson-Boltzmann surface area method (Li et al., 2022b).

3. Results and discussion

3.1. Screening and analysis of influencing factors of PCN endocrine disruption

3.1.1. Determination of the MoA of PCNs on endocrine disruption and the associated influencing factors

In order to analyze the MoA of PCNs on endocrine disruption, the molecular docking was performed to obtain the ability between PCNs and endocrine receptors (2JJ3, 1N46 and 6IN0), respectively. The RMSD value of molecular docking was 0.757–0.980 Å (<2Å), satisfying the modelling requirements. Due to its strongest comprehensive endocrine disrupting activity, CN-71 molecule was taken as an example. The molecular mechanism of CN-71 acting on endocrine hormone receptors (2JJ3, 1N46 and 6IN0) and the associated influencing factors were determined by analyzing the orientation of key groups in ligand binding cavity, non-bond force and molecular crystal structure.

The results of non-bonded interaction between CN-71 and endocrine receptors (2JJ3, 1N46 and 6IN0) (Figs. 1 and S2) illustrated that the hydrogen bonding (H.B.), electrostatic or polar interaction (EPI), van der Waals force (VDWF) affect the binding ability between PCNs molecules and endocrine receptors. The binding of CN-71 with 1N46 and 6IN0 was also affected by the π-π interaction. The MoA between CN-71 ligand and 2JJ3 receptor showed that the formation rates of amino acids in H.B., EPIs, VDWF and hydrophobic interaction were 45.45%, 27.27% and 18.18%, respectively. For the docked complex of CN-71 and 1N46, the formation rates of hydrogen bond, EPI, VDWF, π-π interaction and hydrophobic interaction were 63.63%, 27.27%, 9.09% and 9.09%, respectively. Similarly, the formation rates of amino acids in the docking process of CN-71 and 6IN0 receptor in hydrogen bond, EPI, VDWF interaction, π-π interaction and hydrophobic interaction were 55.56%, 11.11%, 11.11% and 33.33%, respectively. To sum up, H.B., EPIs were the main factors affecting the binding between CN-71 and human endocrine receptors, while the ability of the π-π interaction was weak and negligible. The binding results showed that, the H.B., EPIs were mainly affected by the ligand's electrical parameters (e.g., E_{HOMO}, E_{LUMO}, ΔE, energy gap, q^-, q^+, qH^+, and total energy). The VDWF was essentially relevant to the ligands (e.g., μ, Q_{xx}, Q_{yy}, Q_{zz}, Q_{xy}, Q_{yz}, and Q_{xz}) (Yang et al., 2017). The hydrophobic interaction between the ligand and the receptor was commonly characterized by the n-octanol partition coefficient ($logK_{ow}$), molecular weight (M_W) and halogen atom number (N_{Cl}). Therefore, 18 theoretical molecular structure descriptors were selected to characterize the interaction to explore the endocrine disruption of PCNs.

According to Fig. 1, the main amino acid residues that play a role in the docking process between CN-71 and 2JJ3 are LEU455, SER452 and VAL483. All the main amino acid residues, which belong to non-polar amino acids, will produce hydrophobic interactions. From Fig. 1, it can be seen that the connecting line of the interaction between amino acid and ligand molecule is purple. According to the instruction of Discovery Studio®, purple line and red line represents favorable and unfavorable interactions, respectively. Thus, it is further speculated that the CN-71 molecule is an agonist. Similarly, the amino acid residues that play a role in the docking process between CN-71 and 1N46 include ALA436, TYR409, PRO384, LYS411, and ARG410. Among them, ALA436, TYR409 and PRO384 belong to non-polar amino acid residues, resulting in hydrophobic interactions. The connecting lines are purple; thus, it is speculated that CN-71 is an agonist when it interacts with the above three residues. However, LYS411 and ARG410 are polar amino acid residues, resulting in hydrophilic interactions. The connecting lines are red; thus, it is speculated that CN-71 is an antagonist when it interacts with the above two residues. The amino acid residues that play a role in the docking between CN-71 and 6IN0 are mainly LYS653 and ASP764. Both LYS653 and ASP764 are polar amino acid residues with red connection lines, resulting in hydrophilic interactions. Therefore, CN-71 is an antagonist when it interacts with the above two residues. In summary, we found that hydrogen bonding, electrostatic and polar interactions are the main forces affecting the interaction between ligands and receptors through the formation rate of amino acids. The hydrophobic interactions between ligands and receptors cannot be ignored by the residue analysis.

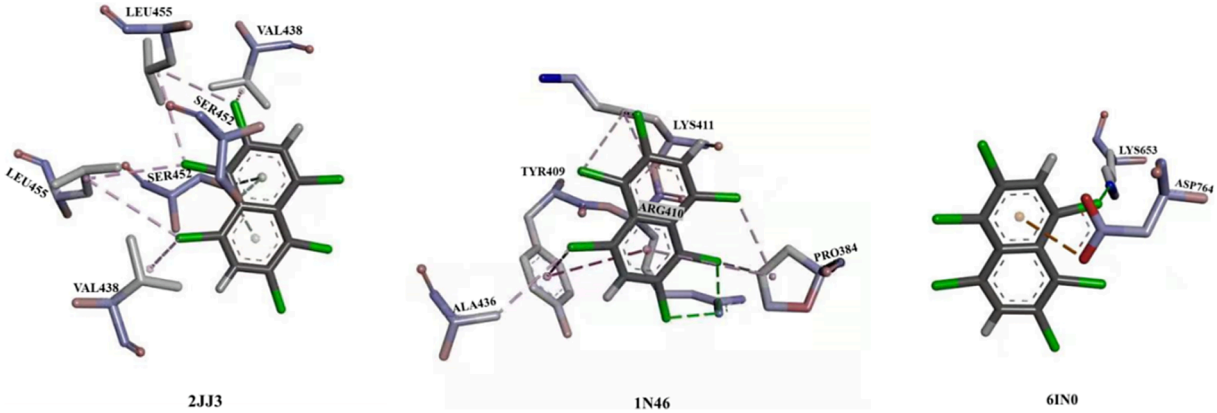

Fig. 1. The amino acids in the binding of CN-71 to estrogen receptor (2JJ3), thyroxine receptor (1N46) and adrenoceptor (6IN0).

3.1.2. Analysis of substituent factors affecting endocrine disruption of PCNs

Fig. 2 and Fig. S3 showed the fitness test of the data of binding ability between PCNs and endocrine receptors (2JJ3, 1N46 and 6IN0) through the fixed effect model. The graph of normal probability and histogram of the residual did not have abnormal points deviating from the normal distribution, indicating that the scheme met the requirements of normal hypothesis. There was no obvious pattern between residual and fitting value, indicating the model satisfied the homogeneity hypothesis of variance. There were also no positive or negative residuals according to the time series in the residual graph, illustrating that there was no pattern violating independence. Thus, the fixed effect model can be applied to analyze the main and second-order interaction effects (Jiang et al., 2017). Table S2 displayed the variance analysis of the main and second-order interaction effects of single and combined binding ability between PCNs and the above endocrine receptors (2JJ3, 1N46 and 6IN0). Since the main effect and second-order interaction effect of above receptors are valued at 0.001, 0.001, 0.009, 0.001 and 0.026, 0.026, 0.045, 0.027 (<0.05), respectively, both effects have a significant impact on the binding ability between PCNs and endocrine receptors. The main effect has a more significant influence.

Fig. 3 shows the values of the main and second-order interaction effects, which met the statistical significance requirements. The estimated effect values of the main effect of PCNs' chlorine substituents are significantly and positively related to their single and combined binding ability with estrogen receptor (2JJ3), thyroid receptor (1N46) and adrenoceptor (6IN0). The main effects of chlorine substituents on the binding ability between PCNs and 2JJ3 and 1N46 are consistent, and the effect estimates of A, H, D and E were significantly positive. In other words, the binding ability of PCNs to estrogen (2JJ3) and thyroid receptor (1N46) is significantly positive. Thus, increasing the *para*-substituent can enhance the binding ability of PCNs to 2JJ3 and 1N46 according to the symmetry of PCNs. The estimated values of chlorine substituents (A and B) were positively related to the binding ability between PCNs and adrenoceptor (6IN0), indicating that their binding ability could be enhanced by increasing the *ortho*-substituent. Similarly, the *ortho*-substituent (AB) and *para*-substituent (EH) could increase the combined binding ability between PCNs and three tested endocrine receptors. In addition, Fig. 3 also showed that the position of PCNs' chlorine substituents had the same second-order interaction effects on their binding ability to estrogen (2JJ3) and thyroid receptor (1N46). The second-order interaction effect values of *ortho*-substituent (BC and FG) were significantly negative with respect to the binding ability of PCNs to estrogen (2JJ3) and thyroid receptor (1N46), indicating that their binding ability could be reduced by reducing the number of ortho chlorine substituents. The estimated values of *para*-substituent (BF and CG) were negatively related to the binding ability between PCNs and adrenoceptor (6IN0), which demonstrated reducing the number of para chlorine substituents could reduce the binding ability between PCNs and 6IN0. Reducing chlorine substituents in *ortho*-position (BC) and *para*-position (BF) can increase the combined the binding ability between PCNs and three endocrine receptors. To sum up, the information of the influence of chlorine substituents on the combined binding ability contained the information of single binding ability between PCNs and the receptors. The ortho- and *para*-positions of chlorine substituents contained the main structural information affecting the binding ability of PCN molecules to endocrine receptors, and the order of the effect was *ortho*-position > *para*-position. It can be seen from 3.1.1 that electrostatic is the main reason affecting the binding of molecules to receptors. As shown in Fig. 3, electrostatic amino acids mainly interact with ortho substituents (such as AB and ah sites) in the docking process of PCNs molecules with three endocrine receptors, while electrostatic amino acids interact with ortho substituents except for ortho chlorine substituents in the process of PCNs molecules acting on 1N46 receptors. In addition to the interaction, it also interacts with para chlorine substituents (such as BF site). It can be inferred that ortho chlorine substituents are the most important groups affecting the binding ability of PCNs to endocrine receptors, followed by *para*-position chlorine substituents. Therefore, the binding ability of PCN homologues to endocrine

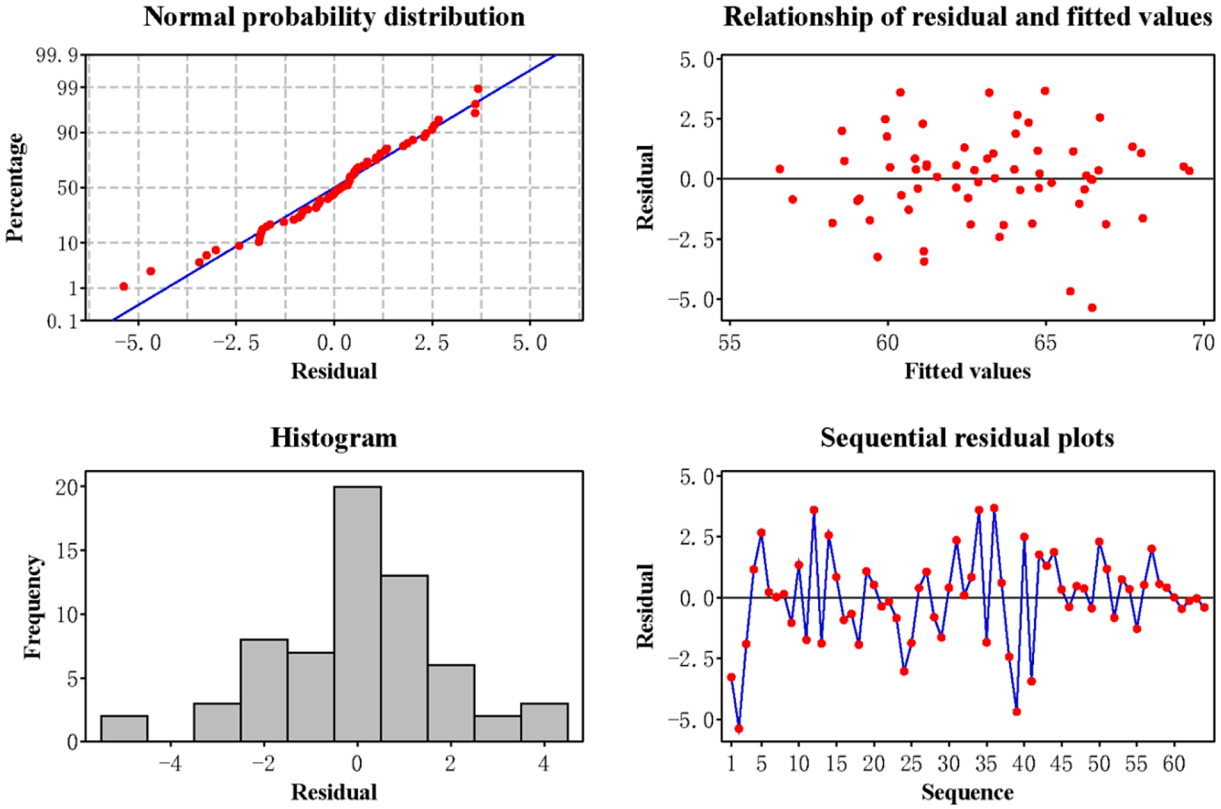

Fig. 2. Residual map of the LibDock score between PCNs and estrogen receptor (2JJ3) adaptability test.

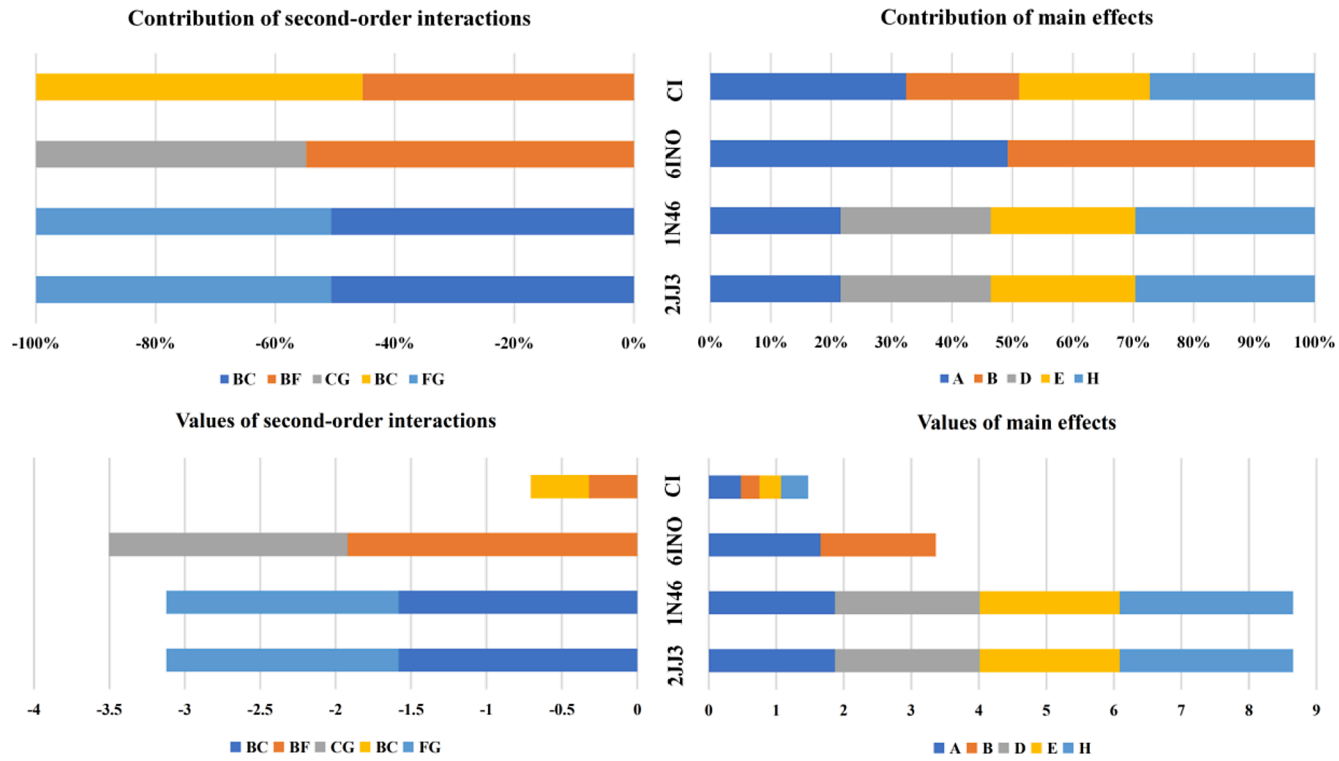

Fig. 3. The main and the second order interaction effect of the position of the chlorine substituent of PCNs on its interaction with endocrine receptors Note: (position-1: A; position-2: B; position-3: C; position-4: D; position-5: E; position-6: F; position-7: G; position-8: H).

receptors can be significantly regulated through adjusting the chlorine substituents in the ortho- and *para*-positions. The logarithms of ortho (N_o) and para (N_P) chlorine substituents were selected to explore the influence of PCN structure parameters on their endocrine disruption.

3.1.3. Screening of key factors affecting PCN endocrine disruption using PCA

The appropriate descriptors were selected to construct the QSAR model of PCN endocrine disruption based on the ligand-receptor interaction mechanism and the information of chlorine substituents determined by the molecular docking and fractional factorial design. It will quantify the main factors on the toxic pathway relating to PCN endocrine disruption, provide theoretical basis for constructing a screening method for PCNs as potential endocrine disrupting chemicals (EDCs), and then supplement the toxic mechanism of PCNs on human through endocrine disruption.

According to the results of molecular docking (3.1.1) and fractional factorial experimental design (3.1.2), physical and chemical parameters ($logK_{ow}$, M_W), quantitative parameters (μ, Q_{xx}, Q_{yy}, Q_{zz}, Q_{xy}, Q_{yz}, Q_{xz}, E_{HOMO}, E_{LUMO}, △E, Energy gap, q^-, q^+, qH^+) and substituent parameters (N_o, N_P, $N_{chlorine}$) were selected as the main factors affecting the single and combined PCN endocrine disruption (2JJ3, 1N46 and 6IN0). It will achieve the combination of macro and micro parameters, so as to more comprehensively cover the characteristic parameters affecting the endocrine disruption of PCNs. It can be seen from Fig. 4 that the single and combined endocrine disruption of PCNs on estrogen receptor (2JJ3), thyroid receptor (1N46) and adrenoceptor (6IN0) can be analyzed through four PCs. The PCA of the main factors affecting PCN endocrine disruption on 2JJ3 and 1N46 showed that most of the parameters (E_{HOMO}, E_{LUMO}, Energy gap, △E, Q_{xx}, Q_{yy}, Q_{zz}, $logK_{ow}$, M_W, N_o, $N_{chlorine}$) in the PC1 had higher load coefficients (0.835–0.997), which explained that the correlation between these parameters and PC1 was better, and the corresponding characteristic root and interpretation degree were also higher, reaching 10.210 and 60.057 (Table S3), respectively. In PC2, q^+ and q^- had larger load matrix coefficients, while PC3 and PC4 were only related to Q_{yz} and μ, respectively. Thus, E_{HOMO}, E_{LUMO}, Energy gap, △E, Q_{xx}, Q_{yy}, Q_{zz}, Q_{yz}, $logK_{ow}$, M_W, N_o, $N_{chlorine}$, q^+, q^- and μ were the main factors influencing PCN endocrine disruption on estrogen receptor (2JJ3) and thyroid receptor (1N46). Similarly, the main factors influencing the effect of PCNs on adrenoceptor (6IN0) (i.e., E_{HOMO}, E_{LUMO}, Energy gap, △E, Q_{xx}, Q_{yy}, Q_{zz}, Q_{xz}, Q_{yz}, $logK_{ow}$, M_W, N_P, N_{Cl}, q^+, q^-, qH^+) and combined endocrine disrupting-mechanism (i.e., E_{HOMO}, E_{LUMO}, Energy gap, △E, Q_{xx}, Q_{yy}, Q_{zz}, Q_{xz}, Q_{yz}, $logK_{ow}$, M_W, N_P, N_o, N_{Cl}, q^+, q^-, qH^+) were shown in Fig. 4.

3.2. Characterization of combined endocrine disruption of PCNs

3.2.1. Correlation analysis between PCN biotoxicity and ligand-receptor binding ability

The chlorine isomers of PCNs consist of eight species (i.e., MoCNs to OCN). The average biotoxicity values ($logEC_{50}$) and LibDock scores between PCNs and target proteins (2JJ3, 1N46 and 6IN0) were calculated. The correlation analysis between PCN biotoxicity and LibDock scores of different receptors was carried out to determine the toxicity intensity of PCNs to different endocrine hormone receptors through endocrine interference effects (Fig. S4). As shown in Fig. S4, the correlation coefficient R of linear fitting between $logEC_{50}$ and LibDock scores of ligands (PCNs) - receptor (2JJ3, 1N46 and 6IN0) was 0.5558, 0.7620 and 0.9534 ($n = 8/8/8$, $P = 0.2/0.05/0.0001$, $r_{min} > r_0$: 0.5070/0.7070/0.9250), indicating that PCNs has observable toxicity to estrogen receptor (2JJ3), thyroid receptor (1N46) and adrenoceptor (6IN0) through endocrine interference effects. The higher the binding ability was, the stronger the toxicity of PCNs to endocrine receptors was. The order of the significance between toxicity and binding ability of ligand-receptor was adrenoceptor > thyroid receptor > estrogen receptor.

In addition, three natural endocrine disruptors: estrone (E1), estradiol (E2) and estriol (E3) were selected as the reference chemicals to compare their endocrine disruption ability with that of the CN-71. The binding energy of CN-71, E1, E2 and E3 molecules with the estrogen receptor (2JJ3), thyroid receptor (1N46) and adrenal receptor (6IN0)

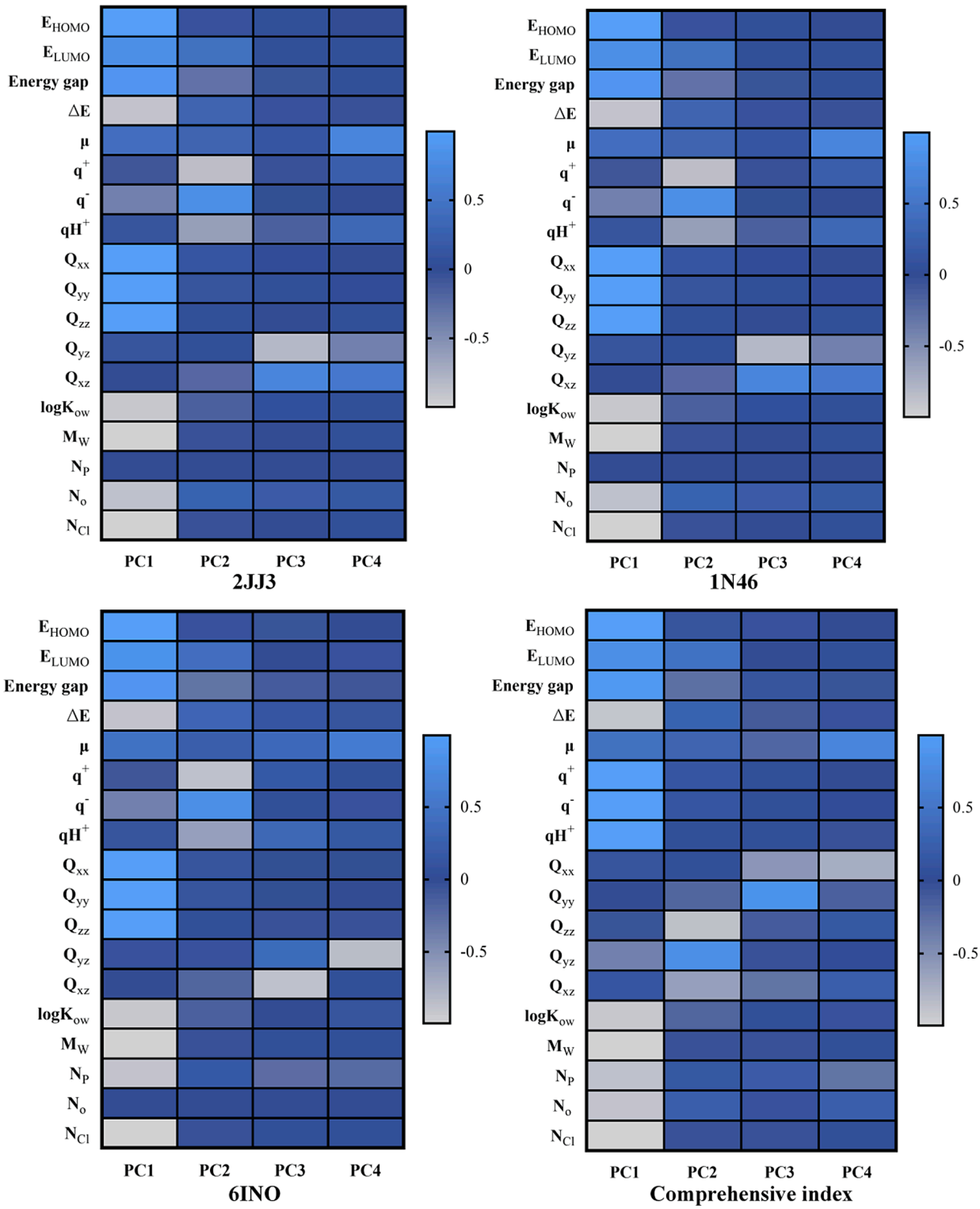

Fig. 4. Screening of physicochemical parameters affecting the endocrine disrupting effect of PCNs on human.

Table 1
Binding energies of CN-71, E1, E2, E3 with 2JJ3, 1N46, 6IN0 receptors.

Endocrine disruptors	Binding energy of 2JJ3 (kJ/mol)	Change rate (%)	Binding energy of 1N46 (kJ/mol)	Change rate (%)	Binding energy of 6IN0 (kJ/mol)	Change rate (%)
CN-71	-127.804	–	-117.643	–	-60.313	–
E1	-33.049	74.14	-101.141	14.03	-42.542	29.46
E2	-43.829	65.71	-57.126	51.44	-12.028	80.06
E3	-123.33	3.50	-91.498	22.22	-43.311	28.19

were calculated, respectively through molecular dynamics simulation method (showed in Table 1). The comparison of their binding energies showed that the binding energies of CN-71-2JJ3, CN-71-1N46 and CN-71-6IN0 were lower than those of E1 (docked to 2JJ3, 1N46 and 6IN0), E2 (docked to 2JJ3, 1N46 and 6IN0) and E3 (docked to 2JJ3, 1N46 and 6IN0), with a decreasing range of 3.50–80.06%. As concluded by Takamatsu et al. (2006), the binding energy between protein and naphthalene molecule represented the strength of the interaction between protein and molecule, and the complex with a higher absolute value of binding energy could have a stronger binding ability. Thus, CN-71 had the stronger interference effects on human internal secretion in comparison with those of the three reference chemicals.

3.2.2. Calculation of the comprehensive index of PCNs' endocrine disruption to human

Comprehensive evaluation is targeting on the comprehensive and holistic assessment of target system described by multi-attribute architecture. In this study, the comprehensive index (CI) was used to represent the endocrine disruption to human of estrogen receptor, thyroid receptor and adrenoceptor. The results in Section 3.2.1 showed that there was a significant positive correlation between the PCN biotoxicity and the above three receptors through endocrine interference effect. The order of PCN biotoxicity produced by endocrine disruption was adrenoceptor > thyroid receptor > estrogen receptor. Thus, a hierarchical model was constructed, and the comprehensive interference mechanism of PCNs on human endocrine hormones was taken as the target layer. The weight was calculated based on the n-order pairwise comparison matrix (Eq. (1)) (Cavallo and D'Apuzzo, 2012) and the paired comparison of different endocrine receptors (Table 2). After performing the consistency test, results showed that the random consistency index = 0.003 < 0.01, indicating the satisfactory consistency was acceptable. Therefore, the weight vector = (0.3292, 0.9295, 0.7490).

The calculation of the weight vector:

$$A=\begin{Bmatrix}1 & 1/3 & 1/5\\ 3 & 1 & 1/2\\ 5 & 2 & 1\end{Bmatrix}\rightarrow\begin{Bmatrix}0.111 & 0.1 & 0.118\\ 0.333 & 0.3 & 0.294\\ 0.556 & 0.6 & 0.588\end{Bmatrix}\rightarrow\begin{Bmatrix}0.11\\ 0.31\\ 0.58\end{Bmatrix}=W \tag{12}$$

$$AW=\begin{Bmatrix}1 & 1/3 & 1/5\\ 3 & 1 & 1/2\\ 5 & 2 & 1\end{Bmatrix}*[0.11 \quad 0.31 \quad 0.58]^T=\begin{bmatrix}0.33\\ 0.93\\ 1.75\end{bmatrix} \tag{13}$$

$$\lambda=\frac{1}{n}\sum_{i=1}^{n}\frac{AW}{W}=3.004 \tag{14}$$

$$CI=\frac{\lambda-n}{n-1}=0.002 \tag{15}$$

$$CR=\frac{CI}{RI}=0.003 \tag{16}$$

The weights of PCN biotoxicity on estrogen receptor (2JJ3), thyroid receptor (1N46) and adrenoceptor (6IN0) were calculated based on the AHP method. The LibDock scores of 75 PCNs with the above three receptors were calculated using fuzzy comprehensive evaluation method. The endocrine disruption (CI values) of each PCN molecule was then obtained. The CI values of 75 PCNs were shown in Table 3. Table 3 showed that the LibDock scores between PCNs and TR (1N46) were the highest, followed by estrogen (2JJ3) and thyroid receptors (1N46), showing that the binding ability between PCNs and thyroid receptor was

Table 2
Paired comparison of different endocrine receptors.

Receptors	2JJ3	1N46	6IN0
2JJ3	1	1/3	1/5
1N46	3	1	1/2
6IN0	5	2	1

Table 3
Calculation of comprehensive binding capacity between PCNs and endocrine receptors based on AHP and fuzzy comprehensive evaluation.

Compounds	Position of chlorine substitution	2JJ3	1N46	6IN0	Comprehensive index
CN-1[a]	1-CN	56.9976	63.5441	43.4219	0.33
CN-2	2-CN	58.2747	62.4574	44.0354	0.33
CN-3[a]	1, 2-DiCN	57.7025	67.6941	42.6140	0.39
CN-4[a]	1, 3-DiCN	62.4144	67.0775	45.6774	0.49
CN-5[a]	1, 4-DiCN	58.9573	65.6848	46.5907	0.47
CN-6[a]	1, 5-DiCN	59.6175	66.8465	41.5154	0.35
CN-7[a]	1, 6-DiCN	62.7280	64.6227	48.0730	0.51
CN-8[a]	1, 7-DiCN	58.1190	68.8081	46.8878	0.53
CN-9[a]	1, 8-DiCN	61.2815	66.1864	42.9829	0.39
CN-10	2, 3-DiCN	56.1351	66.1328	44.9361	0.41
CN-11	2, 6-DiCN	56.4285	64.801	45.5272	0.41
CN-12	2, 7-DiCN	64.0005	65.6248	34.9931	0.18
CN-13[a]	1, 2, 3-TriCN	57.7124	70.1094	46.7195	0.55
CN-14[a]	1, 2, 4-TriCN	63.1095	70.5111	47.1422	0.60
CN-15[a]	1, 2, 5-TriCN	59.3728	67.2935	47.3741	0.52
CN-16[a]	1, 2, 6-TriCN	60.2753	65.3884	48.0062	0.51
CN-17[a]	1, 2, 7-TriCN	62.3059	71.4693	44.9747	0.55
CN-18[a]	1, 2, 8-TriCN	63.3999	70.6400	44.4364	0.53
CN-19[a]	1, 3, 5-TriCN	63.4118	72.0929	46.8523	0.62
CN-20	1, 3, 6-TriCN	68.6501	62.9028	33.9120	0.12
CN-21	1, 3, 7-TriCN	60.7143	69.978	48.9157	0.62
CN-22	1, 3, 8-TriCN	67.0264	69.2464	47.6818	0.61
CN-23	1, 4, 5-TriCN	62.4008	69.0725	43.7622	0.47
CN-24	1, 4, 6-TriCN	61.7414	65.5588	46.9688	0.49
CN-25	1, 6, 7-TriCN	60.279	68.3706	48.2725	0.57
CN-26	2, 3, 6-TriCN	61.8385	66.7249	47.1725	0.52
CN-27[a]	1, 2, 3, 4-TetraCN	61.6518	69.9354	42.3604	0.45
CN-28[a]	1, 2, 3, 5-TetraCN	61.6981	69.3208	48.8832	0.62
CN-29[a]	1, 2, 3, 6-TetraCN	58.3283	72.3018	48.6397	0.64
CN-30[a]	1, 2, 3, 7-TetraCN	63.9918	72.9962	49.0739	0.70
CN-31[a]	1, 2, 3, 8-TetraCN	62.7127	68.0313	45.2836	0.50
CN-32[a]	1, 2, 4, 5-TetraCN	62.9161	70.3820	48.9082	0.64
CN-33[a]	1, 2, 4, 6-TetraCN	65.7831	68.5919	44.4197	0.50
CN-34[a]	1, 2, 4, 7-TetraCN	66.4281	71.4945	44.7353	0.57
CN-35[a]	1, 2, 4, 8-TetraCN	66.7677	67.9290	45.5594	0.53
CN-36[a]	1, 2, 5, 6-TetraCN	65.9767	68.8849	41.5139	0.43
CN-37[a]	1, 2, 5, 7-TetraCN	56.4153	64.6267	49.1702	0.50
CN-38[a]	1, 2, 5, 8-TetraCN	64.7600	69.8079	49.4032	0.66
CN-39[a]	1, 2, 6, 7-TetraCN	63.7254	70.8364	44.8229	0.54
CN-40[a]	1, 2, 6, 8-TetraCN	63.6407	71.8384	44.6091	0.56
CN-41[a]	1, 2, 7, 8-TetraCN	64.5972	71.0631	47.0115	0.61
CN-42[a]		61.3591	66.0832	45.3410	0.45

(continued on next page)

Table 3 (*continued*)

Compounds	Position of chlorine substitution	2JJ3	1N46	6IN0	Comprehensive index
	1, 3, 5, 7-TetraCN				
CN-43[a]	1, 3, 5, 8-TetraCN	61.7471	69.4984	45.8689	0.54
CN-44[a]	1, 3, 6, 7-TetraCN	63.9436	69.5848	46.2756	0.56
CN-45[a]	1, 3, 6, 8-TetraCN	65.2254	72.0022	46.6176	0.62
CN-46	1, 4, 5, 8-TetraCN	62.0250	66.7428	47.3955	0.53
CN-47	1, 4, 6, 7-TetraCN	65.0211	72.3529	46.8255	0.64
CN-48	2, 3, 6, 7-TetraCN	66.8020	69.6515	44.9825	0.54
CN-49[a]	1, 2, 3, 4, 5-PentaCN	60.5485	65.6767	48.1118	0.52
CN-50[a]	1, 2, 3, 4, 6-PentaCN	61.7972	65.8639	50.2029	0.59
CN-51[a]	1, 2, 3, 5, 6-PentaCN	61.1010	64.1537	45.9723	0.43
CN-52[a]	1, 2, 3, 5, 7-PentaCN	69.8848	74.8558	45.6128	0.68
CN-53[a]	1, 2, 3, 5, 8-PentaCN	59.7505	63.5946	47.133	0.45
CN-54[a]	1, 2, 3, 6, 7-PentaCN	69.0956	72.2552	47.7111	0.68
CN-55[a]	1, 2, 3, 6, 8-PentaCN	69.2713	71.6835	41.6567	0.51
CN-56[a]	1, 2, 3, 7, 8-PentaCN	64.4033	72.4009	46.3344	0.62
CN-57[a]	1, 2, 4, 5, 6-PentaCN	66.4374	64.3432	47.6323	0.52
CN-58[a]	1, 2, 4, 5, 7-PentaCN	63.7516	75.9281	44.6465	0.63
CN-59[a]	1, 2, 4, 5, 8-PentaCN	56.5477	63.5217	48.9076	0.48
CN-60[a]	1, 2, 4, 6, 7-PentaCN	64.8573	61.7630	49.2087	0.50
CN-61[a]	1, 2, 4, 6, 8-PentaCN	61.1090	63.3189	47.7704	0.47
CN-62	1, 2, 4, 7, 8-PentaCN	61.2602	71.8369	45.3668	0.56
CN-63[a]	1, 2, 3, 4, 5, 6-HexaCN	69.0895	76.6707	54.8981	0.96
CN-64[a]	1, 2, 3, 4, 5, 7-HexaCN	51.8687	74.3244	54.6770	0.81
CN-65[a]	1, 2, 3, 4, 5, 8-HexaCN	63.8340	66.1507	50.6704	0.62
CN-66[a]	1, 2, 3, 4, 6, 7-HexaCN	61.2153	78.3181	49.1999	0.79
CN-67[a]	1, 2, 3, 5, 6, 7-HexaCN	63.4155	70.7378	54.3551	0.80
CN-68[a]	1, 2, 3, 5, 6, 8-HexaCN	56.9976	63.5441	43.4219	0.33
CN-69[a]	1, 2, 3, 5, 7, 8-HexaCN	58.2747	62.4574	44.0354	0.33
CN-70	1, 2, 3, 6, 7, 8-HexaCN	57.7025	67.6941	42.6140	0.39
CN-71	1, 2, 4, 5, 6, 8-HexaCN	62.4144	67.0775	45.6774	0.49
CN-72	1, 2, 4, 5, 7, 8-HexaCN	58.9573	65.6848	46.5907	0.47
CN-73	1, 2, 3, 4, 5, 6, 7-HeptaCN	59.6175	66.8465	41.5154	0.35
CN-74[a]	1, 2, 3, 4, 5, 6, 8-HeptaCN	62.7280	64.6227	48.0730	0.51
CN-75[a]	1, 2, 3, 4, 5, 6, 7, 8-OctaCN	58.1190	68.8081	46.8878	0.53

[a] Training set.

the strongest, and that of adrenoceptor was the weakest. In addition, it can be seen from Fig. 1 that in the docking process of PCNs with three endocrine receptors, the types and quantities of amino acids that play an important role in the binding of PCNs to 1N46 receptors are the most, followed by 2JJ3, and finally 6IN0. Therefore, it can be inferred that the type and quantity of amino acids involved in the binding process between PCNs and endocrine receptors were the main factors for determining their binding ability. The more types and number of amino acids mean a stronger binding ability.

3.3. Analysis of single and combined endocrine disrupting-mechanism of PCNs on humans based on QSAR model

3.3.1. Establishment of QSAR model of PCN endocrine disruption

In the QSAR model, single binding ability (LibDock scores) and the combined binding ability (Table 3) were used as the dependent variables, and the factors affecting the binding ability of PCNs on estrogen receptor (2JJ3), thyroid receptor (1N46) and adrenoceptor (6IN0) were used as independent variables. The binding energy data were randomly divided into training and test sets for further validation (Li et al., 2022a). This model revealed the mechanical action of both single and combined endocrine disrupting effects of PCNs on three receptors, and quantified the impacts of the main factors influencing PCN endocrine disruption (Table 4).

Results of QSAR models showed that the correlation coefficients were 0.695, 0.709, 0.795 and 0.781 ($n = 51$, $p = 0.001$, $r_{min} = 0.695$ / 0.709 / 0.795 / 0.781 > r_0: 0.447), which met the statistical requirements. The dependent and independent variables met the relationship of 5:1, the Sig. were 0.003, 0.003, 0.000, 0.000 (<0.05), respectively, passing the significance test (Gu et al., 2021). The Equation (17) showed that the coefficients of ΔE, μ, Q_{xx}, Q_{yy}, Q_{yz}, q^+, $logK_{ow}$, N_o and N_{Cl} were positive, whereas the coefficient of E_{LUMO} was negative, indicating ΔE, μ, Q_{xx}, Q_{yy}, Q_{yz}, q^+, $logK_{ow}$, N_o and N_{Cl} imposed a positive effect on PCN endocrine disruption to 2JJ3 expect E_{LUMO}. The binding ability of PCNs onto 2JJ3 can be decreased by reducing ΔE, μ, Q_{xx}, Q_{yy}, Q_{yz}, q^+, $logK_{ow}$, N_o and N_{Cl} of PCNs or increasing its E_{LUMO}, and then the toxic effect of PCNs on 2JJ3 will be reduced. Moreover, E_{LUMO}, △E and q^+ were the main factors affecting the biotoxicity of PCNs on estrogen receptor, that is, the gain and loss of electronic capability was the main factor affecting the toxic effect of PCNs on 2JJ3 ($\Delta E > E_{LUMO} > q^+$). The diminution of molecular ΔE, μ, Q_{xx}, Q_{yy}, Q_{yz}, $logK_{ow}$, N_{Cl} and N_o, or increasing the E_{LUMO} and q^+ will decrease the binding ability between PCNs and 1N46, and then reduce the biotoxicity of PCNs to the thyroid receptor. The binding ability of PCNs to 6IN0 will be decreased with increasing E_{LUMO}, ΔE, Q_{xx}, Q_{yz}, q^+, q^-, qH^+ and N_P or reducing $logK_{ow}$ and N_{Cl} of PCNs, indicating the biotoxicity of PCNs to 6IN0 could be reduced. The single biotoxicity effect of PCNs on 1N46 and 6IN0 was mainly related to their electronic parameters ($E_{LUMO} > \Delta E > q^+$).

As opposed to the single effect models of PCNs to endocrine receptor, Equation (20) showed that the increasing E_{LUMO}, q^+, qH^+, Q_{xy} and Np of PCNs or decreasing their ΔE, Q_{xx}, Q_{yy}, $logK_{ow}$, N_{Cl}. N_o can reduce the CI

Table 4
QSAR model of single and combined effects of PCNs to human estrogen receptor (2JJ3), thyroid receptor (1N46) and adrenergic receptor (6IN0).

2JJ3	$LibDock = 75.479–888.646E_{LUMO} + 1244.759\Delta E + 0.157\mu + 0.190Q_{xx} + 0.102Q_{yy} + 0.436Q_{yz} + 158.566q^+ + 0.629logK_{ow} + 3.773N_{Cl} + 0.432N_o$	(17)
1N46	$LibDock = –32.526–2118.888E_{LUMO} + 1618.221\Delta E + 0.912\mu + 0.531Q_{xx} + 0.552Q_{yy} + 0.593Q_{yz} – 172.649q^+ + 1.182logK_{ow} + 0.634N_{Cl} + 1.126N_o$	(18)
6IN0	$LibDock = –3.644–117.269E_{LUMO} – 151.439\Delta E – 0.159Q_{xx} – 0.786Q_{yz} – 71.411q^+ – 10.878q^- – 31.636qH^+ + 0.327logK_{ow} + 1.241N_{Cl} – 0.162N_p$	(19)
Joint	$CI = –4.001–67.970E_{LUMO} + 48.428\Delta E + 0.011Q_{xx} + 0.016Q_{yy} – 3.070q^+ – 0.898qH^+ + 0.042logK_{ow} + 0.131N_{Cl} + 0.067N_o – 0.009N_p$	(20)

of PCNs to endocrine receptors. This will further reduce the joint biotoxicity of PCNs to human. The QSAR model of PCNs' combined toxicity to endocrine receptors covered the information of each single toxicity model. The PCNs' combined toxicity (10) ($E_{LUMO} > \Delta E > q^{+}$) was affected by the ability of gaining and losing of electrons. E_{LUMO} is related to the ionization potential of compounds, and it characterize the sensitivity of compounds to electrophilic reagents. The compound with higher E_{LUMO} will be easier to give electrons and more reactive (Karelson et al., 1996). Common electrophilic agents inchlorineude cations (such as H^{+} and NO_2^{+}), polar molecules (such as hydrogen halides, halogenated hydrocarbons, acyl halides, and carbonyl compounds), polarizable intermediate electric molecules, oxidants (such as organic peroxy acids), reagents without octahedral electrons (such as carbene and free radicals), and some Lewis acids. However, PCNs, as a halogenated hydrocarbon, belong to electrophilic agents. Thus, E_{LUMO} is the main structural parameter affecting the toxic effects of PCNs on three endocrine receptors. However, in the single effect model and comprehensive model, E_{LUMO} was negatively correlated with the endocrine disruption of PCNs in this study (Equation 17–20), which may be due to the fact that nuchlorineeophilic targets were much more than electrophilic targets in biological organisms (Moosus and Maran, 2011). Based on the above results, the toxic effects of PCNs on human endocrine receptors can be controlled by indirectly regulating the electronic gain and loss ability of PCNs, the number and location of chlorine substituents and their enrichment ability on endocrine receptors.

3.3.2. Evaluation of prediction ability and application domain of QSAR models

According to Fig. 5, the experimental and predicted results of PCNs' endocrine disruption are fitting well in the QSAR models (n = 75, p = 0.001, r_{min} = 0.6876/0.7903/0.5876/0.7744 > r_0 = 0.370). The results further verified the reliability of the above QSAR models for PCNs as a screening method for potential EDCs. Leverage is a technique used to assess the scope of application of the QSAR model. The standardized residual δ and the leverage value h_i help to characterize the application domain of the QSAR model, which can be further used to verify the robustness of the model. Fig. 6 explained that the scope of application was determined by the leverage alert value (h^* and δ), in which the abscissa h^* was 1 and the ordinate δ is ±3 (Yang et al., 2020). All data points of constructed QSAR models were within the applicable range, and innumerable sites deviated from the defined interval. This indicated that four QSAR models had good robustness and wide application range, and they were reliable for EDCs screening of PCN compounds.

3.4. QSAR mechanism analysis for PCN endocrine disruption

The results from QSAR models of PCN endocrine disruption (Section 3.3.1) showed that the combined model of PCNs on endocrine receptors contained the information of single model of PCNs on each receptor. The priority pollution control of 75 PCN homologues was carried out from the perspective of endocrine disruption based on the results of their CI values. It was found that the combined binding ability between CN-71

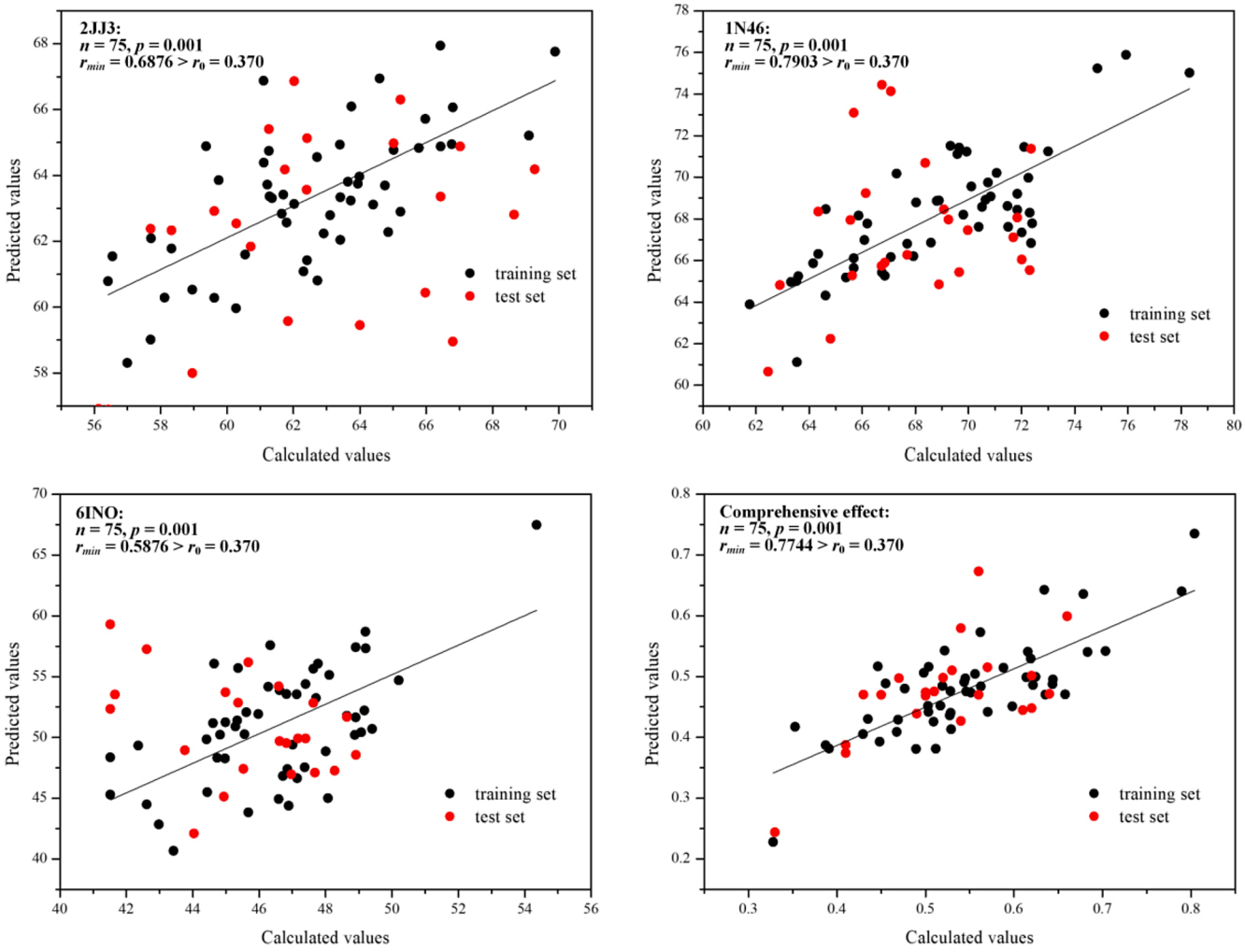

Fig. 5. Linear fitting diagram of QSAR model.

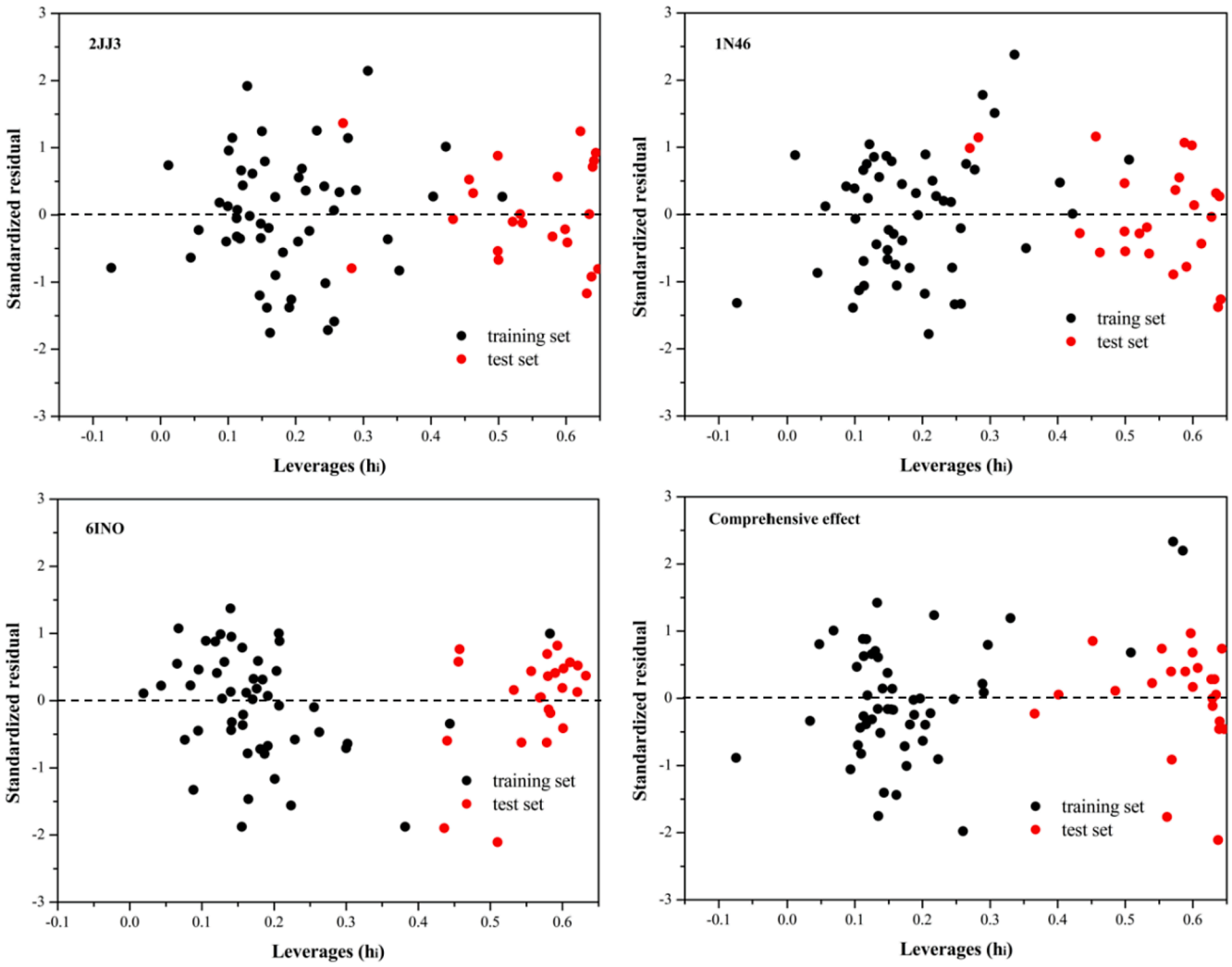

Fig. 6. William's diagram of QSAR model application domain.

compound and three endocrine receptors was strongest, which was considered as the preferred pollutant among the 75 PCN homologues in terms of human health risk. Fig. S5 displayed that the endocrine disruption of PCNs on human was enhanced with the increase of the number of chlorine atoms, indicating the toxic effect of high chloronaphthalene on human was higher.

Previous study showed that the binding between EDCs and endocrine receptors was the key step for them to produce endocrine disruption (Li et al., 2010). The binding affinity of protein and naphthalene were represented by binding energy (Takamatsu et al., 2006). At present, the main source of PCNs is thermal industrial processes, such as metal smelting. Studies have shown that workers in the environment of metal smelting and benzene compounds should eat more foods with high protein, low fat and rich vitamins (Pu, 2010). Thus, the MD simulation was used to simulate the effects of the main components of typical foods (high protein: low-fat milk and eggs; vitamins: apple and kiwi fruit; immunity enhancement: Tea, Lycium barbarum and ginseng) on the binding ability between PCNs and endocrine receptors. The best nutritious diet was selected to prevent the health risk of workers in PCN-contaminated areas.

Firstly, this study used the MD method to simulate the binding process of complexes between protein–protein complex (2JJ3, 1N46 and 6IN0) and CN-71 molecule in the blank group and experimental group (adding the main components of above typical foods), respectively. The change of binding energies between the protein–protein complex and CN-71 (taking the CN-71 as an example) were then obtained. The main components of typical foods which significantly decreased the binding energy were determined. According to the nutritional composition table, it was found that the main components of low-fat milk (whey protein) were β-lactoglobulin (50–55%) and α-lactalbumin (20–25%). The main components of eggs were ovalbumin and ovotransferrin. The main components of apples were carotene and vitamins A, B, C. The main components of kiwi fruit were vitamins C, E. The main components of tea were catechin, caffeine, vitamins C, E, pyrroloquinoline quinone and folic acid. The main components of Lycium barbarum were carotene, vitamins A, C, E, B_2. The main components of ginseng were ginsenoside (rg3, rh3, rb1 and rg1). The results showed that the reduction range of binding energies (-141.305 KJ/mol, -159.623 KJ/mol, -147.931 KJ/mol, -158.192 KJ/mol, -163.668 KJ/mol, -146.352 KJ/mol, -145.036KJ/mol) was -0.81% to 14.36% compared with the blank group (-165.002 KJ/mol), when the main components of the above foods were added in order. When α - lactalbumin, vitamins A, B_{12}, C and carotene were added as vitamins, and carotene, vitamins A, C, E, B_2 and ginsenoside were added as immunity-enhancing substances, the binding energy of the complex decreased significantly (more than 10%). Among them, the binding energy of the complex decreased significantly (>10%) after adding the components of low-fat milk (β-lactoglobulin and α-lactalbumin), apple (vitamins A, B_{12}, C and carotene) Chinese wolfberry (vitamins A, C, E, B_2 and carotene) and ginseng (ginsenoside). Therefore, the binding energy of the complex between protein–protein

complex and CN-71 molecule was calculated using MD simulation under different food collocation schemes generated by Taguchi experimental design (Fig. 7), so as to determine the best nutritious diet for preventing health risk of the workers in PCN- contaminated sites.

The signal to noise ratio (SNR) in Taguchi design identify the control factors during the variable process through minimizing the uncontrollable factors (noise factors) (Bastani and Jahan, 2021). The higher value of SNR, the smaller the fluctuation is, indicating the robustness of the obtained binding energy is better under designed conditions. Therefore, the SNR was used to evaluate whether the proteins-molecule complexes' binding energy meets the standard (Fig. 7). Among the eight experimental groups (Fig. 7), the SNR values of the complexes are consistent with the values of binding energy. It can be seen from Fig. 7 that the binding ability between PCNs and endocrine receptors can be reduced through adding the other recommended schemes except for Experiments 5 and 7. The change rate of binding energies ranged from -2.63% to -16.35% in comparison with that of control group (without any food ingredients mentioned above). What's more, when the main components of apple and Lycium barbarum (carotene, and vitamins A, B_{12}, C, E, B_2) were added at the same time, the binding ability between PCNs and human endocrine receptors was significantly reduced (-16.35%). This indicated the health risk of PCNs to human was significantly reduced. Therefore, it can be speculated that vitamins and carotenes are the main reasons for significantly reducing the binding ability between PCNs and human endocrine receptors. Vitamins and carotenes can be used as the best recommended nutrient combination scheme to prevent the health risk of workers in PCN-contaminated sites. β-lactoglobulin, α-lactalbumin, vitamins, carotenes and Ginseng should not be recommended to intake together in PCN-contaminated areas.

In order to explore the reasons why the supplementary of carotene, vitamins A, B_{12}, C, E, B_2 significantly reduce the binding ability between PCNs and human endocrine receptors, the molecular docking method was used to dock the above nutrients with endocrine receptors (2JJ3, 1N46 and 6IN0). The results showed that except vitamin B_{12} and carotene could not complete the docking process with 2JJ3 receptor, other molecules could bind to three kinds of endocrine receptors. The docking score of these nutrients to target receptors was 0.59–94.26% higher than that of PCNs molecules. A higher docking score means a higher binding affinity between the chemicals and receptors (Li et al., 2022b). Therefore, it can be inferred that the above nutrients can more easily bind to the endocrine receptor, occupy the binding site and reduce or prevent the binding of CN-71 molecule to the endocrine receptor. To further explain the above results, the E_{LUMO} values of the above nutrients were then calculated by DFT method. After calculation, it was found that the E_{LUMO} values of vitamin A (-0.1833), vitamin B_{12} (-0.3116), vitamin C (-0.2313), carotene (-0.1682), vitamin E (-0.1812) and vitamin B_2 (-0.2312) were higher than those of CN-71 (-0.2381). It further reveals the reason that the simultaneous addition of vitamin A, vitamin C, carotene, vitamin E and vitamin B_2 significantly reduces the binding ability of PCNs to human endocrine receptors. The above nutrients are more likely to have electrophilic reactions with endocrine receptors than that of CN-71. Thus, they can easily occupy binding sites and inhibit the binding of CN-71 to endocrine receptors.

To verify that changing of nutrients dose may affect the binding ability of PCN-endocrine disrupting receptor under the complementary nutrition scheme, we adjusted the dose of the optimal nutrition scheme selected in the manuscript. The optimal combination scheme suggested the consumption of apple and wolfberry vitamins (vitamin A, vitamin C, vitamin E, vitamin B_{12} and vitamin B_2) and carotene at the same time. The protein–protein docking of endocrine receptor (1N46, 2JJ3 and 6IN0) was conducted to obtain a new complex using the ZDock method in Discovery Studio® 2021. The binding energy of the new complex was reduced as high as 138.020KJ/mol under the optimal combination scheme. On this basis, the main components of apple and wolfberry were added according to the ratio of 3:1 and 1:3 through the molecular dynamic simulation method. The binding energy of CN-71-complex was -7.750 kJ/mol and -248.482 kJ/mol under the ratio of 3:1 and 1:3, respectively. It is further demonstrated that changing the dose of nutrient has a significant effect on the binding ability between PCNs molecules and endocrine receptors. In our further studies, we will continue to explore the effect of changing the dose of nutritional supplements on the effect of complementary diet schemes.

Moreover, we found the proteins/enzymes related to the absorption (intestinal enzyme: 5UDY), distribution (plasma protein: 6QS9), metabolism (human cytochrome P450 2D6 enzyme: 2F9Q) and excretion (lyase: 1JBQ) of nutrient. The selected proteins from four routes (i.e., absorption, distribution, metabolism and excretion) were docked to the three receptors complex (Complex C) to form Complex A. And then molecular dynamics simulation was used to study the effect of the binding ability of PCN molecules to human endocrine receptors complex under different delivery routes and the best nutrients supplement scheme. The results showed that the binding energy of Complex A was -1.331 kJ/mol, and the absolute value of the binding energy was significantly lower than that of Complex C (without delivery routes) (-138.020 kJ/mol) under the optimal nutrients supplement scheme. The complex with higher absolute value of binding energy will have stronger

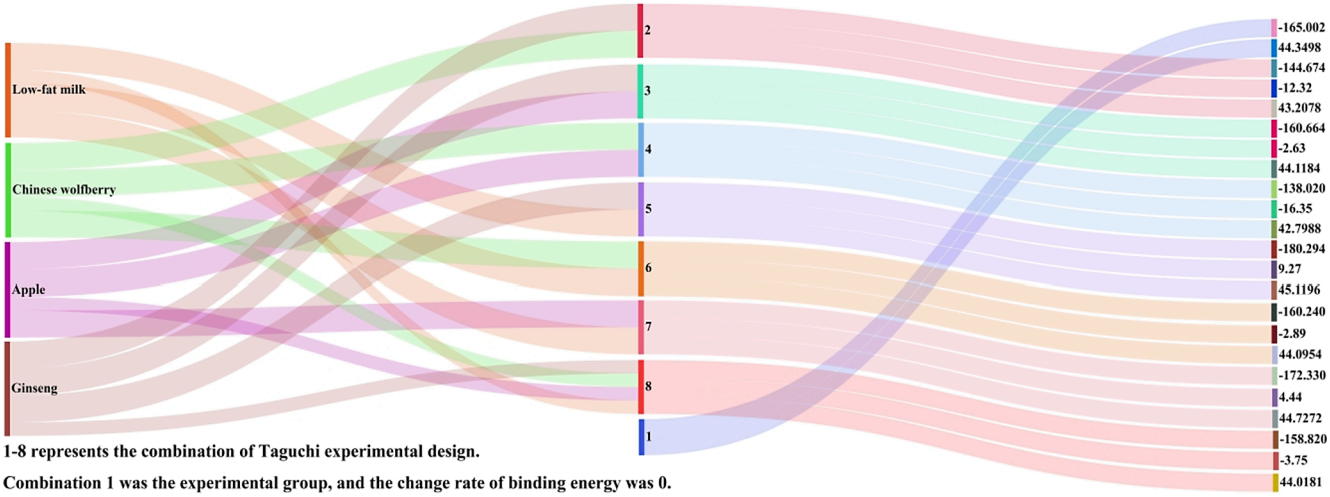

Fig. 7. Calculation of binding energy and SNR of the docking system of endocrine receptor protein complexes and CN-71 molecule under different composition combinations.

binding ability, which further shows that the ADME process has a significant impact on the effect of the nutrients supplement scheme.

4. Conclusion

This study investigated the combined disrupting mechanism of PCNs on human endocrine systems by the QSAR modeling based on the LibDock score. MD simulation was also applied to recommend strategies of nutritional supplementation to help reduce the health risks of workers exposed to PCNs. The findings showed that the lowest occupied orbital energy (E_{LUMO}) was the most important factor affecting the toxicity of PCNs on endocrine receptors, followed by the orbital energy difference (ΔE) and positive Millikan charge (q^+). Among the 75 PCN homologues prioritized from the perspective of endocrine disruption, the CN-71 molecule was considered the priority pollutant to cause endocrine disruption. The binding ability of PCNs on endocrine receptors was significantly decreased by 16.45% with the supplementation of carotene and vitamins (A, B_2, B_{12}, C, and E). These nutritional components were more prone to nucleophilic reactions than PCNs, which could prevent the binding between PCNs and endocrine receptors and further reduce endocrine disruption. Vitamins and carotenes could be used as nutrition candidates to reduce the health risk of the people working in PCN-contaminated sites. In this study, we explored the toxicity mechanism of PCNs on the human endocrine system through in-silico methods and generated the theoretical strategies of nutritional supplements for the reduction of health risks caused by PCNs contamination. Our modeling results could provide a new aspect to advance the theoretical understanding of the health risks of PCNs contamination, particularly given that experimental work on PCNs' endocrine disruption on humans is unavailable mainly due to regulatory, technological, and ethical limitations. The best-recommended nutrient combination scheme suggested in this study presented a feasible modeling approach for efficiently screening the nutrients to reduce the human health risks of PCNs to humans. Meanwhile, the findings of this modeling study would provide insightful knowledge and valuable data and guidance for experimental work, which are still necessary and important for validation and potential application. It is desired to make future efforts in both modeling and experiment particularly through integrating the screen-out nutrient schemes with experiments to find the best options of nutrients and dosage to achieve the goal of the human health risk reduction.

CRediT authorship contribution statement

Xixi Li: Conceptualization, Methodology, Software, Writing – review & editing, Writing – original draft. **Wenwen Gu:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing – original draft. **Baiyu Zhang:** Formal analysis, Writing – review & editing. **Xiaying Xin:** Writing – review & editing. **Qiao Kang:** Data curation. **Min Yang:** Software. **Bing Chen:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Yu Li:** Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107291.

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